3/30/49.

100 mosaics of H168' on lactose streak-dextrin on Lac EMB. If possible, one + and one - obtained from each and tested on other sugars.

Almost all were Xyl - Mtl - and Gal = Lac. Exceptions noted.

		Lac	Gal	
1-20.	A	20-	20-	} 2 - unpaired ≈ A
	B	18+	18+	

#15A is Xyl +/- ; Mtl +/- . Streaks out to purify.
≈ Mtl +/-

21-40.	A	20-	20-	1 unpaired ≈ A
	B	19+	19+	

2A is Xyl +/- Mtl +/- ? Mtl +!

9A ; 9B are Xyl + Mtl + (Conclusion?)

41-60	A pair	14-	14-	All X -
	B pair	14+	14+	M -
	A	3-	3-	
	B	3+	3+	

61-80		~~16~~		4 unpaired.
	A	20-	20-	
	B	16+	16+	All X - M -

81-100	A	20-	20-	All X -
	B	20+	20+	1A Mtl + Xyl - . TS^R

Hold all indicated exceptions.

All above tested on TS, but all appeared resistant. (probably n.s. ?).

81-100 Retested on TS (on Ara EMB).

	A. (Lac-)	15 R	5 S ↓ 86, 89, 90, 92, 94	(Lac+)

B: All 20 showed an S reaction (V_{ic}^R type)

#84 and 85 appeared to be mixed. Streaked these out.

[parentals in excess]

~~Plate 414 c 0881.~~

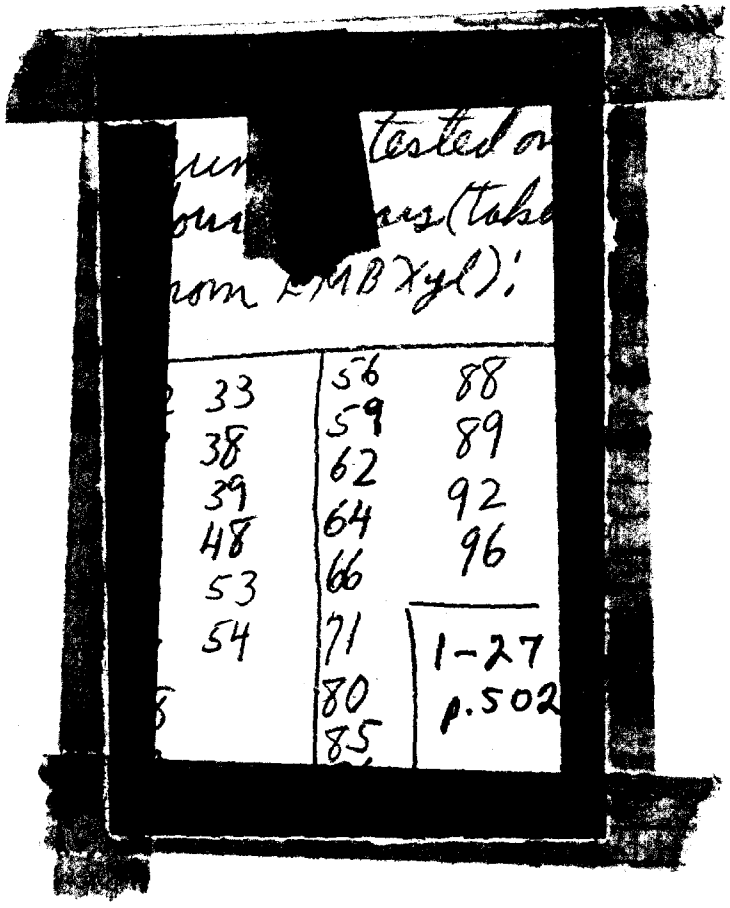
M168 Xyl selections.

5/2/49.

Mosaics picked from Lac selection plates. Streaked out on Xyl EMB. 100 streaked. Separable Xyl+/- in 27 tested first. 17 appeared pure - (replaced c additional mosaics). 56 required to separate a + and -. 49 separated; ∃ 1 came pure + (pair c - corresponding to it). 6 still too crowded.

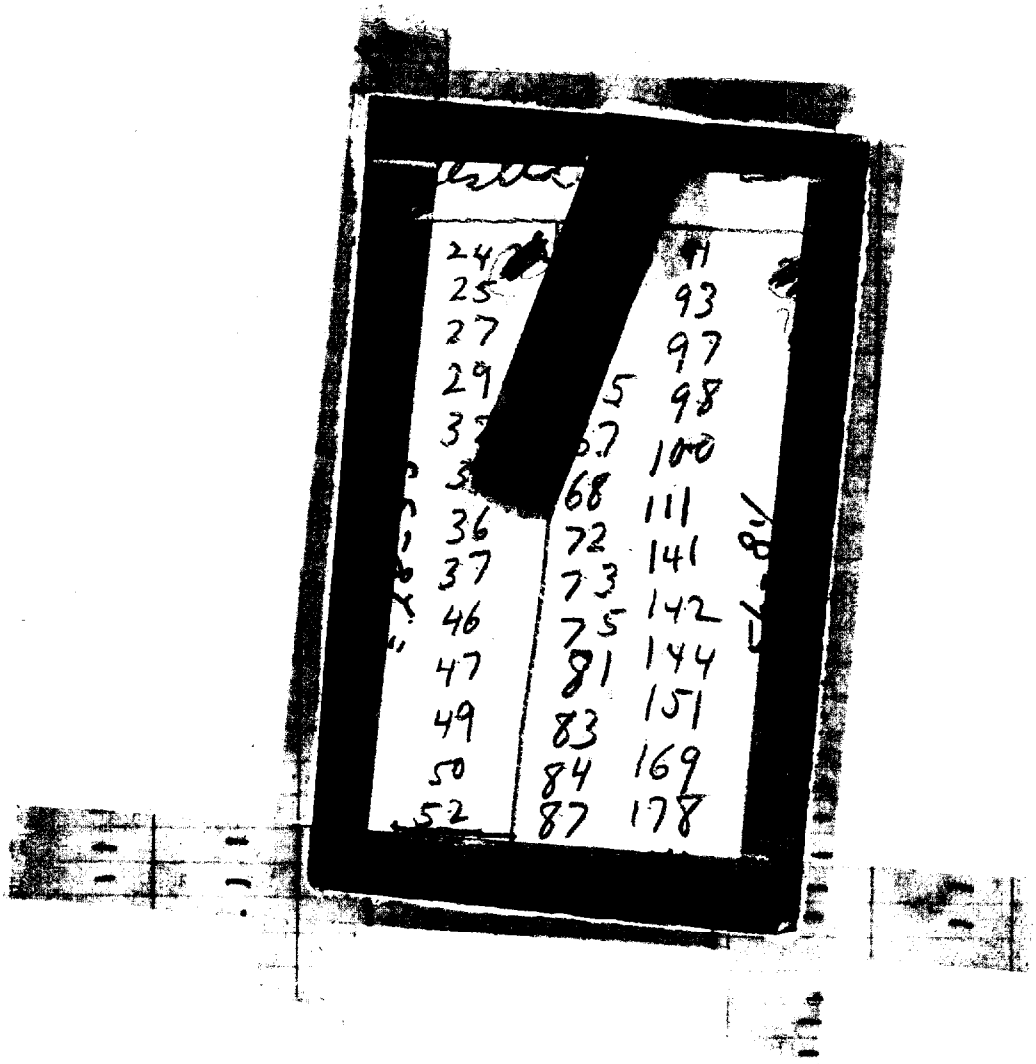
	Xyl- selection: Xyl	lac	Gal	Mtl	Xyl+ selection: X	L	B	Mtl.	
1		−	−	−	+	−	−	+	
2	−	−	−	−	+	−	−	+	
3	−	+	+	−	+	−	−	+	
4	−	−	−	−	+	−	−	+	
5	−	−	−	+	+	−	−	+	
6	−	+	+	−	+	−	−	+	
7	−	−	−	−	+	−	−	+	
8	−	−(+)	− ✓	−	+	−	−	+	
9	−	−	−	−	+	−	−	+	
10	−	+	+	−	+	−	−	+	
11	−	−	−	−	+	−	−	+	
12	−	−	−	+	+	−	−	+	
13	−	+	+	−	+	−	−	+	
14	−	+	+	−	+	−	−	+	
15	−	+	+	−	+	−	−	+	
16	−	+	+	−	+	−	−	+	
17	−	−	−	−	+	−	−	+	
18	−	+	+	−	+	−	−	+	
19	−	−	−	−	+	−	−	+	
20	−	−	−	−	+	−	−	+	
21	−	+	+	−	+	−	−	+	
22	−	+	+ ✓ −	−	+	+	*+ (−?)	+	Gal − ✓
23	−	+	−	−	+	−	−	+	
24	−	+	+	−	+	−	−	+	
25	−	+	+	−	+	−	−	+	
26	−	+	+	−	+	−	−	+	
27	−	+	+	−	+	−	−	+	

Lac: 12 - 115 +.
almost all exceptional Xyl + are Lac +
Gal - Lac almost completely linked
(2 c.o. / 54 tests)


tested o
ns (tabl
rom EMBXyl);
33
38
39
48
53
54
56
59
62
64
66
71
80
85
88
89
92
96
1-27
p.502

~~#5~~ a
502

	Xyl+				Xyl−			
	Xyl	Lac	Gal	Mtl	Xyl	Lac	Gal	Mtl
28	+	−			−	+	+	−
29	+	−				+	+	
30	+	−	−			−	−	
31	+	−	−			−	−	
32	+	−	−			−	−	
33	+	−	−			+	+	
34	+	−	−			−	−	
35		−	−			−	−	
36		−	−			−	−	
37		−	−			−	−	
38		−	−			+	+	
39		−	−			+	+	
40		−	−			−	−	
41		−	−					
42		−	−			−	−	
43		−	−			−	−	
44		−	−			−	−	
45		−	−			−	−	
46		−	−			−	−	
47		−	−			−	−	
48		−	−			−	−	
49		−	−			−	−	
50		−	+			+	+	
51		−	−			−	−	
52		−	−			−	−	
53		−	−			−	−	
54		−	−			+	+	
55		−	−			−	−	



85-92

35
94
99
143
170
174
179
195

93-98

3
82
131
145
155
177

99 76
100 115

	Xyl+				Xyl−			
	Xyl	Lac	Gal	Mtl	X	L	B	M
56	+	−	−	+	−	−	−	−
57						−	−	
58						+	+	
59						−	−	
60						+	+	
61						−	−	
62						−	−	
63						−	−	
64						+	+	
65						+	+	
66						−	−	
67						−	−	
68						+	+	
69						−	−	
70						+	+	
71						+	+	
72						−	−	
73						+	+	
74						−	−	
75						+	+	
76						−	−	
77						−	−	
78						−	−	
79						+	+	
80						+	+	
81						+	+	
82				+		+	+	
83				−		+	+	
84				+		−	−	
85	+	−	−	+	−	−	−	−
86	+	−	−			−	−	
87	+	−	−			+	+	
88	+	−	−			+	+	
89	+	−	−			−	−	
90	+	−	−			−	−	
91	+	−	−			+	+	
92	+	−	−			−	−	
93	+	−	−	+		+	+	+
94		−	−			−	−	
95		−	−			−	−	
96		−	−			+	+	
97		−	−			−	−	
98		−	−			+	+	
99	+	−	−	+	−	+	+	−
100	+	−	−	+	−	−	−	−

(...177)

4/5/49.

22, 23, 12,5, 83 showed unusual combinations:

Retest:

#22: Lac#Gal- (Xyl#). Xyl#: 12 tested. 1 Lac-Gal-
11 Lac#Gal#

Xyl-: 24 tested 16 ##
8 —

No reciprocals found. No other example of the unusual combination.

23: Lac# Gal- Xyl#: 9 tested 1
8 —

Xyl-: 16 13 ##
3 —

#5 ~~12~~: ~~Xyl~~-Mtl# Xyl- 1 Mtl#
5 Mtl-

Xyl# 6 Mtl#

#12 ~~x5~~ Xyl-Mtl# Xyl # 10 Mtl# + 2 Mtl + Xyl+
Xyl- 4 Mtl- Sum: 6 Mtl-
1 Mtl# 3 Mtl#
2 mixed Mtl#,- : Mtl+ : Xyl+
Mtl - : Xyl -

83 Xyl#Mtl-

No clear Xyl# could be found. Xyl- 20 Mtl-.

The streaked sectors from which these colonies were taken were reexamined to determine the prevalence of these unusual types, and the possible occurrence of the reciprocals.

a. Reciprocals were not found at all.
b. Recurrence of rare types was noted. (Total of 2/15 possibilities for Xyl-Mtl#).
Therefore each mosaic colony does represent a limited sample. Size??

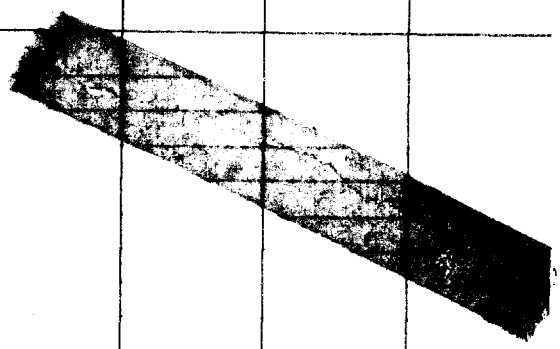
single

See page following 577

Map Xyl-ctr.

4/6/49.

58-161 x W677 on EMS:
Lac, Xyl, Mtl, Mal.
Use .1 ml 4x mixture / plate
Lac B$_1$, use .05 ml or
dilution = .01 ml (D).



Cpunts:

Xyl EMS: #	-
11	1
5	0
8	0
6	2
16	8
7	2
11	1
16	1
14	1
94	17

(sic)

Mtl EMS: #	-
0	54
3	27
2	17
0	14
1	18
6	130

MalEMS #	-
2	17
5	13
3	19
2	45
12	94

Lac EMS #	-
6	26
4	22
4	10
3	17
7	41
24	116

Lac EMS B$_1$ #	-
22	129
23	96
27	113
32	111
57	196
15	98
33	128
209	871

D:	
9	26
21	41
12	31
10	27
16	21
68	146

Xyl "+" picked. */23 were predominantly + on Xyl EMB.

		Xyl	Mtl	Mal	Sal	Lac				
	1	o	o	−	o	o				
	2	+	+	−	−	−				
	3		o	+	+	+				
	4		+	+	+	+				
	5			+		+				
	6			+		+				
	7			+		+				
	8			+		+				
	9			+		+				
*	10			−	+	−	*	+	+	−	−
**	11			+		+		+	+	−	+
*	12			+		+					
	13			+		+					
	14			+		+					
	15			+		+					
*	16			+		+					
	17			+		+					
*	18		−	+	−	−					
**	19		+	+	+	+					
*	20		+	+		+					
	21		+	+		+					
*	22		+	+		+					
*	23		+	+		+					

* Check as (-1)

Mal EMS. + picked.

	Lac	Xyl	MHl	Mal	Gal
1	+	−	−	+	−
2	−	−	−	+	−
3	+	+	+	+	+
4	−	−	−	+	−
5	−	−	−	+	−
6	−	−	−	+	−
7	−	+	+	+	−
8	−	+	−	+	−
9	−	+	−	+	−
10	−	−	−	+	−
11	−	−	−	+	−
12	−	−	−	+	−

MHl EMS. + picked.

1	−	+	+	−	+
2	−	+	+	−	+
3	−	+	+	+	+
4	−	+	+	+	−
5	−	−	+	−	+
6	+	+	+	−	−

Lac + from EMS.

	Lac	Gal	Mal	Xyl	Mtl
1	+	-	-	-	-
2		-	+	+	-
3		-	-	-	-
4		-	-	-	-
5		-	-	-	-
6		-	-	-	-
7		-	+	-	-
8		+	+	+	+
9		+	+	+	-
10		-	-	-	-
11		-	-	-	-
12		-	-	-	-
13		+	+	+	+
14		+	+	+	+
15		-	+	-	-
16		+	-	+	+
17		-	-	-	-
18		+	+	+	+
19		+	+	+	+
20		-	-	-	-
21		-	-	-	-
22		-	-	+	+
23		-	-	-	-
24		-	-	-	-
25		+	+	+	-
26		-	+	-	-
27		-	-	-	-
28		-	-	-	-

Lac – from EMS

	Lac	Xyl	Mtl	Mal	Gal
1	—	–	–	–	–
2		–	–	–	–
3		–	–	–	–
4		–	–	–	–
5		–	–	–	–
6		+	+	–	–
7		–	–	–	–
8		+	–	–	–
9		–	–	–	–
10		–	–	–	–
11		–	–	–	–
12		–	–	–	–
13		–	–	–	–
14		–	–	–	–
15		+	+	+	+
16		–	–	–	–
17		–	–	–	–
18		–	–	–	–
19		–	–	–	–
20		+	+	+	+

Lac –:

	Xyl	Mtl	Mal	Gal
21	—	—	—	—
26				
31				
36				
41				

Lac – Gal relationships.

Among	28 Lac+,	9 Gal+	; 21 Gal–
Among	37 Lac–,	2 Gal+	35 Gal–

Mal relationships.

LacB, Verified Lact.

	Gal	Mal	Xgl	Mtl	26-50	Gal	Mal	Xgl	Mtl
1	−	−	−	−		−	−	−	−
2	−	−	−	−		+	+	+	+
3	−	−	−	−		+	−	−	−
4	−	−	−	−		−	−		
5	−	−	−	−		−	−		
6	−	−	−	−		−	−		
7	−	−	−	−		−	−		
8	−	−	−	−		−	−		
9	−	−	−	−		−	−		
10	+	+	+	+		−	−		
11	−	+	−	−		−	−		
12	−	−	−	−		−	−		
13	−	−	−	−		−	−		
14	−	−	−	−		−	−		
15	−	−	−	−		−	−		
16	−	−	−	−		−	−		
17	−	−	−	−		−	−		
18	−	−	−	−		−	+		
19	−	−	−	−		−	−		
20	−	+	+	−		−	−		
21	−	−	−	−		−	+		
22	−	−	−	−		−	+		
23	−	−	−	−		−	−		
24	−	−	−	−		−	−		
25	−	−	−	−		−	+	−	

checked

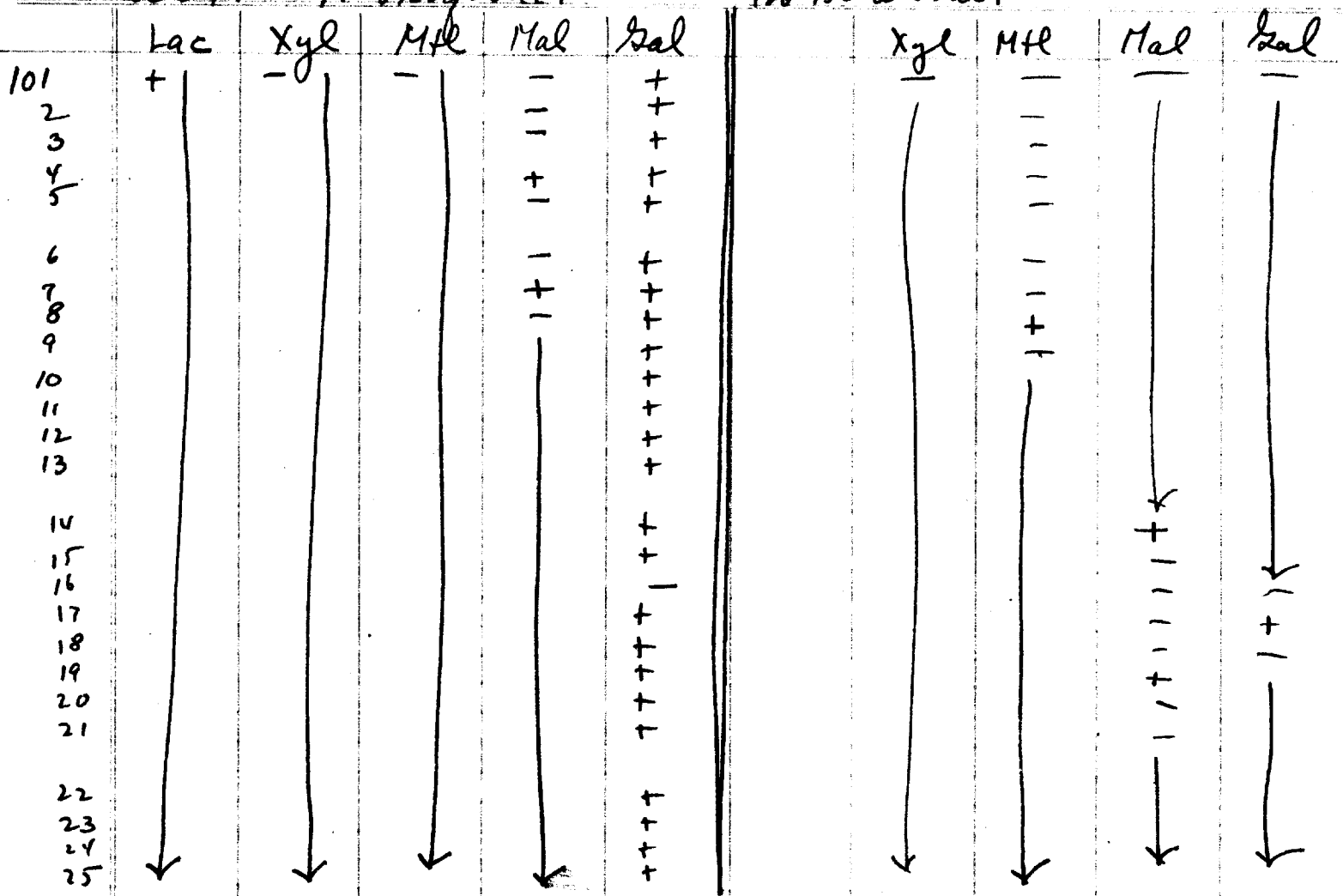
lacB, Verif. Lac+

	Gal	Mal	Xyl	Mtl	76-100	Gal	Mal	Xyl	Mtl.
51	−	−	−	−		+	+	−	−
	−	−	−	−		+	−		
	−	−	−	−			−		
	−	−	−	−			−		
~~55~~							+		
	−	−	−	−					
56	−	−	−	−			−		
	−	−	−	−			−		
	−	−	−	−			−		
	−	−	−	−			−		
	+	−	+	+			−		
	−	−	−	−			−		
	−	+	+	−			−		
~~62~~							−		
	−	+	−	−					
~~63~~	−	−	−	−			−		
	−	−	−	−			−		
	−	−	−	−			+		
	−	−	−	−			−		
	−	−	−	−			−		
	−	−	−	−	Probably		−		
	−	−	−	−			−		
72	−	−	−	−			−		
72	−	−	−	−		−	+		
73	+	−	+	+			+		
74	+	−	−	−			−		
75	−	−	−	−			−		

all opp. Gal+
ufc. #97.
check again. ✓✓

Lac EMS B, . Verif. Lac+

126-150 all Lac+



Gal difficult to score.

probably interacting with B_1 –

Lac- from EMS B, Lac. Lac verified

	Xyl	Mtl	Mal	Gal		Xyl	Mtl	Mal	Gal
1	−	−	−	−	21	−	−	−	+
2	−	−	↓	↓		−	↓	↓	−
3	−	−				−			↓
4	−	−				−			
5	−	−				−			
6	−	−			26	−			
7	−	−				+			
8	−	−	+	+		−			
9	−	−				−			
10	+	+	−	−		−			+
11	−	−			31	−			−
12	−	−				+			
13	−	−				−			
14	−	−				−			
15	−	−				−			
16	−	−			36	−			+ ?
17	−	−			37	−			
18	−	−				↓	↓	↓	
19	−	−	↓	↓					
20	−	−				−			

Summaries.

58-161 x CW671

505 X

Xyl+ EMS:

	Mtl	Mal	Gal	Lac
18	+	+	+	+
1	+	−	−	−
1	−	+	−	−

Mtl+ EMS.

	Mal	Gal	Lac	Xyl
2	−	+	−	+
1	+	+	−	+
1	+	−	−	+
1	−	−	+	+
1	−	+	−	−

Mal+ EMS] Mtl [Mal] Gal Lac Xyl

7	−	−	−	−	−
1	+	−	−	−	+
2	−	−	−	−	+
1	+	+	+	+	+
1	−	+	−	+	−

Lac+ (EMS)

5	+	+	+	+	+
15	−	−	−	+	−
1	−	+	−	+	+
3	−	+	−	+	−
2	−	+	+	+	+
2	+	−	+	+	+

Lac− (EMS)

37	−	−	−	−	−
1	+	−	−	−	+
1	−	−	−	−	+
2	+	+	+	+	+

Lac+ (B1). − − − + −

Gal inducible ē B1 -

Zello's single cell isolates

Pedigree sent.

39 < 79 < 159 het
39 < 79 < 160 – (or het)
39 < 80 < 161 het
39 < 80 < 162 het

49 < 99 < 199 – all mimics
49 < 99 < 200 het
49 < 100 < 201 het
49 < 100 < 202 het

streaks out on EMB Xyl, Lac.
X-18, 19, 19.5, 20.
X-24, 25, 26.

2-159-60-61-62.
-199-200-201-202

Regrow out on EMB to verify mosaicism.

~~Both~~ all are + and – on both media.
No mosaics noted.
X26 is pure Xyl– Lac– (does not grow HZ?)
others are mixture; no mosaics noted.

160 is stated to be pure – ? probably het? but contains a few Xyl+ papillae.
199 is pure Xyl– Lac–; others are mixed.

Disinfection of λ.

4/6/49.

Inoculate Y87 stable "UV 7" (for Esther's mutability program).

A. Pick 100 cols. and test for λ-:

Retest:

5, 6, 7, 15, 18, 19
23, 24, 28, 32, 34
45, 52, 55, 60
61, 78, 79, [illegible], 100

} and renumber 1-20.

4/9: All but 4 were either stable or λ+ on retest from cell suspensions.

Retest 1-4: all 4 λ+, ρ20^R λ^R

B. 4/8. Inoculate W588 10"-20" on EMB Lac.

20 colonies on 25" plate tested for sensitivity to ρ20.

All appeared more or less sensitive to ρ20. When tested on W518, 10 were λ+. This is not, therefore, a reliable criterion for λ-

Check other colonies directly on W518.

20" series: 37 tested none ρ20^s.

15" " 40 " " "

10" " 40 " " " (1 or 2 doubtful. Rechecks: λ+)

Apparent sensitivity may be partly an attenuation phenomenon.

p20: selection as λ+, λ−.

4/8/49.

1+2: Dilute 518 and 811 10^{-7}. Plate .1 ml ea + .1 ml p20 stock.
Control = p20.
Titrate p20 stock on 518.
Plate p20 on W811
Add .5 ml λ 10^9 to .5 ml W518 10^{-7}. Hold 10 mins. Then plate .2 ml
ea p20 to ascertain λ+.

	−P20	+P20	+λ
W518: 10^{-8}	24	0	0.
W811: 10^{-8}	19	31	

Titer p20: 10^{-7}: 253.

Selection for P20l: 10^{-1} ea W811: 173 plaques noted. Occur with a frequency ca. 10^{-6}

∴ P20 can be used to select for λ+. But 10 mins. contact of 518 ea λ does not protect it.

Also, cross-streak λ, p20 ea:

1-4 purified 518/20 isolates

		λ	p20
		1 S	R
5.	K-12	2 S	R
6.	W811	3 S	R
7	W518	4 S	R
8.	B.	5 K12 R	R
		6 811 R	R
		7 518 S	S
		8 R	S

Note: B is resistant to P20. Of B/ types.

P20l. 6 plaques picked from above plated ea W811: heavy plaquing from each, which therefore represent bonafide mutants (cf. p19). Replate single plaques. 3x on W811, once on B. Grow out on B. Loopful from last plaque gave ca. same counts on W811, W518 + B. Essentially same plating efficiency is indicated.

P20l = p21.

p20 mutants; ϕ specificities

4/9/49.

p21 = p20L described above as acting on λ+. [Not excluded that it may be a contaminant].

During titration of p20 on W518, a large plaque with translucent halo was noted. This bred true when picked + plated. Replate on W518; W811, to check identity and grow out on W518 p20 "r" finds true and is inactive on W811.
Material here for × (p20r × p21).

p20 tested ≡ B/ series.

B	S
B/1	S
B/1,5	S
B/2	S
B/3,4,7	S
B/6	R
B/69	S

Cross-reaction ≡ T6 noted.

T6 tested ≡ 518/20 4/test all T_6^R
W518 T_6^S.

V_6^R is therefore related to resistance to p20, but not to λ.

∴ p21 does not override genetic resistance

	p19B	p21
B	S	S
B/1	S	S
B/1,5	S	S
B/2	S	S
B/3,4,7	S	S
B/6	S	R
B/19B	R	S[P]

Lac = reversion.

4/9/49.

96 papillae of H190 kept a week on EMS Lac tested as before.
None were Lac⁻. None contained Xyl+.

Run unsuccessful

Displacement of λ by other φ.

4/12/45.

Test 811/x for maintenance of λ, after purification.

1. 811/~~T6~~ 28 tests all λ+
2. 811/21 8 tests all λ+
3. 811/p9 24 tests all λ+
4. 811/T7 26 tests all λ+.

∴ these phages do not displace λ.

[p14 should be tried as it appears to be lysogenic.]

4/11/49.

494-1 was Lac+ ... T_5^R // Lac- ... T_5^S +R

-2 " " " +R

-4 was indicated as pure S ! (probably +S) but checks.

Anfuist checks of 3, 4, 9, 10 all R was indicated. Recheck.

N12: Streaked out EMS colonies from 4, 5, 6, 7, 8, 12, 13 for further examination.

494:3 Lac+ : 10^R Lac- 9S 1^R

494:8 Lac+ : 10^R Lac- 10^S +R

:9 Lac+ : 10^R Lac- 10^S +R

:10 Lac+ : 10^S Lac- 4^R 6^S +S !

Most are entirely segregated, e.g. Xyl brush tests :

4	abc- d ±
5	abcd-
6	abc-
7	abcd-
8	acd- b ±
12	abc-
13	abc- d+.

4d and 8b are only hopeful.

restreak these.

4.9. 8 recoverable, but throw out.

Partial analysis of 502X. 8 % in each group.

a)	Xyl −	Lac +	36	43%
		Gal +	35	42%
		Mtl +	2	2.5%
	Xyl +	Lac +	1	1
		Gal +	0	0
		Mtl −	1	1

∴ Lac segregation is ca 40%.

Possible intype.

Xyl	Gal	Lac	
+		+	V_1
−	②	①	

		%
no c.o.	X−L−	40
①	X−L+	55
②	X+ L−	3+
①.②	X+ L+	$\ll 1$

Map Mal in Het + normal strains

4/12/49

~~EMS Mal~~ ~~Mal~~

-1 58-161 x W677 — A T(0) B T(B) C T(B.)

-2 W478 x W677.

Pick colonies randomly to EMS Lac (ē pigeon supply)

Recover from these plates to test segregations.

Summaries:

	Lac+	Lac-	Σ
(1). A	14	34	48
C	7	8	15
B	1	7	

~~Also:~~

Lac+	Lac-	Σ	
13	38	51	
7	7	14	
8	26	34	
28	71	99	28% +

(2). A

28	12	40
29	10	39
16	16	32
31	19	50
16	6	22
30	31	61
13	7	20
33	20	53
16	17	33
27	10	37
28	13	41
267 ✓	161	428

62.4% +

Plated on EMS Lac

15	11
12	15
10	14
8	10
17	20

B

A

69	40	109
31	20	51
37	21	58
137	81	218

62.8% +

C

46	67	113
36	70	106
82	137	219

37.4% +

EMSB, plates badly faded, but pink presumed + and - to Lac + Gal

EMB for conclusion:

	Lac	Gal	
1C:	30 -	29 -	1 +
	13 +	10 -	3 +
2C	19 +	13 -	6 +
	20 -	20 -	0 +

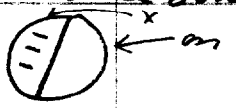
Gal + is simply infrequent and may not necessarily be linked directly to Lac; may be corresponding to Mal.

Mal difficult to score May retread ε B, -

Checks on λ- and λ^R

4/12/49.

Test each of the following for λ on W518 and sensitivity to λ, p20, p21



1. 482-1 carries R grossly.
2. 451-1 "λ^R"
3. 451-6 "λ^R" ✓
4. 449-D1 "λ- prototroph"
5. W877 carries p14

1. ~~s~~ sensitive to λ. Strongly lysogenic. ~~p~~ $p20^s$ $p21^s$
2. resistant to λ, $p20^s$ $p21^s$ not lysogenic Lac+
3. ? resistant to λ, $p20^s$ $p21^s$ not lysogenic. Lac+
   resistance should be rechecked λ^R. λ-
4. λ^s $p20^s$ $p21^s$ λ- Confirmed as λ- product of a cross.
5. λ^R $p20^s$ $p21^s$ (gross colony.). Not tested for λ+ as it carries p14.

Check single colonies: 8 all λ^R $p20^s$. Cf. 2 and 3., but these selected ☆ p14.

Check ~~4~~ 451-1 for antiphetic agent resistant to λ.

Search for λ mutants

4/12/49.

1. Mix 451-6 as λ^R & W518 as λ^S. After 2½ hr. of growth in K2, streak out to separate, & look for effect of λ-mutant possibly carried by 451-6. After first purification, test K12/21 isolates as T6 (20) and mix & W518 (50) for transmission, & as 20 Lac- (W518 λ-) recovered from mixed culture. Each one was λ^S. ∴ resistance is not transmitted extracellularly & is probably genetic. Keep 451-6 as W887

2. Displacement of λ: plate p14 & K-12; W518. No lysis noted. ~~Streak out K12~~ occasional pap. conc. noted. K12 did not show this.

Streak out p14 & K-12. No evident reactions.

p21-resistants. On the selection plate it was noticed that some of the colonies seemed to be somewhat weak fermenters. Some of these appeared to be $T6^S$. All K/21 noted were λ+. Touch of colony directly from picking needle may be a suitable method of testing λ on a large scale. Recover λ+ Lac- from lytic margins, and test for resistance to λ, p21.

Tests on ~~—~~ from 43 ~~colonies~~ gradients tested. As many as could be conveniently picked ~~from~~ cleanly were pooled for each test to ensure a thorough sample. None were $p21^R$; 3 showed signs of a λ^S component.

Conclusion Resistance to p21 is not infective; only bacterial mutations were found; no λ mutants.

~~Titrate p20s and p21.~~

4/13/49.

100 colonies of Y70 10" UV picked and dept tested for λ on W518.
1 apparent λ-. Pick, streak out, and retest.
λ+ λ^R $p20^S$. [Check on this].

	p20	λ
520-1	partial clearing	R
Y70	R	R
W518	S	S
W811	R.	R

520-1 thus shows some deviation from Y70 from which it came. (partial attenuation of λ?)

4/21/49. 219 additional tested. All were λ+.

W-1: Gal- mutants and λ disinfection attempts.

4/21/49.

20 plates W-1 on EMB Gal for - mutants. Ca 300/plate = 6000.

4 Gal- mutants noted. Unequal sizes. Purify and check Lac instability

		Glu	Ara	Gal		Lac	
1	W494	+++	+++	—		—	stable!
2	495	+++	+++	—		—	
3	496	+++	+++	—	1 papilla noted	—	
4	497	+++	+++	—		—	

A few doubtful Lac papillae noted in (1) ~~Lac+~~ ~~found~~. Isolate W502. Gal-Lac+

Disinfection. Test individual colonies by picking & needle and leaving on a ribbon of W518. (24) tested from 7 and 20 see treatments.

2 uncertain λ- Streak out on Mal EMB to verify W-1 origin and recheck.

#1 Mal+, undoubtedly W518, pushed out by stiff bunch

#2 Pure Mal-. On check was λ-. Recheck individual colonies for λ^s: All λ^R λ- ρ20^s

518

A23: 420 tested from 7 second runs: 22 possible λ- noted. Recover and recheck. Number 3-24

A24. 110 additional tests. 2 possible λ-. Streak out as 25-26.

4/24/49

R23: Test Isolates 3-24 and subculture responses.

	Mal EMB streak for purity	W518	ρ20	λ
3	Some Mal+ I.	Occ. λ plaq	S	R
4	—	λ −	R^{ρ}	
5	—	Occ. λ plaq.	S	
6	—	λ −	S	
7	—	λ −	S	
8	—	λ −	S	
9	Some Mal+	λ −	S	
10	—	λ −	S	
11	—	λ −	S	
12	—	λ +	S	
13	—	λ − diff. +	R	
14	—	λ −	S	
15	—	λ −	S	
16	—	λ −	S	
17	—	λ ±	R	
18	—	λ −	R	
19	—	λ −	R	
20	—	λ ±	R^{ρ}	
21	—	λ [illegible] −	[illegible] S	
22	—	λ −	S	
23	—	λ −	S	
24	—	λ −	S	
W-1		λ +	R	R
W518.		λ −	S	S

λ seems to have reappeared in some cultures.

Note #18, 19 which are $\rho 20^{R}$ λ −

25		R	R
26		S	R

4/16. Retests:

	ρ^{+40}	λ	$(\lambda)/518$
W-518	S	S	-
W-898	S	R	-
W-899	R	R	-
W 900	S	R	-
W 901	S	R	-
-25	R	R	-
-26.	S	R	-

cultures OK again

April 24, 1948.

P24. 4 individual colonies of W898 tested:

All are λ^R, $p20^s$. When cross-streaked with W518, there is some mild apparent discoloration; no definite plaques or inhibition.

Keep and compare # 18, 19 as $\lambda - p20^R$;
22, 23 $\lambda - p20^s$;

Picked 8 individual colonies from Mal EMB for λ. Do not check those already λ+.

W518.

	4
2	6
3	7
4	8
5	9
6	10
7	11
8	13
11	14
12	15
13	16
14	17
15	18
16	19
17	20
18	21
19	22
20	23
21	24

no lysis observed.

Note culture which was used as W518 4/25 is apparently unmixed as it tests $p20^R$ λ^R on suitably controlled plates. All cpts. which this might affect have been streaked and present minds are correct

2 Telemas W898.
18 W900
19 W901
4 W899

c. Streak out conjunction of W900 and W901, & W518 above, on Mal EMB to determine whether resistance to p20 is transmissible.

Disinfection of λ.

4/21/49.

A) Prepare T(m) Glu. Add 1:5 deoxypyridoxine (Dopy) 25 mg/ml to make final level of 5 mg/ml. Inoculate lightly ~W677

1. P21 A23: no growth. 10/10 λ+
2. P24 faint turbidity. Transfer to second tube and streak out on Lac EMB.
3. A25. turbid — 3d transfer.
4. A26: Streaks out P26. 15 tests all λ+ very thick growth in thick streak. Isolated colo. OK.

B) Grow W677 at 44-45° in # Y2 Glu, repeated transfer.

1. A22
2. P22
3. A23
4. P23
5. Late P23
6. A24
7. P24
8. A25
9. P25
10. A26

P26. Streak out B10 for examination of single colonies

15 tests all λ+!

Neither deoxypyridoxine nor high temp. succeeded in desinfecting λ.

Diffuntial phages.

4/22/49

Test plaques from Chi. sewage for diffuntial on 518, 811 +887

	λ−	λ+	λ^R

These were scored on bac EMB; as W887 is bac+, they scored very poorly.
These tests are for λ+/− diffuntial only:

A22: No λ+/− diffuntials noted in (100) tests

A23 Test plaques on λ−, λ+ and λ^R (on W3A) (160) tests. No diffuntials
1 appeared lysogenic (filled center). Streaks out as 527−1.

A24. As above. (60) tests No diffuntials.

→ Bacteria isolated not lysogenic; ~~dk~~ clear & plaques sem.
Probably resistant bacteria were picked along ~~i~~ phage from initial plaque.

A25	(67) tests.	No diff.
P26	65 tests	No diff.
P27	(28) "	" " .

420 tests No diff.

Map Mal.

4/23/49.

A B C

1. 58-161 x W677 — Mal EMS (0), (B,), (B+B,).
2. W478 x W677

Pick Mal+ to EMS DB, Lac — Additional — Totals:

Lac:		+	−	Σ	
1	A	7	4	11	} Pool
	B	38	60	98	} Pool
	C	41	44	85	
		86	108		
2	A	14	27	41	} Pool
	B	48	72	120	} Pool
	C	38	99	137	
		100	198		

Mal. Ratios from plate counts: (by D.A.S.)

		+	−		+	−		+	−	
1A		3	16	10	19	146	1C	9	19	
		2	6		17	173		8	22	
		7	10		26	114		14	22	
		1	6		77	396		12	26	
		2	14		43	248		8	32	
	2	15	52	24% +	182	1077	14.5% +	51	121	30% +
2A		9	19		85	232		51	248	
		8	22		47	208		58	244	
		14	22		60	192		65	228	
		12	26		71	244		70	~~26~~ 236	
		8	32		86	332		59	192	
		51	121	30% +	349	1208	22% +	303	1148	21% +

Picks Mal+ Lac+ and Lac- from pur. tests separately to other sugars, and confirm Mal reaction.

1 A

		7 L+ Gal	3 L- Mal	Mtl	Xyl
L+	1	+	+	–	–
	2	+	+	–	–
	3	+	+	–	–
	4	+	+	–	+
	5	+	+	–	–
	6	+	+	+	+
	7	–	+	–	–
L-	1	–	+	–	–
	2	–	+	–	+
	3	–	+	–	–
	4	–	+	–	–

1 B. L+

	Gal.	Mal	Mtl	Xyl
1	+	+	–	–
2	+	+	–	–
3	+	+	–	–
4	+	+	–	–
5	+	+	+	+
6	+	+	–	–
7	+	+	–	–
8	+	+	–	–
9	+	+	–	–
10	+	+	–	–
11	+	+	–	–
12	+	+	–	–
13	+	+	–	–
14	+	+	–	–
15	+	+	–	–
16	+	+	+	+
17	+	+	~~+~~	–
18	+	+	–	–
19	+	+	–	–
20	+	+	+	+
21	+	+	–	–
22	+	+	–	–
23	+	+	–	–
24	+	+	–	–
25	+	+	–	–
26	+	+	–	–
27	+	+	–	–
28	+	+	–	–

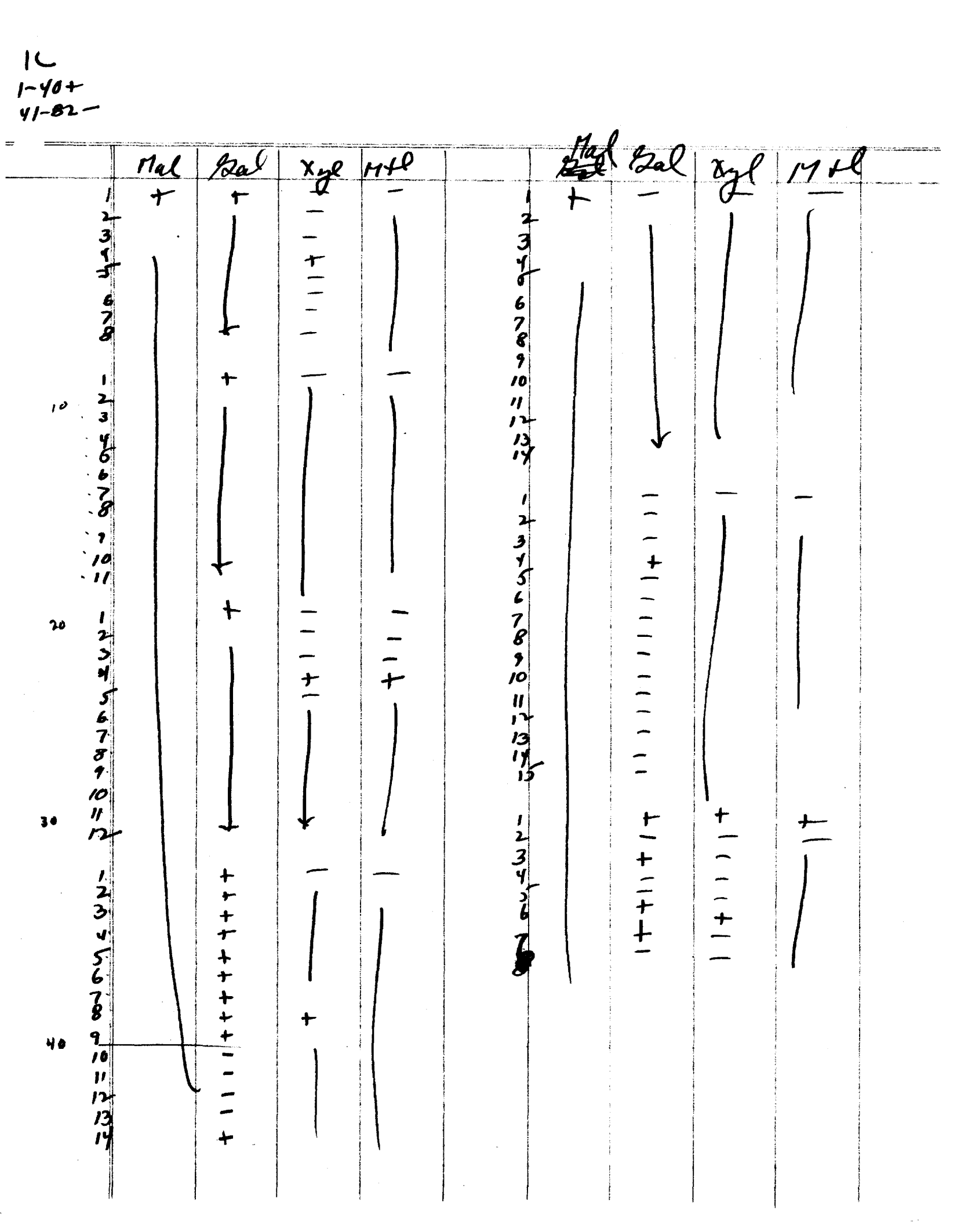
	Mal	Xyl	Gal	Mtl
29	+	–	+	–
30	+	–	+	–
31	+	+	+	+
32	+	–	+	–
33	+	+	+	–
34	+	–	+	–
35	+	–	+	–
~~36~~	~~+~~			
~~37~~	~~+~~			
~~38~~	~~+~~			
~~39~~	~~+~~			

1B
L + 36-77
L - 41 -

	Mal	Gal	Xyl	Mtl
36	+	+	−	−
37		+	−	−
38		+	−	−
39		+	+	+
40		−	−	−
41		−	−	
42		−	+	
43		−	−	
44		−	−	
45		−	−	
46		+	−	
47		−	−	
48		+	−	
49		−	−	
50		+	−	
51		−	−	
52		−	−	
53		−	−	
54		−	−	
55		−	−	
56		+	−	
57		−	−	
58		−	−	
59		−	−	
60		−	−	
61		−	−	
62		−	−	
63		−	−	
64		−	+	
65		−	−	
66		−	−	
67		+	−	
68		−	−	
69		−	−	
70		−	−	
71		−	−	
72		−	−	
73		+	−	
74		−	−	
75		−	−	
76	-?	−	−	
77		−	−	

	Mal	Gal	Xyl	Mtl
78	+	−	−	−
79		−	−	−
80		−	+	+
81		−	−	−
82		−	−	−
83		−	−	−
84		+	−	−
85		+	−	−
86		−	+	−
87		−	−	−
88		−	−	/
89		−	−	−
90		−	−	−

1C
1-40+
41-82−



2A
. 41L+
15-41L-

	Xyl	Gal	Xyl	Mtl						
1	+	+	+	+						
2			−	+						
3			+	+						
4			+	+						
5			−	−						
6			+	+						
7			+	+						
8			−	−						
1	+	+	+	+						
2			−	−						
3			+	−						
4			+	−						
5			+	+						
6			+	+						
1	+	−	−	−						
2		−	−	−						
3		+	+	−						
4		−	−	−						
5		−	−	−						
6		−	−	−						
7		−	+	−						
8		+	−	−						
9		−	−	−						
10		+	+	+						
11		−	−	−						
12										
1	+	−	−	−						
2		+	+	+						
3		+	−	−						
4		+	−	−						
5		+	+	+						
6		+	+	+						
7		+	+	+						
8		−	−	−						
9		−	−	+						
10		+, −	+	+						
1	+	−	+	+						
2		−	+	+						
3		−	+	+						
4		−	−	−						
5		−	+	+						

thick plate grows well!

2B

1-45+ (Lat)

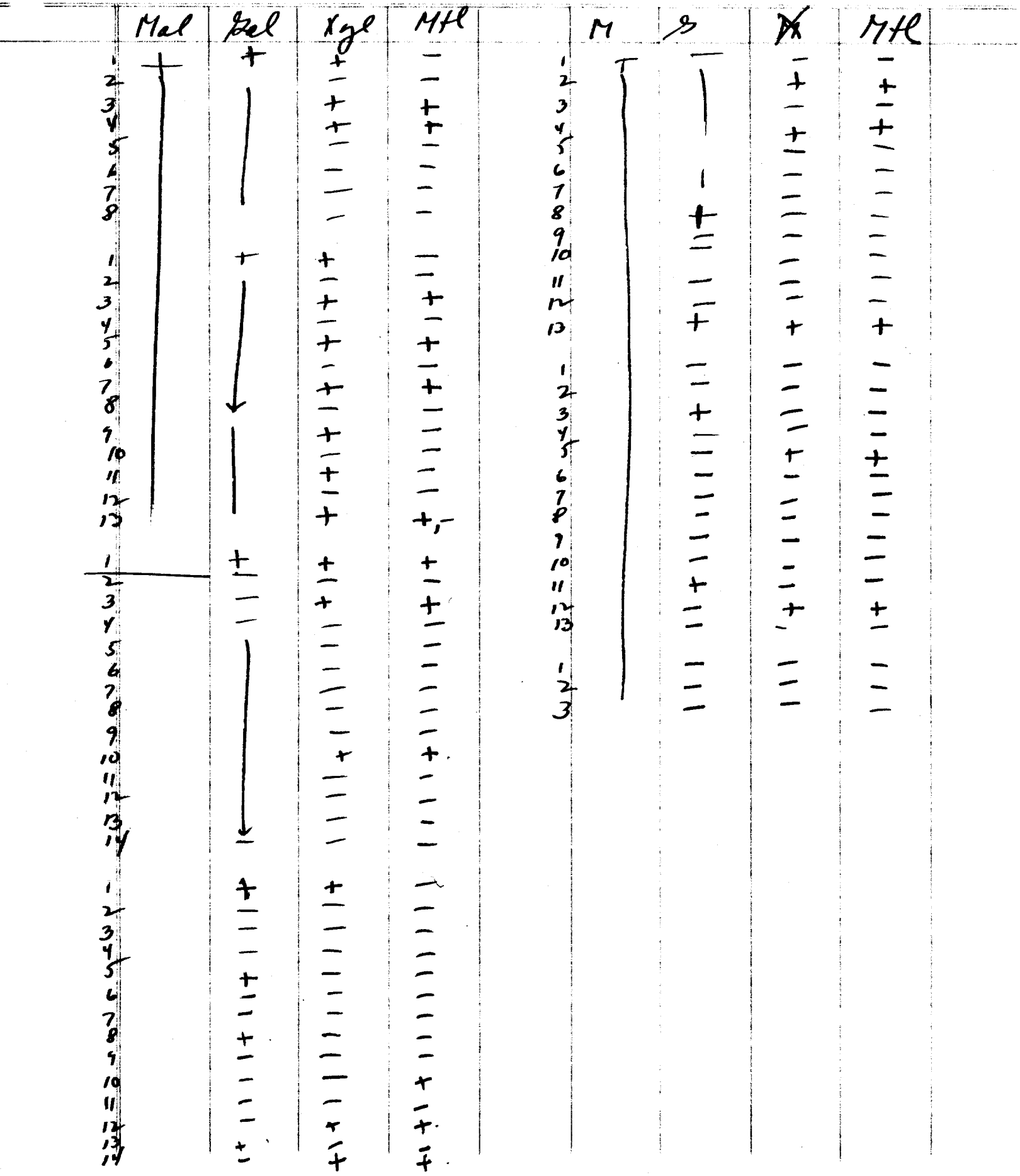
46-83-

	Gal	Xyl	Mal
1	+	+	−
2		−	−
3		+	−
4		−	−
5		+	−
6		−	−
7		−	−
8		+	+
9		−	−
1	+	−	−
2	+	+	+
3	+	+	+
4	+	+	+
5	−	±	−
6	+	−	−
7		−	−
8		+	−
9		+	−
10		+	+
11		+	−
12		−	−
1	+	−	−
2		−	−
3		−	−
4		±	−
5		−	−
6		+	−
7		+	+
8		+	+
9		+	+
10		+	+
11		+	−
12		−	−
13		+	−
14		+	−
1	+	+	−
2	+	+	+
3	+	−	−
4	−	−	+
5	+	+	−
6	+	−	+
7	−	+	−
8	+	−	+
9	−	+	−
10	−	+	−
11	−	−	−
12	−	+	+
13	−	−	+
14	+	+	+

40

	Gal	Xyl	Mal
1	−	+	+
2	−	+	+
3	−	−	−
4	−	−	−
5	−	−	−
6	−	−	−
7	−	−	−
8	+	−	−
9	−	−	−
10	−	−	−
11	−	−	+
12	−	−	−
13	−	+	+
14	−	−	−
15	−	−	−
1	−	+	−
2	−	−	
3	−	−	
4	−	−	
5	−	−	
6	+	−	
7	−	−	
8	−	+	
9	−	−	
10	−	−	
11	−	−	
12	−	−	
13	−	−	
1	−	−	−
2	−	−	
3	−	−	
4	−	−	
5	−	−	
6	−	−	

N.C
1-22L+
23-70L-



all Mal+

1A.	Lac+	Mtl-Xyl- 4		Mtl+Xyl+ 1		Mtl-Xyl+ 1	
	Lac-	3		0		1	
1B	Lac+	All Gal+	Mtl-Xyl-33	++5	−+	1	3 9
	Lac-	8 Gal+ 43 Gal-	−−	++1	−+	3	55
1C	Lac+	All Gal+	−− 37	++1	−+	2	40
	Lac-	6 Gal+ 35 Gal-	−−38	++1	−+	2	41

No apparent linkage of Mal to Lac.

2A	Lac+	All Gal+	−−3	8++	−+2	+−1
	Lac-	9+ ascor Xyl	−−14	13++	−+2	+−1

2B.	Lac+	40 Gal+ 5 Gal-	−−19	12++	−+14	+−0
	Lac-	3+ others −	−−30	5++	−+2	+−1

2L Lac+ 22 Gal+ --11 ++7 -+4

Lac- 8 Gal+ 37-- ++9 -+1 +-1
40 Gal-

Clearly the Het cross has a higher proportion of Xyl+.

Gal- and Ar- mutation runs.

4/25/49.

Y10 50 x 300 Gal EMB 4V 7 secs. 15,000 8 mutants.

A.

		Gal		Ar		Glu		Lac		Mal		Xyl	
W 909	1	−	−	++	✓	++	+	+	✓	+	−	+	
911	2	−		−	✓	−	⊕	−	✓	−	✓	+	−
912	3	−		±	✓	++	+	±	+	+	✓	+	
913	4	−		−	✓	−	−	−	✓	−	✓	−	
914	5	−		−	✓	−	−	−	✓	−	✓	−	
910	6	−	−	++	✓	++	+	+	+	+	−	+	
915	7	±	+	±	+	+	+	−	−	−	✓	−	good growth
916	8	−	-thin	−	✓	−	−	−	thin	−	✓	-thin	

B. W894 25 x 400 = 10,000. Ar EMB

W 904	1	−	✓	−		+	+	−	✓	−	✓	+
905	2	−		−		+	+	−		−		−
906	3	−		−		+	+	−		−		−
907	4	−		−	pmp?	−	+	−		−		+
908	5	−		−		−	−	−		−		−

A1 and A6 are the only ones suitable for independent matings and B1

Use 904 for conjugations

Analysis of λ− : W898; 899.

4/27/49

1. Cross W898 × W518 ; 899 × 518. mEMS Lac. All Lac −.
   11 (898×) tested. All p20^S 10 λ^R 1 λ^S
   32 (899×) " . All λ^R. 2 p20^S; 30 p20^R.
   Apparent segregation.
   Test for Mal., V. linkages.

2. Pick 898 at cross streak ± λ. Streak out. Test 20 single colonies all are λ−.

3. Test infectivity of λ^R; p20^R : Streak out portions of W518 and W899 on Mal EMB. Test Mal+ (W518):
   10 from W898 all λ^S p20^S
   10 " " " " . ∴ Resistance of W899 is not infective.

2a. Do 899 All λ− ε one doubtful possible exception. Streak this out as #533-2a. 6 colonies retested were λ−.

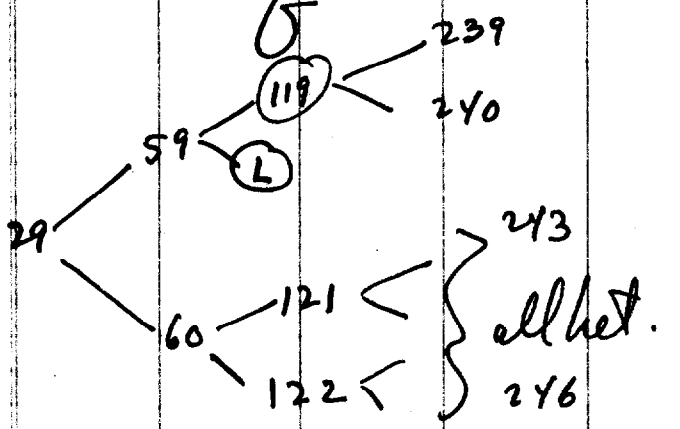
898 × #1 λ^S } Test Mal, T1.
2-11 λ^R } Mal+ V,^{R} 6 Mal− V,^{R} 4 Mal− V,^{S}

899 × 1-2 p20^S } Mal−V,^{R}; Mal−V,^{S}
others p20^R } 5. 3 Mal+V,^{R};
30 Mal−V,^{R}
13 Mal−V,^{S}
46

Zelle's series 5.

4/28/49.

Letter of 4/26



239, 240 } Pure Lac+ Xyl- Mtl- Gal- Ar- .
Check nutrition, & responses. V_1^R V_1^S (LB, BM+ . / proto)

Xylose - mutant run
Mannitol - " "

April 30, 1949.

40 x 500 = 20,000 in XylEMB. W904 7secs UV.

a) First sequence, readily isolated.

12 mutants, all Xyl- Mtl+ Glu+. W917-928.

1-12 Use 1-6 for Mtl- mutants.

b. Second sequence W929-944

1-16

May 2, 1949. Use 1-8 above (W917-924) for Mtl EMB - UV 7 sec. irradiations

Mannitol Mutants:

Spleter each x 300 = 1500 x 8 = 12,000

Tentative No.	From			
1	1	Slow		
2	1	+		
3	2	Mucoid		
4	2	"		
5	3	+		
6	3	slow		
7	3	slow		
8	4	+	Glu	Mtl
9	5	slow		
10	5	slow		
11	5	slow		
W945 12	6	—	from W922	
13	6	slow		
14	7	+		
15	8	+		

8

differential phages.

5/5/45 ff.

See:

58 test

0 acteri on W887 inhibited on T2 plates!

21 tests.

Het outcross

538

5/7/49.

W826 x

#1 WY66 — 40 tested 5/9: 2 heterozygotes: 538/11, 12. 60+ cols. tested. No heterozygotes.

2 W477 — 100+ cols. " . 5 hets. 108 tested 5/9 " 8 suspected.

Heterozygotes in cross 2 confirmed.

538: 1-5. 8 additional in second set. 6-13

W826 x WY77

~~Label~~

Het in and out cross

5/7/49

W478 x EMS lac and B_1.

a W477

b W945

	a (B) +	a (B) −	a (O) +	a (O) −
	147	40	59	5
	25	11	95	8
	244	56	71	1
	9	2	72	3
	47	11	123	2
	103	27	85	3
	51 ~~318~~	18	76	2
	226	47		
	854	212 / 1066	581	24 / 605

	b (B_1) +	b (B_1) −
	309	26
	177	49
	283	13
	364	48
	221	24
	1354	160 / 1514

	+
	163
	239
	278
	115
	179
	158
	84
	1216 / 1289

Pick + colonies and test for lac v.

1-11 12-20

A 100 11 certain 9 doubtful lac v. 1-20. off 12-20 #16 ab het others probably not.

B 148

Almost every streak has colonies which are doubtful lac v: light + variegated, mostly central. Vaguely mottled appearance reminiscent of het plates. Select most likely lac v.

Many lac stars prototrophs! probably Lac+ G− when G− is expected!

W478 x W945.

539B.

5/13/49.

8 possible heterozygotes picked and tested on var. sugars:

	Lac	Mal	Gal	Xyl	Mtl	Glu
1			−		−	+
2			−	+ v?	+ v?	+
3			−	+ v?	+ v?	+
4			−	ø v	−	+
5			+	+	+	+
6			−	+V	+	+
7			+	+	+	+
8			+	+V	+	+

no clear explanation of the lac-slow segregants.

see 553

Sorbitol Mutation Run.

May 10, 1945

W945

40 plates × 300 = 12,000 tests. UV 7 secs Sorbitol E-413

Chalcone glucose.

	Glu	T5	Sorb.
1	±	P	± or − ?
2	±	P	"
3	++	S	"
4	±	S	"
5	++	S	"
6	±	S	"
7	++	S	"
8	++	S	

Zelle's 6 and 7 series

5/11/49.

7: Sent "Mass" culture of 159-161; 175-181; 187-190; 368-8.
and "Mos. Cl." " " 175-190.
Study on EMS Xyl; EMB Xyl; EMB lac:

		Zelle Cl.	EMS Xyl	Lac	Xyl.
1	159		not		
2	160		mostly		only — recovered
3	161		all-		"
4	181 Mass		q.		"
5	181 Mos. Cl.		q.		only — recovered
6	184	Sq.	n.q.		
7	368	Sq.	nq		
8	367	Sq.	nq.		
9	195	Sq.	nq		
10	186	Sq.	nq		
11	187	Sq.	nq	—	—
12	188	Sq.	nq	—	—
13	189	Sq.	n.q.		
14	190	Sq.	n.q.		

		EMS lac		
6:	56	nq		
	58	q.		only — recovered.
	111	q.	8 1-6	only — recovered
	112	q.	7,8	lac v

Is 6 a H168 alt?

last not Xyl v !!

5/22. Recover Xyl+ colonies from EMS and S.O. EMB Xyl to check on heterozygosis.

lac v recovered from 6:111 (7,8) Streak out 7a, b 8a, b
a. from EMB lac v; b from EMS Xyl bush.

37 - segregants

		Lac	Xyl	Mal	Mtl	Ar	Gal	T5		
1.	6-58	—	—		—		—	R		
2.	7-184	—	—		—		—	R		
3.	185	—	—		—		—	"		
4	186	—	—		—		—			
5	187	—	—		—		—	"		
6	180	—	—		—		—			
7	189	—	—		—		—			
8	190	—	—		—		—	"		
9	367	—	—		—		—	"		
10.	368	—	—		—		—			

all alike.

544.

Test Het x Het

544

May 21, 1949.

W477 x

	1	W978	Gal -	477 = Mal- W478.
C	2	979	Gal -	
D	3	980	Gal -	
E	4	981	Gal -	

1L = A 1-8 1M = B. 1-4.

C, D, E = 2, 3, 4 Lac

Test only 4 from 2 in C.

E3 = C5.

		Lac	Mal	Gal
A	1	V	V	+
	2	V+	V	+
	3	V	V	+
	4	V	+	+
	5	V	V	+
	6	V	V	+
	7	V	+	+
	8	V	V	+
B	1	V	V	+
	2	+	V	+
	3	-	V	+
	4	-	V	+

		Lac	Mal	Gal
C	1	V	V	-
	2	V	V	+
	3	V	V	+
	4	V	V	+
	5	V		+
D	1	V	V	+
	2	V	V	V
	3	V	V	V
	4	V	V	V
E	1	V		V
	2	V+		+

Streak out from EMS Lac

A6 H205

D4 H206

I. 48 on Lac EMB. 8 distinctly Lac v.

*. 12 colonies from Mal EMS to Mal EMB. 5 Mal − (microscoped as EMS). 3 Mal + 4 Mal v !

plates are pregnant. Material is roundabouting.

II Some Lac v rather indistinct, probably owing to weak Lac + Gal −.
56 EMS lac + to EMB. 5 definite Lac v picked
5 additional held for further incubation

III ~~44~~ 56 Lilac II pigmented compartment
Pick 4
Hold 8

IV 44. Pick 2
Mold 2

Reject "Hold"

Glu − × Gal −

May 22, 1949.

A. W990 (Y10 Glu −) × W618 (BM Gal −) (later appears to be Lac strain).

canal.

Inhibition Gal - BM - strains:

	Glu	Lac	Gal	
619	++	−	−	
625	++	++	−	} suitable for cross!
626	++	++	−	
990	−−	++	+	

Test 990 harvested on Lac for fermentation: washed cells

		10m.
3:55	Lac	+
	Glu	−
	Gal	++

5/24/49. Cross W625 and W626 ≅ W990 on EMS Lac.

5/24/49.

(A.) Mg. Following Knaysi, inoculate 58-161 + W677 heavily into NSB + M/2 (1) $MgSO_4$. Shake 3 hours., wash and plate.
(2) Controls: saline. Streak out on EMB Lac. (+, − no sectored colonies.)

(B) Use A2 above. Plate on EMS Lac and T(0) and incubate at various temperatures. 30, 37 and 44.

30°	9 Lac+	15 Lac−
37°	12 +	12 −
44°	10 Lac+	14 Lac−

(C) Plate on T(Mg M/3) 37°

12 Lac+ 12 Lac−

Counts / 4 plates

		1 plate
A1	573	143
A2	106 (x ½)	27
B 30		23
37	105	26
44	93	23
C	48 (⅓)	12

Streak out samples of prototrophs on EMB to test for segregation.

A2	8 Lac+	16 Lac−	A1.	14 Lac+	11 Lac−

No Lac ± noted in these tests. The effect of Mg showed a threshold

Gal+	Lac+	Mal-	Xyl-	Mtl-	Ar-	47
	"	"	"	"	Ar+	4
Gal-	Lac-	Mal-	Xyl-	Mtl-	Ar-	68
"	"	"	"	Mtl+	Ar-	1
"	"	Mal+	"	Mtl-	Ar-	1
+++++						7
+++++	Ar-					1
Lac-	Gal+	---				1
"	"	--	Ar+			2
Lac-	Gal+	Mal+	Xyl+	Ara+	Mtl-	1
Lac+	Gal+	Mal+	---			1
"	"	"	Ar+	--		1
Lac+	Gal	----				2

An difficult to score. Lac Gal; Xyl Mtl generally limited as ____.

Retest the 7 full +++++ for diploidy tests. "546A"

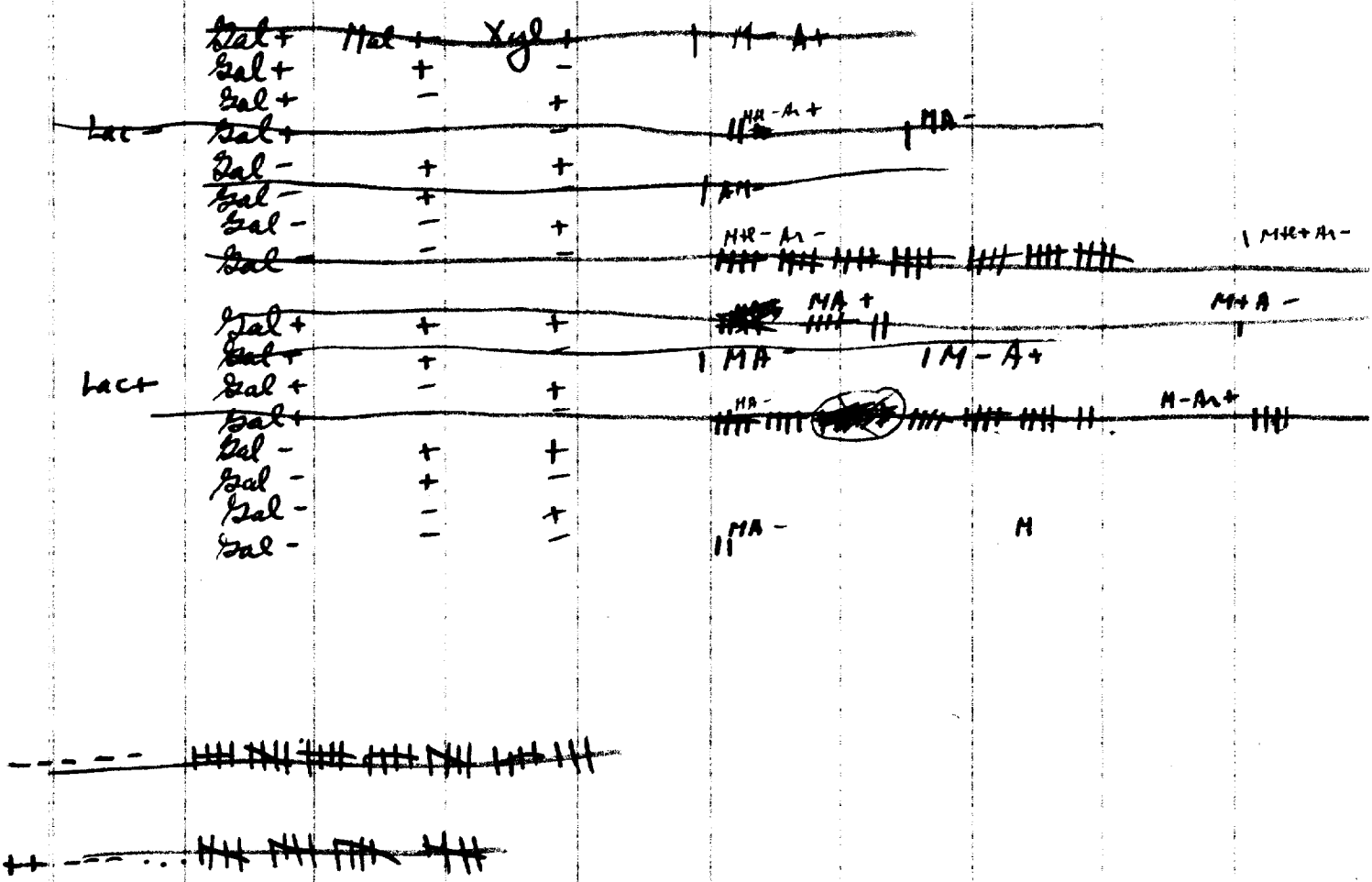
		Lac	Gal	Xyl	mtl	Mal	Arab
$MgSO_4$	1	+	+	−	−	−	−
	2	+	−		−		
	3	−	−		+		
	4	−	−		−		
	5	−	+				
	6	+	−				
	7	−	+				
	8	+	−				
	9	+	+				
	10	+	−				
	11	−	−				
	12	−	+				+
	13	+	+				−
	14	+	−				−
	15	−	+				+
	16	+	+				−
	17	−	−				−
	18	−	−				−
	19	+	+				
	20	+	+				−
NaCl	1	−	−	−	−	−	−
	2	−	−				
	3	+	+				
	4	−	−				
	5	−	+	+	+	+	
	6	+	+				
	7	−	−	−	−	−	
	8	+	+				
	9	+	+				+
	10	−	−				−
	11	+	+				
	12	−	−				
	13	−	−				
	14	−	−				
	15	−	−				
	16	−	−				
	17	−	−				
	18	−	−				
	19	−	−				
	20	−	−				
	21	+	+				
	22	−	−				
	23	+	+				
	24	+	+				

*

		Lac	Gal	Xyl	Mtl	Mal	Ara				
B-30°	1	−	−	−	−	−	−				
	2	−	−								
	3	+	+								
	4	−	++				+				
	5	−	−				−				
	6	+	+								
	7	−	−								
	8	−	−								
	9	+	+								
	10	−	−								
	11	+	+								
	12	+	+								
	13	+	+								
	14	+	++	+	+	+	+				x
	15	+	+	−	−	−	−				
	16	+	+								
	17	−	−								
	18	−	−								
	19	−	−								
	20	−	−								
	21	−	++				+				
	22	−	−				−				
	23	−	−								
	24	−	−								
B-37°	1	−	−	−	−	−	−				
	2	−	−								
	3	−	−								
	4	−	−								
	5	+	+								
	6	+	+								
	7	+	+								
	8	+	+								
	9	+	+								
	10	−	−								
	11	−	−								
	12	−	−								
	13	+	+								
	14	+	+	+	+	+	+				x
	15	−	−	−	−	−	−				
	16	−	−	−	−	−	−				
	17	+	+	+	+	+	+				x
	18	−	−	−	−	−	−				
	19	+	+	−	−	−	−				
	20	−	−	−	−	−	−				
	21	−	−	−	−	−	−				
	22	+	++	+	+	+	+				x
	23	+	+								
	24	+	+	−	−	−	−				
	X	+	+	0	0	−	0				

B-44°	lac	Gal	Xyl	Mel	Mal	arab				
1	−	−	−	−	−	−				
2	−	−								
3	−	−								
4	+	+								
5	−	−								
6	+	++				+				
7	−	−								
8	−	−			+					
9	−	−								
10	−	−								
11	−	−								
12	+	+								
13	+	+								
14	−	−								
15	−	−								
16	−	−								
17	+	+								
18	+	++			+	+				
19	−	−								
20	+	+								
21	−	−								
22	+	+								
23	−	−								
C 1	−	−	−	−	−	−				
2	+	+								
3	+	+								
4	+	+								
5	−	+				+				
6	+	−								
7	−	+								
8	−	−								
9	+	−			+					
10	+	+	+	+		+				
11	+	++			+					
12	+	+	−	−		−				
13	−	−								
14	+	+								
15	−	−								
16	−	+								
17	−	−								
18	+	+								
19	+	++	+		+	+				
20	−	−								
21	−	−	−		−	−				
22	−	−								
23	+	+	−		−	−				
X	+	+	0	0	−	0				

Pool data from prototrophs secured in various treatments.



Fermentation Tests

	W	Mal	Lac	Glu	Gal	Mtl	Xyl	Arab
	958	−	s	+	−	s	s	+
	959	−	+	+	−	−	−	s
	960	−	s	+	+	+	+	+
*	961	−	+	+	+	+	−	+
	962	−	−	+	−	−	+	+
	963	−	−	−	+	−	−	+
	964	−	−	+	−	−	+	+
*	965	−	+	+	+	+	+	+
	966	−	s	+	+	+	+	+
*	967	−	+	+	+	+	+	+
	968	s	+	+	+	+	−	+
not pure	969	−	s	+	+	−	s	+
	970	−	s	+	+	−	+	+
*	971	−	+	+	+	+	+	+
*	972	−	+	+	+	+	−	+
	973	−	−	+	−	−	+	+
*	974	−	+	+	+	+	−	+
	975	−	−	+	−	−	−	+
	976	−	−	+	−	−	−	−
	977	−	s	+	+	s	+	+

* suitable as markers.

	W	Gal	Lac	Glu	Mtl	Xyl	Mal	Arab
	978	−	s	+	s	+	−	+
	979	−	s	+	−	+	−	+
	980	−	−	+	−	−	−	+
	981	−	−	+	−	−	−	+

	W	Glu	Gal	Lac	Mtl	Xyl	Mal	Arab
	990	−	+	+	−	+	−	+
	991	−	+	−	−	+	−	+
	992	−	−	−	−	+	−	+
	993	−	+	s	−	+	−	+ *
	994	−	−	−	−	+	−	+
	995	−	+	−	−	+	−	+
	996	−	−	−	−	+	−	+
	997	−	−	−	−	+	−	+
	998	−	+	−	−	+	−	+
	999	−	+	−	−	+	−	+
	1000	−	+	−	−	+	−	+
	1001	−	+	s	−	+	−	+

* Light centres, also in others to some extent.

Segregation of H206

Mosaics from (Lac ↓) streaked out either on Lac or Mal EMB.
+, I− tested from each, on Lac, Mal, T5.

	Lac+	Lac−		Lac+	Lac−	Lac+	Lac−		Lac+	Lac−
Mal, T5: → 1	+	−		+	+	+	−		+	−
2	+	+		+	−	+	−		+	+
3	+	+		+	−	+	−		+	−
4	+	−		+	−	+	+		+	+
5	+	−		+	−	+	+		+	+
6	+	+		+	−	+	−		+	+
7	+	+		+	+	+	+		+	+
8	+	+		+	+	+	+		+	+
9	+	+		+	−	+	−		+	−
10	+	+		+	+	+	−		+	+
									+	+

	Mal+	Mal−				Mal+	Mal−		
Lac+	41	0	41			16	0		16
Lac−	28	18	41			20	36		56
	64	18				36	36		

	Mal+	Mal−				Mal+	Mal−	1	Mal+	Mal−
1	−	−							−	−
2	−	−	+	−	−	+	−		−	−
3	+	−	−	−	+	−	−		+	−
4 Lac →	−	−	+	−	−	−	−		−	−
5	+	−	+	−	+	−	−		−	−
6	+	−	−	−	−	−	−		−	−
7	−	−	+	−	−	−	−			
8	−	−	−	−	+	−				
9	−	−	+	−	+	−				
10	+	−	+	−	−	−				
					−					+
					Mal+					Lac−

all $T5^R$ in these tests!

From these data the adjusted table is:

	Mal+	Mal−	
Lac+	31	0	31
Lac−	38	31	69
	69	31	100

i.e., Mal+ = 69%
Lac+ = 31% = Mal−

7/31 This analysis is liable to vitiation if Mal− is epistatic to Lac!

W977 Gal mutations

5/31 ff/49.

45 EMB } Galactose. 150 scorable colonies.
30 TZ }

1 good Gal – mutant

~~W97~~

W977 x W677 (Mal alleles
16 possible + | 493 − . Many Mal$_x$. Restricted → 4/m Mal S.

9, 12, 11, 1, 2, 6, 8, 3? Het
13, 19, 5, 7 −

others? There may be Mal$_1$ + Mal$_x$ ±. Hold momentarily.

551A4 recreated from brush on EMS Lac.

No Mal+ colonies, but papillated background.
Mal+ probably contaminant, not part of heterozygote.
Ca 90% Lac v

Check 5 single cols. from Lac EMS.
4 ⇒ Lac v pure Mal-

June 6, 1949.

A. W478 × W677 on Lac, Mal EMS.

B. W45 × W677 on Lac EMS. 15 plates. No yield.

(A'). Test for Lac and Mal ν.

180 Lac+ tested for Lac ν. 16 possible Lac ν.

120 Mal+ tested for Mal ν. No likely Lac ν.

	Lac	Mal	Xyl	MH	Gal	
Alac 1	−,+ ν	−	− ✓		✓	} ν? Check from EMS.
2	(−,+)	− and +	−,+ ✓	−,+	−,+	
3	ν✓	−	−	−	−,+	
← ν 4	ν✓	(−,(+))	ν✓	ν✓	ν✓	
5	ν✓	−	−	ν	ν	
6	++	−	−	−	++	
7	++	+	+	++ ✓	++	
8	ν+,−	−	ν✓	− ✓	++	
9	ν✓	−	ν✓	ν✓	ν✓	= H211
10	++	++	++	++ ✓	++	
11	++	−	++	− ✓	− ✓	
12	ν	−	ν	ν✓	ν✓	= H212

#s 1, 2, 3, 4, 5, 8, 9, 12 (8 in all) are likely heterozygotes. All are Mal−. Some may be suitable for further outcrossing. = (9, 12)

Check on Mal+ component in #4.

June 9, 1949.

Second batch picked from A. 100 tests (Lac).
6 possibilities tested. #13-18.

		Lac	Mtl	Xyl	Mal	Gal
1	13	V	V	V	—	V
2	14	V	+	+	+	V
3	15	V	V	V	—	V
4	16	V	V	V	—	V
5	17	V	+	V	—	V
6	18.	V	+	+,- v?	+(-)	V
7	19.	V	V+	+	—	V-

Second batch (200) from MalEMS: Ca'h missed and are Mal+
8 possible Mal v, "v" colonies picked for retest. 1, 2 looked definitely variegated, 3-8 only somewhat irregular.
Only #1 is Mal v. W 2 13.

New and sp. heterozygotes

June 7, 1949.

(B) W45 × W677 on Lac EMS. No. 4. No yield, even ē biotin + thiamin

(A) W478 × W677 on ~~Xyl~~ Xyl EMS. (unlabelled) – high yield 125–/40+

(C) W478 × W595 on Lac EMS, Xyl EMS. v. poor yield diff. to score

(D) W~~+~~677 × W826 on Mal EMS. unl. – high yield 133–/31+

A. Xylose. 80 tests. Miscored –'s : ~~HH~~ III

72 tests. 2 possible Xyl v. #1 Xyl v Lac v
#2 Xyl slow Lac – not Lac v

C. Xylose 40 tests Mostly –'s. 1 ? No v.
Lac 7 " No v.

D. Maltose 100 tests. 2 unlikely Lac v. Not v.

6/12/49

A Xyl. 100 addnl. tests.
3 possible Xyl v.

	Xyl	Gal	Mal	Mtl	Ara	Lac
1 ~~Xyl~~	v	v	—	v	+ ?	v
2	+, slow	v	+	+	+	v
3	v	v	—	v	v ?	v

#2 is apparently Xyl + but it has a mottled appearance which may be related to Gal v.

5393 tests of heterozygosity

W478 x W945

6/9/49

	Lac	Mal	Gal	Xyl	Mtl	Ara	
1.	V	—	—	—	—[redacted]	V	
2.	V +faded	—	—	+	V++	V=	
3.	V ok	—	—	V	V	V	= H210
4.	V faded+	—	—	V	—	V	
5.	V+	—	+pure?	+	V	V+	
6.	faded V?	—	—	V	V	+	
7.	V+	—	+	+	V	V+	
8.	V-	—	+	V	V	V-	= H209

For general segregation studies, #8 is apparently best suited, as it shows stubborn variegation on four sugars. The Gal factor here resembles that of W583. #3 might also be useful as a Gal - heterozygote.

Segregation of W209, ~~Reversion of Hel~~

6/9/49

	Lac	Xyl	Mtl	Ara	Lac	Xyl	Mtl	Ara
Lac selection.	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	+
	+	−	−	+	−	−	−	+
	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	+
	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	−
Mtl Selection	−	+	+	−	−	−	−	−
	−	−	+	+	−	−	−	+
	−	+	+	−	+	−	−	+
	−	+	+	−	−	−	−	−
	−	+	+	−	−	−	−	+
	−	+	+	−	−	−	−	+
	+	−	+	−	+	−	−	+
Ara select.	+	−	−	+	−	−	−	−
	−	−	−	+	−	−	−	−
	−	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	−
	−	−	−	+	−	+	+	−
	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	−
	+	−	−	+	−	−	−	−
Xyl sel.	−	+	+	−	+	−	−	+
	+	+	−	+	−	−	−	−
	−	+	+	−	+	−	−	+
	−	+	+	−	+	−	−	+

Mg on recombination

6/14/49

58-161 x Y10 ca 8x from Y2. Mix 1 ml c

		Colonies:	
A	1 ml H_2O	13, 12, 6, 2	8 = av.
B	1 ml M/1 $MgSO_4$	3, 4, 7, 0	3.5 = av.

Incubate 2:30 PM — 10:20 PM. Dilute 1:10 and plate .1 ml on T(B,)

$MgSO_4$ alone did not augment recombination.

Gal, Mal reversions of H209-210

6/11/49.

Pick single colonies from EMSLac to EMBLac, EMS Mal ± EMS Gal for reversion study.

209 is Gal+Mal- Each of 4 isolates gave ca 95%+ Lac v.

210 is Gal-Mal- " " " " " ".

Hold on EMS for reversions.

A few papillae which appeared were not reversions, nor Lac v! [H210 presumed + 3/21/50].

6/17 ff. Mal+ and Gal+ isolated respectively.

A. Single Mal+ purified on Mal EMS. Each of 6 s.c.i. tested on EMB Mal, EMB Lac: Each of 6 isolates is Lac v. Mal+ Many colonies on Mal EMB, however, show extreme mottling as if segregating at least for a modifier of Mal (2n vs. n ??) No Mal- colonies seen, but this might be due to close linkage of Mal+ to lethal. Retests on 6 suspicious colonies: all Mal++ pure, Gal-

B. Gal+. Two papillae isolated. Not yet purified

6/20 but predominantly Lac v, almost pure Gal+ ± no signs of Gal segregation.

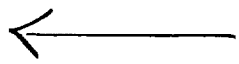
6/21. 8 s.c.i. from Gal EMS. All preponderantly Lac v. No Gal- or marked Gal v seen. Some colonies mottled on Gal EMB, like Mal above. Restreak the most suspicious for a Gal- component. None of 24 colonies tested yielded a Gal-. ∴ Gal also hemizyg

Conclusion: Mal and Gal both hemizygous.

B5, B7 reverted for T, L. test on

	BM	B, M	BB,	BB, M
5, 7	++	++	–	++

Both are only M-astases demonstrable.



6/11/49.

Lac v streptomycin Lac - and + selections.

	+select. Lac#	MtL ~~Mtgl~~	Gal	Xgl		-select. Lac	MtL ~~Mtgl~~	Gal	Xgl	
A H211	+	—	+	—		—	—	—	—	
	+	↓	↓	↓		—	↓	↓	↓	
	+					—				
	+					—				
	+					—				
	+					—				
	+					—				
					Nutr					Nutr
	+	—	+	—	+	—	—	—	—	MTL
	+	↓	↓	↓	+	—	—			MTL
B	+				T	—	—	↓	↓	MTL
H212	+				+	—	—			TL *
	+					—	+		+	MTL
	+					—	—			(T) +
	+					—	+	↓	↓	MTL
	+					—	—			TL

* 556 B4. = T-L- Lac- Mal- Gal- Xgl-. Het? No! W1022

6/12/49 ff. Attempt Reversion of B5, 7, 8. Add several drops from ++ medium to each. -T and -L tubes all grew after 48h! ← -M tubes remained clear. Transfer from B5T, B7T, B1T.

6/21/49. No M reversions noted. B1, B5 appear to have reverted on T, L. Reisolate and check reactions

H 211 - 212 segregations

6/14/49.

	Lac	Mtl	Xyl	↔	Lac	Mtl	Xyl	
(B)								
H211	+	−	−		−	−	−	20 pairs
Lac sel.	+	−	−		−	+	+	1 pair
					−	−	−	3 unpaired.
(C)								
Xyl sel.	−	+	+		−	−	+ −	17 pairs
	● −	+	+		+	−	−	6 pairs
	Xyl+ relatively				−	−	−	9 unpaired
	scarce!				−	+	−	1 "
					+	−	−	3 "

Note scarcity of Xyl+ Lac+.

(D1) 212								
Lac	21	+	−	−	−	−	−	#34 Lac v
	2	+	−	−	−	+	−	Also?
	1	+	+	−	−	−	−	1, 2, 4, 6 16?
								Do not include ←
?	6	+	+	+	−	−	−	
	5	+	−	−	−	+	+	
	1	+	+	+	−	+	+	
		+	+	+	−	−	−	1 unpaired
(D2)								5 "
		See next page.						
		+	+	+	−	+	+	#7 Lac v
								#24 Lac v

See next page.

Each of these cultures was Lac v on retest. Pick, as possible, single Lac+ and check on Mtl, Xyl. #1–9 were

Lac+ Mtl− Xyl−. #2 ? ~~Lac v Mtl v Xyl v~~ vv: Xyl− Lac+

#10 ? Lac+ Mtl+ Xyl+

Restreak these.

H212

	Lac		Mtl		Xyl		Lac		Mtl		Xyl		Lac		Mtl		Xyl	
Lac sel.	A	B																
D2. 1	+	−	−	−#	−	−	+	−	−	+	−	+	+	−	−	−	−	−
2	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−
3	+	−	−	−	−	−	0	−	0	−	0	−	+	−	−	−	−	−
4	+	−	−	−	−	−	+	−	−	−	−	−	+	−v	−	−	−	−
5	+	0	−	⊖	−	⊖	+	−	−	−	−	−	+	0	−	0	−	0
6	+	−	−	−	−	−	+	−	−	+	−	−	+	−	−	−	−	−v
7	v	−	v	+v	v	+v	0	−	0	−	0	−	+	−	−	+	−	+v
8	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	+
9	+	0	−	0	−	0	+	−	−	+	−	+	+	−	−	−	−	−
10	+	0	−	0	−	0	+	−	−	−	−	−	+	0	−	0	−	0

		Unpaired:	
+−−//−−−	17 pro. 14	+−−	5 4
+−−//−++	4 pro. 5	−−−	2 0
+−−//−+−	1 pr. 2		

The Lac+ Xyl+ exception (#7) is obviously V.
Discount Lac+ Xyl+ exceptions.

D1 1	v	−	+	+	v	+	+	−	−	−	−	−	+	⊖	−	0	−	○
2	v		±		v		+	−	−	−	−	−	+	−	−	+	−	+
3	+	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	+
4	v	−	+	−	v	−	+	−	−	+	−	+	v	−	+	−	+	−
6	+	−	−	−	−	−	+	−	−	+	−	+	+	⊖	−	○	−	○
6	v	−	+	−	v	−	+v?	−	+	−	+	−	+	−	−	−	−	−
7	+	−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−	−
8	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−
9	+	−	−	+	−	−	+	−	−	−	−	−	+	−	−	−	−	−
10	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−

Lac		Mtl		Xyl	
+	−	−	−	−	−
+	0	−		−	
+	−	−	−	−	−
v	−	+	−	+v	−
+	−	−	−	−	−
+	0	−		−	
+	−	−	−	−	−
+	−	+	−	v	−
+	−	−	−	−	−
+	−	−	−	−	−

Summary above is —

Check on "+" of:
1, 2, 3, 4, 6, 16, 24, 34, 38 of D1 and T of D2.
#1-10 556 D1

← #10 is pure Lac+ Xyl+. Nutritional
← Prototrophic, but Mal+, therefore a contam.
Odor resembles Proteus, but not a spreader
T1, T6, T7 – resistant

E 212 Xyl selection

Lac		Mtl		Xyl		Lac		Mtl		Xyl		Lac		Mtl		Xyl	
−	−	+	−	+	−	−	−	+	−	+	−	−	−	+	−	+	−
−	−	+	−	+	−	o	−	o	−	o	−	−	+	+	−	+	−
−	−	+	−	+	−	−	−	+	−	+	−	o	+	o	−	o	−
−	+	+	−	+	−	−	−	+	−	+	−	−	−	+	−	+	−
−	−	+	−	+	−	−	−	+	−	+	−	−	−	+	−	+	−
−	−	+	−	+	−	o	−	o	−	o	−	−	−	+	−	+	−
−	+	+	−	+	−	o	−	o	−	o	−	−	−	+	−	+	−
−	−	+	−	+	−	−	−	+	−	+	−	−	+	+	−	+	−
−	−	+	−	+	−	o	+	o	−	o	−	o	+	o	−	o	−
o	−	o	−	o	−	−	−	+	−	+	−	−	−	+	−	+	−
												−	+	+	−	+	−
												−	−	+	−	+	−
												−	+	+	−	+	−
												−	+	+	−	+	−
												−	−	+	−	+	−
												−	−	+	−	+	−
												−	−	+	−	+	−
												−	−	+	−	+	−
												o	−	o	−	o	−

X+M+L− //−−− 25 pro.

X+M+L− //−−+ 7 pco.

−−− 5 ind.

~~Lac~~

−−+ 3 ind.

No M/X c.o. No Xyl+ Lac+.

Among Xyl−, 28 L− : 10 L+.

Xyl+ all L−

H213 analysis

6/11/69.

Streaks of single Mal v colonies show:
Lac−, Gal−, Xyl v, Mtl v, Ara+.

Test for ~~zone~~ heterozygosity of Lac. Four single cols. from Mal EMS to Lac EMS; Mal EMB ~~0~~ No Lac+ found.

3 Mal selections. Mal+ were Xyl+ Mtl+; Mal− were Xyl− Mtl−

Test all 3 pairs for mutations.

1+	(T)+	1−	M (T)
2+	TLB,	2−	M (T)
3+	TLB,	3−	M.

3− x 3+ would be suitable for crossing!

Note that (B)M− is Mal+
TLB,− is Mal+

Test

1	557a:	B3		TL	Mal− Mtl+ Xyl+	557 B3
2	(1)	B7		++	Mal− Mtl+ Xyl−	
3		B40		TL	Mal− Mtl+ Xyl+	557 B40
4		A20		M	Mal+ Mtl− Xyl−	557 A20
5	(2)	6B	✓	TLB,	Mal− Mtl+ Xyl+	557 B6
6		13 B	✓	TLB,	Mal− Mtl+ Xyl+	557 B13
		19A		~~BTLA~~	++	
		22A		~~TLB,~~	++	

213AAA. Xyl selections.

23pairs +++ / ---

All Xyl+ were Mtl+;

Xyl	Mal	Mtl	Xyl	Mal	Mtl	
+	−	+	−	−	+	2
+	+	+	−	−	+	1
+	−	+	−	−	−	8

Nutritional tests.

(A1) 1+ TL 2+ TL
1- M(T) 2- M−

6/14/49.

+, - isol. from single crossed colonies.

A. Maltose selections

(1).	34	paired selections were	Mal+	Xyl+	Mtl+	//	- - -
+	19	Unpaired	+++	1 + 0	/ 1		
	53		- - -	2 + 2	/ 4		

	Mal	Mtl	Xyl	Mal	Mtl	Xyl	
#3	+	+	+	—	+	+	
7	+	+	+	—	+	—	
40	+	+	+	—	+	+	
20	+	—	—	—	—	—	
(2) #19	—	+	+	—	—	—	} To be checked! ✓ Scored properly +
22	—	+	+	—	—	—	
6	+	+	+	—	+	—	
13	+	+	+	—	+	+	- Must have been picked as + on Mal. (from Mtl??)

53 parental pairs

6 + 2? had one recombinant.

Total crossing over, $< \frac{8}{2 \times 61 + 5} = \frac{8}{107} = 8\%$

Mannitol selections.

AA.

	Mtl	Mal	Xyl	Mtl	Mal	Xyl
3 pairs:	+	+	+	—	—	—
2 "	+	—	+	—	—	—
1 unpaired	+	+	+			
1 "	+	—	+			

←

Test H212 segregant for Het. 558

6/13/49.

556 B4 x 58-161 on Lac, Mal EMS.

Almost pure Lac+. Cal 2% − 61+ : 2− . ~~Mal ±~~.

Mal 40+ : 6−

100 Lac+ prototrophs tested. None were Lac v

35 Mal+ None Mal v

556-B4 does not carry "Het"

Reversions of H138.

~~559~~
559
(558)

6/14/49.

H138 is Lac_1/Lac_2 MtlV Mal- spont. heterozygote. Is it Mal-/Mal- ?

Streak purified LacV on MalEMS. Pick papillae after 3-4 days.

8 "papillae" tested. None were Mal+ at all. Abandon this trial
nor LacV

6/19/49. Repeat on T(us) Mal.

6/15/49.

Cross 557-3+ x 557-3- on Mal & lac EMS.

No yield! 1 Lac+ colony in 25 plates Not v

Different colony isolates of
H37Ra are inconsistent

Spontaneous Lac^{v} Mal hemizygosity

6/15/49.

W45 × W677
W67 × W677
} Lac EMS.

W45 gave almost no yield: 1 + colony / 15 plates
W67 gave numerous Lac+, ca 2+/~~600~~ Lac−

Pick all +'s and check for heterozygosity.

W67. A ✓ Lac$^{v\pm}$ Mal^{v} Xyl^{v} Gal^{v} Mal−/Mal− H214 See infra.
B. Lac^{v} Xyl^{v} Mal^{v} Gal^{v} Mal^{v} H215 = mixture! Now Xyl− ? or Xyl+ ! Mal+
W45 C. Not Lac+.

H214 streaked on ~~Mal (Tr Xan)~~. T(us) Mal gave numerous papillae. Pick as H214M+ for hemizygosity test.

6/20. 1-4:

	Mal	Lac
1a	+	v
b	+	v
2a	+	v
b	+	v
3a	+	v
b	+	v
4a		
b		

apparent Mal^{v} is modifier mutting. No. — segregants isolated

6/17/49.

Cross W1014 x W588 on EMS, Lac, Mal for percentage of Mal V in heterozygotes.

9 Lac+ / 408 Lac −
31 Mal Lac+ / 191 Mal Lac −

Mal− Mal+
DM Lac− x TLB, Lac+
Should give excess Lac+!!
Note deviation!! Should also be Het.

These ratios maintained on reincubation!

29 Lac tests. No Lac V
48 Mal " " Mal V

After 24 h. reincubation, additional +'s picked:

Mal.	4	No V.
Lac	18	10 likely heterozygotes

Check for other factors
#1-8 Lac V colo. picked OK
9, 10 mass pick.

	Lac	Mal	
1	V	+	Reisolate from Lac EMS.
2	V	+	
3	V	−	
4	V	+,− V?	
5	V	−	
6	V	+	
7	V	+	
8	V	−	
9	+,−	−	
10	+,−	+,−	Ref. colonies rather small.

Reisolate H168 from Jelle transfer. 583

6/18/49.

MRZ. sent "H168": 5a1, 5a2, 5a3, "Swis 8":
Also "8 - 215, 216, 217.

1. 5a1, "8" both show no Xyl+ component when streaked out from plant.
Lac v reisolated from each of these 4 isolates. Recheck from Lac EMS for Xyl, Mtl, Lac v components!

#3 = 5a1
#6 = 5a2
#9 = 5a3
#13 = "8"

} Lac v Mtl v Xyl − !!!

28: 215, 216 both Lac+, — 217 Lac+ 216 is scarcely prototrophic. Lac v isolated from 215, 216 prototrophs on Lac EMS. 217 is pure Lac+, reverts fairly readily to prototrophy

H213 Segregant XXX.

6/19/49.

1. W1014-1 × W1015-1 Mal v F2
2. " -2 × " -2 "
3. W478 × 566-B4 Lac v F2 97 Lac+ : 3− ; 102 M+ : 11− Note reversal of linkage relationship to 566-B4!
~~4. W1014-1 × 566-B4 BF-(Mal−) × F1 ♂~~

1, 2 gave very low yields! - colonies could like + or intermediate.

1. Mal: 10 tests all Mal−.
   Lac 10 " " Lac− ! (of course! both parents lac−)

2. Mal 8 tests 6 Mal− 2 Mal+
   Lac 18 " all −

(3) 100 tests each for Lac v and Mal v (200 total)
2 likely heterozygotes on lac ~~total~~ None on Mal.

1	Lac v	Mal+	✓
2	Lac v	Mal+	✓

Note. These heterozygotes Mal+. This cross also gave 1 Mal+ prototroph in excess!

v. infra.

6/20/49.

Papillae from W108 in Gal EMB picked and purified. Four isolates tested (may be same):

	1	2	3	4	
Gal	+	+	+	+	(fades)
Glu	—	—	—	—	
Tre	—	—	—	—	
Mannose	—	—	—	—	
MH	—	—	—	—	
Sorbitol	—	—	—	—	
Mal	—	—	—	—	
Lac	—	—	—	—	
W	1016	1017	W1018	1019	

4/546

Loss of Xyl+ from H212.

6/21/49.

Brush Lac, MAL v on Xyl EMB, Lac EMS to find possible illustrations of losses of Xyl v.

A.	All Xyl v.	#14 mostly Xyl−.	Reisolate from Lac EMS.	40
(B)	"	#7 "	" " " " "	12
(E).		16 tests. all Xyl+. #9 Xyl− but no growth on EMS. ∴ a segregant streaked out on Lac EMB		16
(C)		V12. #3.	24 tests.	24
(D)		V12. #26.	40 tests	40

Restreak E9, C3 and D26 on Lac EMB.

C, D pure Lac+

E pure +, − No v.

(F).	ca. 60 tests	1/~~La~~ Xyl− were pure Lac+	60
(X) }	80 tests	No Xyl−	80
(Y) }			
		Σ	272

∴ 272 tests of Lac v, no Xyl− (i.e. Xyl+ losses) were found.

Crosses (fertility tests)

6/21/49.

1. Y10 × W1014 — V. good yield.
2. W588 × W1014 — No yield!
3. W1015 × W1014 — Good "
4. W1015 × 58-161 — "
5. W1015 × W478
6. W478 × W677 (for Xyl ± Lac −).

H. 50 Lac ± tests: No Lac ± — 237 Lac+ : 2 Lac−

5. " " — 1408 Lac+ : 13 Lac−

[illegible]. 100 Mal ± tests " [Mostly Mal+ as expected].

100 Lac ± tests. " 547 Lac+ : 143 Lac− as expected.

1014 = BM Lac Mal 1015 = TLB, [redacted] Lac

6-23-49

H213	Mal (3 plates)									
	+	−	V							
A	42	397	38	(2 plates partially smeared)						
B	65	394	24							

H215	Mal (4 plates)			Lac (6 plates)			Xyl (2 plates)			
	+	−	V	+	−	V	+	−	V	
C	cā 460	0	0	0	1112	174	0	cā 320	0	
D	cā 640	0	0	0	290	131	0	cā 300	0	3 lac plates no good
E	0 (C)	cā 480	0	0	435	196	0	cā 240	0	
G	cā 430	0	0	0	719	122	0	cā 480	0	1 lac plate no good

Mal Segregation of H215

568

6-23-48

H215 was a Lac+ prototroph from W67 x W677 on Lac EMS. This prototroph was streaked out initially on EMS Lac, and on other EMB sugars, where it was scored as Mal v, Lac v etc. The EMS plate & the initial streaking was preserved as 568-1.

16 single colonies from 568-1 picked and streaked on EMB Lac, EMB Mal and punched on EMS Lac.

	Lac	Mal		Lac	Mal
1	v	−			
2	v	+			
3	v	+			
4	v	+			
5	v	−			
6	v	+			
7	v	−			
8	v	−			
9	v	+, − v?	17	v	−
10	−	+	18	v	
11	v	+	19	v	+
12	v	+ (−)	20	v	+
13	v	+			
14	v	−			
15	v	+			
16	v	+, v?			

Streaked out en masse from −1.

Mal EMB Many +, − colonies. Some + colonies had a variegated appearance but not distinctly Lac v. Looks for Mal / Xyl crossovers.

59 Mal+ tested were Xyl −

42 Mal− tested. 41 Xyl+ 1 Xyl−? → TLB −. Probably W677! Restreak as 568-2

Mal EMS. 100+ tested for Mal v. None Mal v. 7 Mal−.

May be mixture of Mal+ Xyl− and Mal− Xyl+.

W112 + W811 λ-disruptions.

6/23/49ff.

Irradiate W811 on EMB bac 10 secs. Rel 200 colonies and brush against W578 to test for λ+.

Due to high incubator temperature, readings sometimes indistinct. 5 isolates selected are possibly λ−. Streak out on bac EMB

All but #2 are λ−. This may be mixed.
Check remaining 4 for λ⁵: (using W811)

These 4 cultures are resistant to W811.

Ditto E 487. ca 300 tests on W578
Lytic tests very sharp. 1 λ− found in 281 tests.

=570-6 Also λ-sensitive. = W1025

Test for induced lysogenicity.

7/3ff/49 Do. W112 142 tests.
38
"
180

7/8/49 79 2 might be mixed.
259 All λ+

7/15/49. Do. 470 200 tests. 2 λ− found. W1026-27
both λ− λᴿ

6/29/49.

20 plates, heavily seeded, EMS Lac. and +.

7/3/49. 30 plates. ca 50/ = 1500. 1 Lac+ prototroph. #1

1. [redacted] Lac v (principal) Xyl- Mal-. Occasional + colonies * purified to determine whether parentals.

3 Xyl+ were Mal + Lac- Sal+ Mtl+, probably parental.

4 Mal+.

7/8/49. 35 plates ca 30/ = 1000

7/9 2 Lac+ and 1 mucoid Lac+ prototrophs. } 4 Lac v
35 plates 1 Lac+. } #471:2-5

1. Lac v ⊙ Mal- Xyl- [redacted]

2 Lac v ⊙ Mal+ Xyl+ Mtl+
3 Lac v ● Mal- Mtl- Xyl-
4 Lac v ⊛ Mal- Mtl v Xyl v
5 Lac v ◎ Mal- Mtl- Xyl-

] Completely inviable 1/23/50 (Bu.y.)

3-5 are predominantly Mal-. A few Mal+ colonies were seen, but were undoubtedly parental or other prototroph contaminations.

2-5 are homogenously as above., as tested by comparing p.c.i with intire original.

Modified linkage-ratio test. 572

6/29/49.

A.	W1022 556 B_4	x 58-161	}	Lac, Mal, Gal EMS ± B_1
B	W677	x 58-161	}	

A + B rather badly contaminated in 2 runs. Parents should be repurified, but this is likely due to medium or training.

But on uncontaminated plates, A shows excess Mal+ B shows ca 10%. A also almost pure Lac+

Counts:

A). Lac EMS:

+	−
3	0
4	0
36	0
6	0
43	1
37	2
26	1
49	1
204	5 / 209
97.6%	

Mal

+	−	
19	1	
27	2	
10	0	
29	0	
16	1	1$
67	3	
139	7	/ 146.
95.2%		

Gal (scoring??)

+	−
38	1 sect.

B)

34	36	
29	39	
23	35	
33	53	
24	34	
26	27	
39	73	
203	297	/ 500
40.6%		

5	32	
9	66	
15	135	
6	82	
35	315	/ 350
10%		

scoring different but ca 30% +.

Test all exceptions of A on various media.

		Mal	Lac	T1	MR	Xyl	Gal
Mal-	1	−	+	S	−		+
	2	−	+	S	+	+	+
	3	−	+	S	+	+	+
	4	−	+	S	−	−	+
	5	−	+	S	+	−	+
	6	−	+	S	−	−	+
	7	−	+	S	+	+	+
	8	−	+	S	−	−	+
Lac-	1	+	−	S	+	−	+
	2	−	−	S	−	−	−
	3	+	−	S	+	+(−)	+
	4	+	−	R	+	+(−)	+
	5	+	−	S	+	+	+

Note: Mal exceptions are not related to Lac exceptions. ~~MR~~-Xyl linkage still firm. V_1 and MR data should be recorded from the Mal+Lac+ populations.

Test Lac+ on Xyl EMB, T1 / EMS Lac

40 tested. #14, 18, 30 are Xyl−
other 37 Xyl+.

Indications of Mal ÷ Xyl linkage.
#33 is a mixture of V_1^R, V_1^S (on Lac EMS).
All others are V_1^S.

Note uniformity of characters from 58-161!

7/1/49.

Lac EMS. Counted 110+ : 36− Test + for lac V.
200 tests. 10 kept for recheck.

In previous work, single V colonies were usually taken to isolate a diploid strain. Now, the broad streak was used to determine whether any diploids are duplex with respect to Mal or to Gal. Streaked out on Lac EMS, and EMB Lac, Gal, Mal

	Lac	Gal	Mal
1	+,−	++	−, +
2	++,−	++	++
3	V	++	++
4	V	++ −	++, −
5	V?	++	+, −
6	V	++	+, −
7	V	++	=, (+)
8	V?	++,−	=, (+)
9	++	++	++ (−)
10	++	++	−

} not V

Mostly Lac − prototrophs.

Lac V Mal+
Lac V Mal+
Lac V Mal−
Lac V Mal−
" "
" "

Retest Lac+ prototrophs from 4, 5, 6 to find any duplex types.
8 from #4 are Mal+
8 ea. " #5, 6 " Mal− } all Lac V

Antibiotics, λ and K-12

7/1/49.

a. Determine sensitivity ranges by inoculating one drop into 10 ml Penassay + indicated material.

P1

W478. Chloromycetin/ml — 1 mg — 100 γ — 10 γ — 1 γ
A2 — — — — — — +++
P4 — — — — — +++
Aureomycin — 1 mg — 100 γ — 10 γ — 1 γ
— — — — — — +++
P4 — — — — +++ streak out as Amp^R
Bacitracin — 100 u — 10 u — 1 u — 0 u. (Control)
P4 — ± — +++ — +++ — +++
— — +++ — +++ — +++
— —

W112 Nitroalkridin — 1 mg 7 sat. — 500 γ sat'd. or — 100 γ
— — — —
— — — —

2,5, aminonitroalk. — sat'd. —
— — —
— — —

P2 Streak out all tubes at lowest inhibitor concentrations to find a possible λ−. Bactericidal − or + ?

Aureomycin and Chloromycetin are bacteriostatic at 1 γ/ml or below

Bacitracin and the acridines are bactericidal at these conc.

Test +++ cultures (isolates) for presence of λ.

8 each, chloromycetin and aureomycin survivors (not resistant) all λ+

Reisolation of M168 from Zello.

7/8/49

16 cultures tested, streaked on Xyl EMB.
All streaks are Xyl +, − mixtures. Occasional ± seen. Pick for propagation on Lac EMB.

Xyl ± recovered. Streak out on MH EMB for material to test Xyl loss.

(320) MH ± colonies touched on Xyl EMB; Lac EMS.

3 Xyl − colonies noted. Grew very poorly on EMS lac, probably segregated, but streak out on MH EMB.

7/17/41

Does buffer activation increase enzyme?

cells harvested from 100 cm Y2 Lac (36h.) to 5 ml water.

A) 2.5 ml cells + 2.5 ml water

B) " " NaP M/5. Incubate 12 20 PM — 215.

Assay ___ samples. Wash treated cells with water, save supernatants, and dry sediments over P_2O_5.

		Di		
Super .5 ml	A		016 (20 mins.)	No activity is extracted by water. Ca 8-10 fold activation by buffer, and ca. 1/3 activity is extracted!
	B		420 (5 mins.)	
cells .05 ml	A	080	111 271 (20 mins)	
	B	056	490 (5 mins)	

∴ Total sediment of B) should have activity of $4.5 \times \frac{1}{.05} \times \frac{450}{100} \times \frac{20}{5}$

= 1,160,000 u.

and A) should have $4.5 \times \frac{1}{.05} \times \frac{200}{100}$ = 180,000 u

Recover dry cells.

A 7.5 mg.
B. 11.0 mg.

B formed a much more compact pellet than A, of which ca 1/3 was estimated lost in preparing for drying and scraping from slide.

Calculate on predicted basis of 12 mg. cells in each.

Triturate each in 5 ml H_2O to prepare for assay.

Predicted activity, ca. 15000 u/mg for A and 13,000 u/mg for B.

Note / These Lac cells also gave brown purple color ± iodine.

Lysogenicity: conclusions.

Two kinds of phage apparent:

φ_A carried by 7, 13, 15, 18, 24, 32, 34, 36. type: W1029.

φ_B 17, 19, 23, 31

	φA^+	φB^+	φO^+
A^s	—	—	6, (14), 27, 29,
B^s	7, 15, 18, (24), 32, 34, 36	—	(10), 16, 21
O^s	(13),	(17), 19, 23, 31.	++

Of 40 cultures, 8 carried A, 4 B = 12/40 = 30%

Hence A^s; 7, B^s = 11/40.

The strongest phage action was that of #17.

#24 responded moderately to λ—

11/29/30: Throw out all except 10, 13, 14, 17, 24

Lysogenicity tests on Shapiro's E. coli cultures. 580

7/16/49.

Test by seeding E each strain as indicated, then spotting each strain on surface. Active combinations indicated:

Host			
1			
2	sparse growth		
3	"	"	
4	"	"	
5			
6	#7, 13, 15, 18		
7		17±	19±
8	[illegible]		
9	s.g.		
10		17±	19±.
11	s.g.		
12	s.g.		
13			
14	7, 13, 15, 18		
15		17, 19	++
16		17, 19	
17			
18		17, 19	
19			
20			

Following groups noted.

6, 14 sensitive to 7, 13, 15, 18

7, 10, 15, 16, 18 sensitive to 17, 19.

Note: 19 → 18 → 14.
6

$(7,15,18)\ P_a^+ P_b^s$

$(6,14)\ P_a^s$

$(17,19)\ P_D^s - P_b$

$(10,16)\ P_a$

Key:

Shapiro			
835	ω 1028	#6	sensitive to ρ7
866	ω 1029	#7	" ρ17, carries ρ7
848	ω 1030	#10	" ;
116	ω 1031	#17	carries ρ17

Should include λ-λ^s; λ-λ^R and λ+ in these tests.

λ tests, continuation

7/19/49.

Check, intense, #21-40.

21	v.sth. 23, 31
22	—
23	—
24	23, 31
25	—
26	—
27	24, 32, 34 (all weak), 36.
28	—
29	24, 32, 34 (v. wk), 36.
30	—
31	—
32	23, 31 v. strong.
33	—
34	23, 31 wk.
35	—
36	23, 31 ++
37	—
38	—
39	—
40	—

21, 24, 32, 34, 36 ← 23, 31

27, 29 ← 24, 32, 34, 36

N.B. 24, 36 → 29 2 or 3 λ's indicated.

↑ ↗

31

Test 21-40, λ, W1029 and W1031 on:

27	24, 32, 34, 36, 1029	
32	no lysis.	λ?? exceedingly weak if at all.
1028	24, 32, 34, 36, 1029	
1029	no lysis.	
1030	no lysis	
518	no lysis	(even λ did not show up).

7/15,17/49.

C) 1. W478 × W1022. 635 Lac+ : 1 / 14 Lac −.
200 Lac+ tested for Lac v. None found.
100 additional tested. Select those that appear weakest Lac+
5 suspicious colonies reincubated. 3 are Lac v.
Picks 1) Lac' Lac v and 2) Broad streak to LacEMS, LacEMB, MalEMB.

what are data?

A) (2)(1) W67 × W1022 1 Lac+ / 3000 prototrophs.
Lac v Mal+ Xyl+ Mtl v?

B) 8. Y40/6 × W1022. 10 plates LacEMS. To determine whether the prototrophs from this cross were diploid. Test 20 Lac+, 12 Lac− as T(o) with T6.

Lac+ :	19 R	1 S	
Lac− :	6 S	4 mucoid, unscoreable.	2 too dilute.

Indicates that Lac+ are not Lac+ U_6^R/U_6^S, and therefore that they are haploid.

A)

W1033, 1034 received from Cavalli 7/21/49.
Both are ~~F+~~ Lac+. 1033 is (B)M 1034 is BMB, ? as labelled.
Cross ♀ W677, cf. 58-161
Harvest and concentrate ca 5:1 as usual. Call concentrated cells 1ml = .1, and plate various dilutions of 1033 on EMS Lac or T(0) plate smeared with 677 (= 1ml).

Count: 10^{-6} ml 1033 on EMB Lac gave 580 colonies.
Count = 5.8×10^8.

No prototrophs appeared at 10^{-7} or 10^{-6}.
10^{-5} (on T(0)) gave 1 10^{-4} gave 30.
Talac rate ca $\frac{30 \times 10^{-4}}{580 \times 10^{-6}}$ = ca .05% = 5/10,000.

but 58-161 at this dilution gave 34 colonies !! (mixup?)
on EMS, No colonies were seen at 10^{-4} from 1033 x 677.
10^{-3} gave 18 (5+ 13-)
However streak of 1033 on 677 background on EMS Lac B, gave very much higher yield than did 58-161. The effect must be largely based upon microcolony formation. Efficacy of 58-161 should be checked!

7/24/49

Inoculate 677 and 1033 heavily together in Y2 broth (1:5 ea.) After 24h. streak out on lac EMB and test single +, − colonies on Xyl.

21 Lac− were Xyl−
of 8 Lac+, 6 were Xyl+, 2 were Xyl−.

Rechecked these 2 (583B1, B2) on lac, xyl, and mutation.

B1 and B2 are both Lac+ Xyl− Mal− LB1−. Stock as W1048, 1049

Additional tests of same plating:

13 Xyl− :→ 10 Lac−, 2 Lac+, 1 mixed.
36 Xyl− :→ 29 Lac− 5 Lac+, 2 mixed

49 Xyl−	:	39 Lac− 7 Lac+	3 mixed.
5 Xyl+	:	5 Lac+	
14 Lac−	:	14 Xyl−	
24 Lac+	:	18 Xyl+. 5 Xyl−	1 mixed.

There are exceptional Lac+ Xyl− present. 1033 is pure, but 677 needs re-verification. Streak out on lac EMB to check! OK!

7/25.

Cross check plated mixture of supra. Xyl and Mal.

39 Xyl+. 36 Mal+ 3 Mal−. } 1-5
5 Xyl+ 3 + 2 − }
35 Xyl− 34 Mal− 1 Mal+ 6.

6 Xyl-Mal recomb.
79 keto

		Lac	Mal	Xyl	Gal	Mtl	V	Nutr.	W
	1	+	−	+	−?	−	R	L	1060
	2	+	−	+	−?	+	S	MT	1061
	3	−	−	+	−	+	R	TLB$_1$	1062
	4	−	−	+	−	−	S	B$_1$	1063
	5	−	−	+	+	+	R	MTL	1064
	6	−	+	−	−	−	R	TLB$_1$	1065
	677	−	−	−	−	−	R	TLB$_1$	1066
"Hz" =	1033	+	+	+	+	+	S	M	1067

25 Xyl− 1 Mal+
15 Mal+ 1 Xyl−
20 Mal− All Xyl−

~~# 7 1066~~
~~# 8 1067~~

7	−	+	−	+	−	R	TLB$_1$	1066
8	+	+	−	−	−	R	ML	1067

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A	1014 x 1015	T(0)	
B	1014 x ~~88~~	233+ : 110 −	Lac EMS.
C	1033 x 477		

A. Yield quite low, ca 5-10/plate instead of > 100.
Streaks out on EMB Mal. 31− : 7+ No Mal v.
Note: BM Mal − x TLB, Mal+ should be excess of Mal +.
Cf. W1022.

B. 100 tests Emphasis on streak+ prototrophs.
4 distinct, 5 uncertain Lac v. → (7) likely Lac v.
Streaks out a) isolated Lac v and b) broad bands on Lac EMB, Lac EMS, Mal EMB.

52 addnl:

C. 100 tests
1 distinct Lac v. ~~1,3~~ ?? Lac v. all Lac v.

B)

	Lac EMB	Mal EMB.	
1	v	+	
	v	+	
2	v	+	
	v	+	
3	v	+	
	v	+	
4	v	+	
	v	+	
5	v	+	
	v	+	
6	v	+	
	v	+	
~~7~~			
8	v	+	1 v+ colony?
	v	+	
11	v	+	
	v	+	
12	v	+	
	v	+	
13	v	+	
	v	+	
14	v	+	
	v	+	
15	v	+	
	v	+	
16	v	+	
	v	+	

None Mal v. All Mal+

C)

1	v	
2	v+	
3	v+?	v+?

Crossean double-sugar
Mfr.

JUL 25 1949

(A) (B) (C)
W1048, or W1049 mixed with Y87/6 and inoculated
1:10 into Y2 3PM

~~3PM~~. Streak out ~~A, C~~ A, B, C on Lac, Mal and ~~Lac Mal~~ Malac

Plate out AC and BC on EMB Malac

Repeat P25.

A, B were pure Lac+ Mal- ; C was Lac- Mal+ All (A, B, C) were pure + on Malac.

BC. 4 plates ca 150 scorable. 1 Malac - colony noted.
AC. 5 plates ca 250 (= 1250) 4 Malac - colonies? [might be a contaminant.] Isolate to analysis.

Repeat:
AC' (2 × 100) No -.
BC' (3 × 150) No -.
These might ~~be~~ not be Mfr.

See 589

24 hr heavy mixed cultures plated on EMB Malac

A	Y87 +	W814	0
B	1056	"	✓
C	1088	"	(✓)
D	1059	"	✓✓

Y87 + W814 pure lac−, lact resp.

4 plates each.

A). 1600 cols. No Malac −

B) 4 Malac − 2 sectorial. 1 sectorial not isolable.

C) 3 − and 2 sect ??

D). 7 − and 6 sectorial, not all isolable

Lac EMB. (2 plates).

D). 2 sectorials

B. 1 " + 2 unisolable

C. 1

A. 0 (4 plate).

JUL 2X 1949

Align

Sh 1		(5)	HC, YE, (A5)	YNA + A6 ±	W1045. Proline
Sh 2		(5)	"		"
Sh 3		(5)	"		
Sh 4	42	K-12	+ all AA		
Sh 5	45	"	± all AA	A12, A4 ++	
Sh 6	46	"	+ " 48hrs.	+ all AA.	
Sh 7	47	"	± all amino acids	A4, A12 ++	
Sh 8	50	"	like 11		Tyr
Sh 9	51	"	+ or ± all amino acids		Tyr
Sh 10	53	"	± in minimal 36h.	do. 11	Tyr. (Φ ±
Sh 11	54	"	24h: HC, YE, (YNA),	A4, (A12) ± others	Tyr.

→ All grow promptly on tyrosine, later adapt.

Sh 1-3 were 3 mutants / 20 tests.

47 additional colonies tested. 6 mutants found: # 4, 22, 26, 35, 40, 43.

Each also showed up very well on minimal agar.

4, 22, 35 were prototrophs. T.O.

		W
Random remaining 3:	A: A12	1050
	B: cystine	1051
	C: histidine	1052

of Sh 4–11.

Summary. 9/67 from treatment with penicillin on the wild type, W1045, were mutants. Of these 9, 6 were prototrophs. Only one was preserved as W1046

The others were W1050–1052, respectively, :

1050
1051
1052

A series of 8 "mutants" were isolated from a K-12 exp. All proved to be rather reversible tyrosineless. Exp # Sh 11 as W1053

"Coso" single mutants from W1045. 586

JUL 25 1949

W1050 = A

1051 = B

1052 = C.

Plate separately and in combination on T(0) plates.

Also inoculate into Y2.

At 36 hours, A-C were blank., AB and AC had numerous prototrophs. BC had a few.

A27.(48h.) A revertants noted. Not so numerous as in AB and AC. ?? Reversions or recombinants ??

Check 1050 with various 4 strokes. Cf. 1033. In Vegas.

	T/	2/	3k	4	5	6	7
1050	R	R	R	R	R	R	R.
1033	S	S	R	S	S	S	S

↑
N.G.

Need double mutants for conclusive result

New penicillin mixes gave low yields of double mutants

#1 from 1050 = W1069
lys + methionine tryptophane

#3 from 1051 W1070

Cystine. Gave growth responses to A12 also on Vits.

16 hrs: +++ on methionine or lysine

Also give smaller responses on:

Vitamin mix
B_1
Pant
Inositol
K
B_{12}

} Probably reversions or tube contamination

24 hours – growth as T10. Just a "slow" mutant.

Hfr : Lac- mutation sus.

Crosses of Lac- mutants.

7/24/49

Irradiate 1033 on 18 Lac EMB plates. Survival high (N_y^{R}!)
6 mutants recovered. Ca 6000 colonies.

	Lac	Mal	Glu	Gal	
1	—	—	—	±	W1054
2	—	+	+	+	1055 – 1059.
3	—	+	+	+	
4	—	+	+	+	
5	—	+	+	+	
6	—	+	+	+	"5 – 9"

Cross 5, 7, 8, 9 × W677 for Lac alleleism, on EMB Lac and in Y2, then to EMB Lac!

5 ×	All Lac –	None very suitable as recombination markers.
6 ×	2-3% Lac+	
7 ×	All Lac –	
8 ×	20% Lac+	
9 ×	All Lac –	
Parents	All –	

EMB Lac. (Mixtures plated out after 24 h.)

5:	0/400 +.
6:	0/500
7:	0 /50
8	0 /800
9:	0 /25.

Lac, × Lac, recombination unfeasible.

Comparison of 1033 & 58-161 HSt.

7/27/49.

As supra. 677 background, on T(B,).

1 ml ≐ 10^9 cells.

Dilution	58-161	1033
0	>1000	
3	11	>1000
4	2	200
5	4	20+
6		4

[Prototypho J.

3/21/50: Conclusion is ca 100-fold augmentation in HSt.

Analysis of 585a.

7/29/49

Streak out sectorial colonies on EMB Lac. (see 585a)

1. D Lac
2. D Lac
3. B Lac
4. C Lac
5. D Malac
6. B Malac
7. C ? Malac
8. C? Malac x
9. D Malac
10. D Malac
11. D Malac
12. D? Malac
13. D Malac

Also, 4- from B, 4- from C and 6- from D

		Lac	Mal	Sal	Xyl	Mtl	V_1
B	1	—	—	+	—	—	R
	2	—	—	+	—	—	R
	3	—	—	+	—	—	S
	4	—	—	+	—	—	R
C	1	—	—	—	—	—	S
	2	—	—	—	—	—	R
	3	—	—	—	—	—	R
	4	—	—	—	—	—	R
D	1	—	—	—	—	—	R
	2	—	—	—	—	—	S
	3	—	—	—	—	—	R
	4	—	—	—	—	—	S
	5	—	—	—	—	—	R
	6	—	—	—	—	—	S
Parents:		+	—	—	—	—	R
		—	+	+	+	+	S

10- from 589a D.

3 Lac- Mal- Sal- Xyl- Mtl- V_1^R #3,4,5

6 " " V_1^S

1 Lac- Mal- Sal- Xyl+ Mtl- V_1^S #9

A-D plated at 12h to EMB Lac. Pick +, − to thin sugars for random test.

A). 36 Lac+ : 34 Mal-Xyl- " 2 Mal+Xyl+" Recheck!
Both were Lac-
1 Lac- 1 "

40 Lac- : 40 Mal+Xyl+

B). 10 Lac- : 10 Mal+Xyl+
40 Lac+ : 40 Mal-Xyl-

D) 40 Lac+ : Mal-Xyl-
20 Lac+ : Mal-Xyl-

60

16 Lac- : Mal+Xyl+

C) 36 Lac+ Mal-Xyl-
20 Lac- Mal+Xyl+

∴ no "recombinations" except in A. 12 hours too short?
Recheck:

589 Analyses.

	Tests.								
1. Lac−	13	All V_1^S :	5 Mal−Xyl−	8 Mal+ Xyl+					
			1, ~~4, 5, 6, 7,~~						
			~~9, [redacted]~~						
			2, 3, 8, 11, 13						
Lac+	22	All V_1^R	All Xyl− Mal−					!! No Recom. Mal+	
2. Lac−	16	V_1^S ± 25 V_1^R	All Xyl− Mal−						
Lac+	14	All V_1^R	All Xyl− Mal−					No Mal+!	
3. #10 artifact									
Lac+	14	V_1^R	Mal+ − Xyl−					(No) recombination here.	
Lac−	6	V_1^S	Mal+ Xyl+						
	29	V_1^R	Mal−	Xyl−	} 37				
4. Lac+	8	V_1^R	Mal−	Xyl−				No Mal+	
Lac−	4	V_1^R	Mal−	Xyl−					
5. Lac+	8	V_1^S	Mal−	Xyl−			2 Recombinants		
Lac−	2	V_1^R	Mal−	Xyl−					

6.

Lac+	13.	V_1^R	Mal-	Xyl-
Lac-	8	V_1^S	Mal-	Xyl-

8.

Lac+	25	V_1^R	Mal-	Xyl-	
Lac-	11 indistinct	}	Mal+	Xyl+?	No - colonies on 'Malac'-Not segregating

9.

Lac-	10	V_1^R	Mal-	Xyl-
Lac+	11	V_1^R	Mal-	Xyl-

10.

Lac-	12	V_1^R	Mal-	Xyl-
Lac+	23	V_1^R	Mal-	Xyl-

Summary: Parents are ~~+~~ Lac, Mal, V_1]: + − R, − + S

①	− − S	− + S	+ − R	Selected as Lac ± .
②	− − S, − − R		+ − R	
③		− + S	+ − R	
④	− − R		+ − R	
⑤	+ − S, − − R			
⑥	− − S		+ − R	
⑨	− − R		+ − R	
⑩	− − R		+ − R	

7/30/49

Replate 589D, after 24hrs. additional at room temperature, on EMB Lac and Malac.

10 Lac plates Lac+ > Lac−. ca 500 colonies.

2 ? Lac ±

20 Malac plates. ca 1000 colonies.

~~HH HH~~ 10 Malac −

11 2 Malac v (+/−)

11 2 ? Malac v (may be +/±)

14 1.4%

14 D Lac ~~9~~ Pure Lac+! Mucoid margins.

15 D Lac + Test +, − on Mal. Pure Mal−! 2 L−M− 11 L−M+ ; 10 L+M−

16 D Malac

17 D Malac

18 D Malac? (+, −)

19 D Malac? (+, −).

may be +, −, ±..

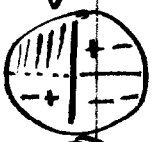
[40 Lac+ tested. All Mal−].

Lac segregation seems to be a good criterion of recombination.

17. 5 Lac+ : Mal−

35 Lac− : 22 Mal+ ; 17 Mal−

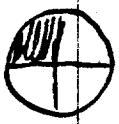
No Lac+ Mal+!



18. 20 Lac+ Mal−
20 Mal+ Lac−

Looks for recombination of other factors — all epidemiale re perm + v, R

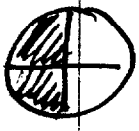
19. 30 Lac+ Mal−
3 Lac− Mal−
7 Lac− Mal+



14. 8 Lac− : 3 Mal−
5 Mal+

33 Lac+ : all Mal−.

16: 40 Lac− : Mal−
40 Lac+ : Mal−



7/29/49

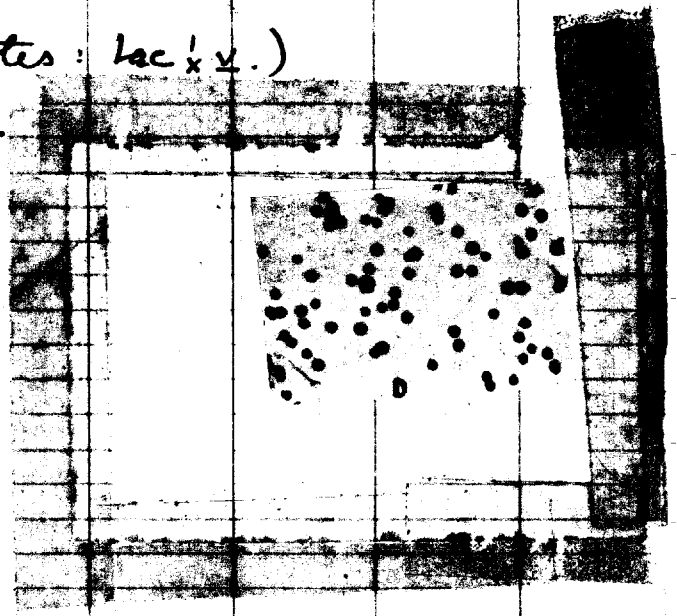
W1034 x 677

100 lac+ tested for lac−. All lac+

Many prototrophs were sectored on EMS Lac!

January 31, 1949.

A) W1058 + 477 (for persistent heterozygotes: lac + x v.)
B) W1058 + 814 24hrs. 72h. Room temp.
C) W1056 + " " " "
D) W1059 + " " " "
E) W1022 + 1033 (Reverse symmetry)
8/1 F) 487 + 814. [like B-D + 24h.]



A: W10 x 200. No lac+ noted.
B-D: high proportion of lac- Mal-
Select segregating colonies from lac EMB:
But frequency of Mal- lac- makes it difficult to decide what parents are!

F. 8/2. Ca 300 colonies: No Malac-. Antac, +, - sem =
E' p. 8/2 1 lac v colony. Also Xgl, Mal v. Cross-hat.

G. 8/2. 1033 + 595 24h.
E" p2. 8 x 100. lac EMB. Almost all +. No v noted.
B: ca 200/plate 10 lac: - second. Contamination unavoidable
Several weak + colonies noted (9) may be Mal- lac+. 1-6 lac v.

2 Xgl	1 v	7
2 Mal	1 v?	8
5 Mal	2 v?	9-10
1 Xgl Mal	1 v ⊕	11

E: ~~75~~ 300 lac+ Mal+ 18 lac- Mal-. No recombinations! (Do 1022 anti Hfr?)

August 4-6, 1949

89. (W1033+ W595).

selection

	Lac	Mal	Gal	Xyl	Mtl	T1 (Lac)		
1	+−	++	++	++	+	SR	4L+ : MG+	NoL−!
2	+−	+−	++	++	+	SR	8L+ : 6MG+ (2MG−)	
3	+−	+−	++	++	+	SR	10L+ : MG+	8L− : MG−
4	+−	+−	++	++	+	SR	10L+ : M+G+	10L− : M−G− (1) M±
5	+−	+−	++	++	+	SR	10 "	7 "
6	+−	+−	++	++	+	+S−R	10L+ → M+G+	6L− → M−G−
7	+−	++M	−+	−+	+(−)			
8	−	++	−	−	−	−R	10M+ → L−	10M− → L−
9	+−	+−	−+	−+	+(−)	SR	10L+ : M+G+	10L− : M−G−
10	+−	+−	−+	−+	"	SR	10L+ : M+G+	10L− : 6M−G−; 5M+G−
11	+−	=+	−+	−+	"	SR.		

Lac
Xyl v
Mal v
Gal v
Xyl Mal

#1 → 10 Xyl+ → LMG+ 10Xyl− → 9 LMG− 1LG−M±?

#8 is Mal v all else −R!

#7 → 10 Xyl+ → LMG+ 10Xyl− → 10 L+G−M− sic?

Summary:

#1 was "pure" Lac+M+G+ (parental). − not 32 complete but were there!

#3,5,6,9,11 parentals only (re Lac Mal Gal)

2: Lac+M+G+ (parental) and (Lac+ M−G−) (recomb.)

4: Both parents and (?) L−M+G−?

7: L+M+G+X+ (par.) and (L+G−M−X−) (recom.)

8: LMGX− (par) and (M+LGX−)

10 LMG+ (par); LMG− (par). and (M+L−G−)

~~11. LMGX+ (par) and LMGX− (par.)~~

Only 4 likely recombinants. Exhaustively checks all of these. (2,7,8,10)

8/7/49

Maltumated!

#7. 10 Lac+ (Mal−) Xyl+ Gal+ Mtl+ V_1^S] M? Complementary? Recombinants
10 Lac− Mal+ Xyl− Gal− Mtl− V_1^R] TLB1

Mal scoring questionable. Also some inconsistency. Colonies which originally scored (Xyl+) Lac+ and (Xyl−)(Lac+) → Lac−! Recheck! and mutation.

#10 (A) 10 Lac+ Gal+ Xyl+ Mtl+ (Mal− ?) V_1^S Recomb?
(B) 5 Lac− Gal− Xyl− Mtl− Mal+ V_1^R ~~Recomb.~~ Recomb.
(C) 5 Lac− Gal− Xyl− Mtl− (Mal−) V_1^R Parental

These appear quite difficulty. a may be Mal+. Do these an interaction between Lac or other factors on Mal expression?

#8 10 Mal+ Lac Gal Xyl Mtl− V_1^R TLB1 Recom.
10 Mal− Lac Gal Xyl Mtl− V_1^R TLB1 Par.

#2. 6 Lac+ Mal+(?) Xyl+ Mtl+ Gal+ V_1^S
2 Lac+ Mal− Xyl− Mtl− Gal− V_1^S

Mal+ was miscored when examined.

8/10 Confirmations: 1) Lac+ is weak Mal+
Lac− is strong Mal+] Both Mal+!
2) Gal+ is Mal+ (slow)
Gal− " "

10) A Mal slow
B Mal+
C Mal−

Interpretation of Mal slow??

P4. Replate 6.

20 Lac. ca 150/ Lac- streak. 2 v (difficult to ascertain)

15 Mal 3 v Many not isolable or curtain

5 Xyl 4 v Clear cut plates.

5 Gal No v

Note —: + turned higher on Xyl and Lac than on Mal! (i.e., exc Mal+ Lac-?)

	Selection	Lac	T1	Mal	Xyl	Gal	streak
1	Lac	+S	-R	+-	+	+	Lac+ -
2	Lac	+S	-R	"	+	+	Lac+(-)
3	Mal	+S	-R	"	—	—	Mal+ —
4	Mal	+S	-R	"	+-	+	Mal+ —
5	Mal	+S	-R	"	"	"	Mal+ -
6	Xyl	+S	-R	"	"	"	Xyl+ -
7	Xyl	+S	-R	"	"	"	Xyl+ -
8	Xyl	+S	-R	"	"	"	Xyl+ -
11 5926X	[illegible]	−S		"	+	"	Xyl+, - All Lac-S; 3 Xyl-Mal+; 7 Xyl+Mal+
12 5926M1		−S		"	+	"	[illegible] Mal++ Not recomb. (T.O.)
13 5926M2		++SR		"	+	"	[illegible] Mal+ -

Streak on selection sugar. Brushall on Lac, Mal, Gal, Xyl, T1

Patent Recomb: #3: Mal+- : Lac-Xyl-Gal- (R?)

Lac↓

11 (592B Xyl±)		Xyl+	Lac-	Mal+ V_1^S	Parental
		Xyl-	Lac-	Mal+ V_1^S	Recomb.
13 Mal±	10	Mal+	Lac-	V_1^S Xyl+	ζ Parentals only.
	10	Mal-	Lac+	V_1^R Xyl-	

P4. Plate again from "B" on various: Ca 100/plate

20 Lac	16 v.	[2BL]	
15 Mal (joint selection on EMB)	2 v	MI-2	} see 591b for analysis
5 Xyl	1 v	X	
5 Malac	4 –	No v.	

of 1-16, #4,7,8,11 were pure Mal– but Lac+,–
Analyze others for recombinants. (4?)

a) Verify 4,7,8,11 as having Lac+ Mal– and Lac– Mal–

b) Sample ca 10 each side

1	Lac+ Mal–	and Lac– Mal+
2	"	"
3	"	"
5	"	"
6	"	"
9	"	"
10	"	"
12	"	"
13	"	"
14	"	"
15	"	"
16	"	"

} Parentals only. ie Lac; Mal

c) Reanalyze 4,7,8,11.

4	Lac+	Mal –	Sal	+ ↓	Xyl	–	MR	–	V,	R TLB,
	Lac–	–		–		–		–		R TLB, Rec
7	Lac+	–		+		–		–		R TLB,
	Lac–	–		–		–		–		R TLB, Rec
8	Lac+	–		+		–		–		R TLB,
	Lac–	–		–		–		–		R TLB, Rec
11	Lac+	–		+		–		–		S TLB, Rec
	Lac–	–		–		–		–		R TLB, Rec
Parents		+		–		–		–		R TLB,
		–		+		+		+		S M

Include Sal char!

August 3, 1949

A) Mix 4 ml ca. 24h. grown cultures of 814 and 1059

B) Mix 1 ml ca. in 10 ml 42.

Plate after 12h.

A) Malac 2×50. No –

Lac ca 150. No Lac v.

B). Malac 1×100 No –

Lac 2×100. Several Lac v.

Streak out and bunch on Lac, Mal, Xyl etc.

	Lac	Mal	Xyl	Mtl	T1. (Mal)	
1	+ –	–	–	–	R	
2	+ –	– +	– +	– +	+S – R	Lac-Mal+ 27; 16 +- All Mal-
3	+ –	–	–	–	R	
4	+ –	–	–	–	R	
5	+, –	– +	– +	– +	+S – R	10 L-M+ 25 L+M-
6	+, –	– +	– +	– +	+S – R	15 L-M+ 13 L+M-

∴ 1, 3, 4 are pure Mal-Xyl-Mtl-Lac- V_1^R.

2, 5, 6 are mixed, but show parental combinations only (No Mal+Lac+)

Plate B after 36 hours for further segregation material.

P2. 14 Lac EMB. 3 Mal EMB 2 Mal Xyl EMB. ca 150 ca. plate

3 Mal v. Only 2 isolable (7, 8) No Mal Xyl v

Few Lac +/+ some were distinctive v, others were 9,10 ⊙, and some were either v or conjunctions

11 – 19 were distinctive Lac v. 20 – 24 simply appeared + and – a common radius. #12 may have a fortuitous Lac –; #13 Lac +

Analyses of 7-24.

August 4-6, 1949

	Lac	Mal	Ryl	Mtl	T1		
Mal						(MR)	
+ −	7 = +	+(−)	++	+(−)	+S −R	10L+ : M+ X+ ;	2L+ M−X−
+ −	8 = +	"	+	"	"	10L− "	8L+ M− X−
	9 + −	"	+(−)	"	"	10L− M+	10L+ M−
	10 + −	"	"	"	"	"	"
	11 + −	"	"	"	"	L−M+	L+M−
	12 + −	"	"	"	"	L−M+	L+M−
	13 +, −	"	"	"	"	L−M+	L+M−
	14 + −	"	"	"	"	L−M+	L+M−
	15 + −	"	"	"	"	L−M+	L+M−
	16 + −	"	"	"	"	L−M+	L+M−
	17 + −	"	"	"	"	L−M+ ;	L+M−
	18 ++ tog cold	"	"	"	"	10L+M−	6L−M+
	19 + −	"	"	"	"	L−M+	L+M−
	20 + −	"	"	"	"	L−M+	L+M−
	21 + −	"	"	"	"	"	"
	22 + −	"	"	"	"	"	"
	23 + −	"	"	"	"	L−M+	L+M−
	24 + −	"	"	"	"	"	"

None of these (7-22) were recombinations of Lac with Mal!

X-	Lac+Mal-	Lac-Mal-X+
3A	W814	+ W1062
3B	"	W1063
3C	"	W1064

Plate on EMB ~~Mal~~ Lac and Xyl. 24h. 37°
B, C had one Lac$^{\pm}$ each. (ca 5×100 cols)

(no recombinants)

B' had several Lac$^{\pm}$ colonies. One of these streaked out and cross-tested Lac··· Xyl. All parentals: B1: 14 Lac-Xyl+
12 Lac+Xyl-
C1: 20; 23 resp.

A-C' sub plated after 48 hours.

1 ~~Mal~~ from Lac EMB 2,3 4 ⊙ from MalXyl EMB.

3A-C. Ca 300 cols. ca. No Lac-Xyl-. Probably not Hfr! unless Xyl+ is favored in these combinations.
1 Lac$^{\pm}$ stem 3A Streak and analyse as 593-5.

593:1-5. Cross test.

	Lac	Xyl	
1	+ −	+ ±	All Lac- were Xyl+
2	++ −	+ −	Lac+ were Xyl-
3	++ −	+ −	
4	++ −	=, +	

No Recombinants apparent

W1062-1064 apparently not Hfr

Conditions of Hfr recomb.

August 4, 1949

GPM. Mix overnight 814 + 1059 (20X)

A) hi water (saline)

B) mi = vol. T(0)

C) mi = vol Y2.

Final suspensions ca 5X.

A6: dilute and plate out on Malac and Lac EMB (36 hours). (3 plates ea.)

A. Malac: ca 100 cols. total No –
Lac +, – : No ☐.

B: No colonies

C: Numerous Malac – ! 15 – / 40 +
Numerous Lac ☐. ca Y/5a

∴ Recombination occurs in heavy Y2 suspensions but not in saline

Cross of #1045 derivs.

August 8, 1949.

A = 1069 (LyMeth + typ)
B = 1077 (hist + iso-val)
C = 1078 (cyst + isoval)
D = 1081 (cyst + Ly meth)
K = 1033 (BM Htr)

Incr. separately P7, and also AB, AC, BD, and BK

	48h.
A	—
B	—
C	—
D	—
K	—
AB	3-4 diffuse colonies *
AC	—
CD	—
BK	—
A + B	—
A + C	—
A + K	—
B + K	—
B + D	—
C + K	—
D + K	—

Pick + streak on T(0)

Hfr crosses

~~W1073~~

W1073 × W909

August 8, 1949

W1073 W909

BM Hfr Lac-Mal- × Y10 Gal- plated after 36 hrs. on Y2 Lac

ca 100/plate

16 Lac ~~+~~ possible Lac+. 1-19 fairly distinctive. 20-32 ◑

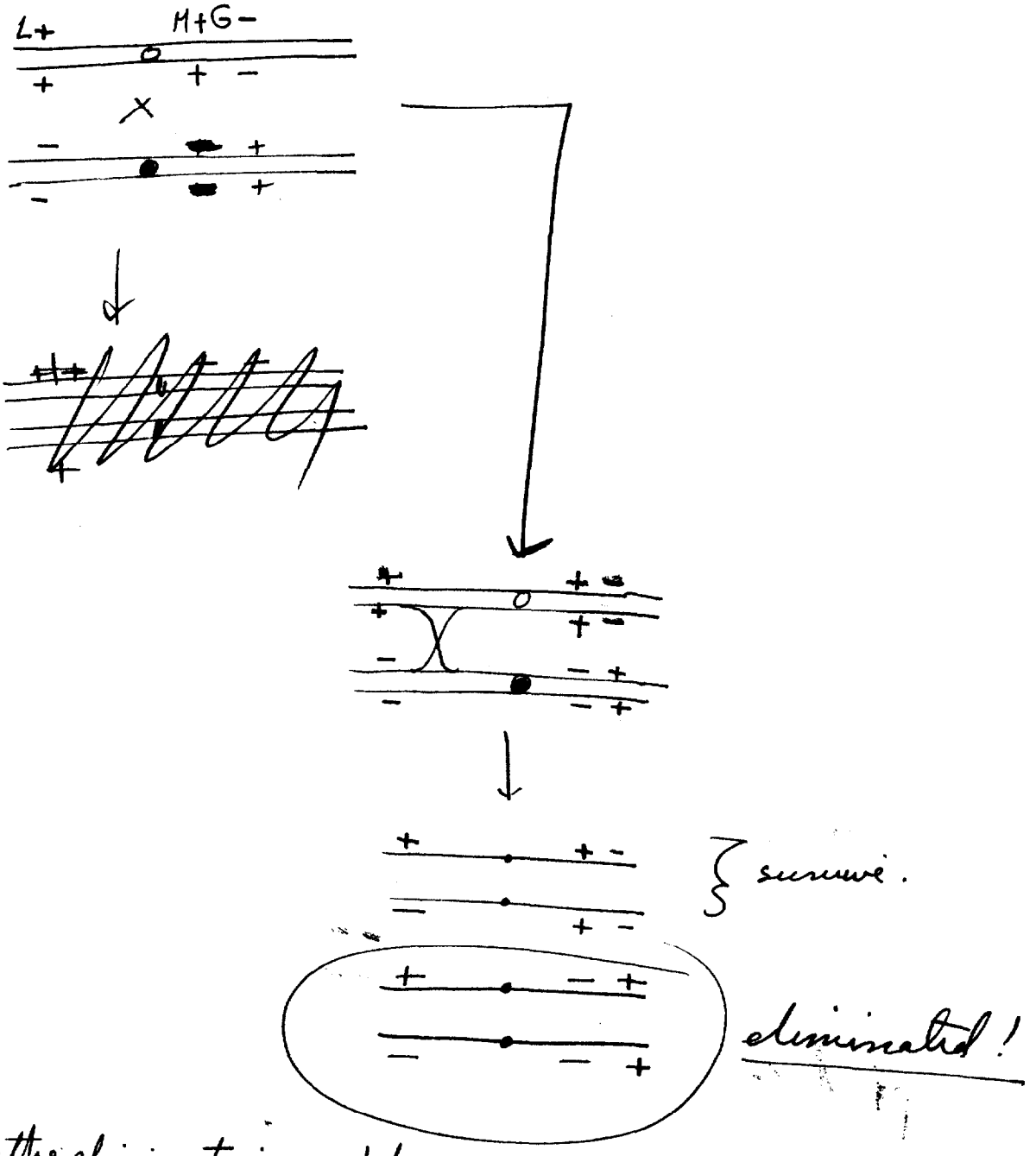
Ca 10 tests each (L^{10-}, L^{10+})

5 Mal 2 Mal+? 33-34

5 Gal 1?? Gal+. 35. | Reincubate.

	Lac	Mal	Gal		
1	All L+	+	−	L+M+G−	
2			−	− + −	+ + −
3			−	− + −	+ + −
4			−	− + −	+ + −
5			−	− + −	+ + −
6			−	− + −	+ + −
7			−	− + −	+ + −
8			−	− + −	+ + −
9			−	− + −	+ + −
10			−	− + −	+ + −
11			−	− + −	+ + −
12			−	− + −	+ + −
13			−	− + −	+ + −
14			−	− + −	+ + −
15			−	− + −	+ + −
16			−	− + −	+ + −
17			−	− + −	+ + −
18			−	− + −	+ + −
19			+ −	− − +	+ + −
20			−		
21	ogive + one papillate	− +	+	− + −	(+ + −)
22		+	−		+ + − , − + −
23			−	− + −	+ + −
24			−	− + −	+ + −
25			−	"	"
26			−	"	"
27			−	"	"
28			−	"	"
29			−	"	"
30			−	"	"
31			−	"	"
32			−	"	"
33				+ + −	+ − −
34			+?−	− + −	+ + − − − +
35			+?−		

P9: ca 5% Gal+ −, or Gal+ +



But the elimination must be due to deficiency, as deficient chromosomes can be kept in heterozygotes!

596B
D.G.

8/17/49

Recheck of results of 596. One + and one − picked from each of 1-34 and rechecked on lac, mal, gal.

	LMG	LMG	
1	~~+ + −~~	+ + −	(no 1 −)
2	− + −	+ + −	
3	− + −	+ + −	
4	− + −	+ + −	
5	− + −	+ + −	
6	− + −	+ + −	
7	− + −	+ + −	
8	− + −	+ + −	
9	− + −	+ + −	
10	− + −	+ + −	
11	− + −	+ + −	
12	− + −	+ + −	
13	− + −	+ + −	
14	− + −	+ + −	
15	− + −	+ + −	
16	− + −	+ + −	
17	− + −	+ + −	
18	− + −	+ + −	
19	− − +	+ + −	
20	− + −	+ + −	
21	− − +	~~+ + −~~	+ + − (no 21 +)
22	− + −	+ + −	
23	− + −	+ + −	
24	− + −	+ + −	
25	− + −	+ + −	
26	− + −	+ + −	
27	− + −	+ + −	
28	− + −	+ + −	
29	− + −	+ + −	
30	− + −	+ + −	
31	− + −	+ + −	
32	− + −	+ + −	
33	− − −	+ + −	
34	− − +	+ + −	− + −
W909	+ + −		Vector characteristic of W909 weak.
W1073	− − +		

W 1084 x 1059.

August 9, 1949.

Plate 24h. cultures on Lac, Mal, Malac. (M-L+ x M+L-).
ca150/

NIO:

6 Malac : No — found.
8 Lac : No ~~v~~ found.
8 Mal : 2 ~~v~~, unreadable.

Replate 3P/10.
ca200/
5 Malac No —
10 Lac
10 Mal

Replate 1A14:

No Malac —
No Lac ~~v~~

Replate from 8/14 on 8/20: (Malac)

DG
598a

August 15ff. 1942

Mutant yields not recorded.

W	Sh 3			
1097	A.	histidine		
1098	C.	"		
1104	D.	aromatic?	mutant!	Any 2 of (tyro; Val; esp.) tryp
1102	E.	threonine		
	~~F.~~			
~~1099~~	G.	histidine		
1101	H			
	~~K~~	Valine		
1105	L	Thramine or	any pair from	Arg Lys Meth Cyst.
1100	M	histidine		

Arg+Lys	Cyst+M.	1K	A	2K	L	C	M

	Sh 4	
1106	B	histidine
1107	D	histidine
1109	E	histidine Val 200
1108	G	histidine
1110	K	Val 200.

8/23/49

Nutritional tests of 596 B

	+				+			
	M	T	L	B, +	M	T	L	B, +
1								
2	+	−	−	− +	+	−	−	− +
3	+	−	−	− +	+	−	−	− +
4	−	−	+	− +	−	−	−	− ±
5	+	−	+	− +	−	−	−	− +
6	+	+	+	− +	+	−	−	− +

Hfr mixed cultures

5986

August 20, 1949.

9P20. Mor heavily in Permassay:

D. W1084 L+M− * W1059 L−M+ (Hfr × Hfr)

E. W1084 + ~~W847~~ W842 (Hfr × +)

1) plated P22. (ca 40 hours).

D Malac 10/100 plates No — (all ~~one~~ two plates!)

E 30/ No —.

2) Plated P23. Yield very low 0-2-16/plate

No —.

3 Plated P24 10^{-6} ml 5 plates Malac each.

D No Malac — // 400 cols.

E 1? or No Malac — / 600 cols. (Cratio Lac- : Mal-??)

Hfr in- and out-crosses

August 16ff. 1949

W1073 ×	Mal: +	Mal: −	Plates (sample)	Lac: +	Lac: −	
A. W1033	29	24	0	15	25	1
B. 58-161	28	61	4	45	77	9
C. K12.	22	26	1	12	27	3

Incubated together 48 hours in ~~Erg~~ Prunasseay. Plated on 10 plates each of Lac and Mal EMB. Possible sectored colonies streaked out on homologous medium.

[sectored colonies in 10 plates in table above ↑]. ca 10 tests each −, +.

A: Lac v. L+M+; L−M−.

B. 9 Lac v. All: L+M+; L−M− 9 Mal v. 9: L+M+ L−M−

C. 3 Lac v. All L+M+ L−M− 1 Mal v. ✱ L+M+ L−M−; L+M− L−M−

No Recombinations!

Plate 122. 10 Lac each. Cultures are badly chimyxed.

A). ca 25 ea. + > −

B) ca 2 ea.

C) ca 150 ea.

3 Lac v.

10 colonies on EMS: all +.

#3 all Lac+Mal+

Plated P23. Yields v. low
10^{5} Lac, 10^{5} Mal ea.

2. A). ca 20/.
3 Lac V.

High variance of +/− points to clumping
but Lac+ >> −

B) ca 5/
No V.

C) ca 2/

Colony yield too low for any conclusions

---

3 Plate P24 ca 10^{-6} ml./plate.
10 Lac 10 Mal ea. ca 200/. for A, B ca 150/ C.

A 3 Lac V 4 Mal V

B 3 Lac V 11 Mal V

C 3 Lac V

All parentals only

Mtr crosses.

August 25, 1949.

In NB mix hourly as follows: Plate 10^{-7} ml: 8/27.

A	1033 × 677	Lac 15	Mal 10	
B	1059 × 814	Lac 10	Malac 5	
C	1059 × 1084	Lac 10	Malac 15	~~Malac~~ Mal 10
D	1073 × 1033	Lac 15	~~Malac 1~~	
E	1073 × 8161	Lac 15		
F	1073 × K12	Lac 15	EMS Lac 5 (2 drops)	

80

A ca 80/ Lac+ >>> − (20:1)

B ca 30/ No Malac −

C ca 20-50/ No Malac − Mal − = Lac −

D ~~Ca 200~~/ Lac+ >> Lac −

E ~~Ca 150~~/

F Ca 100/

A. 1 Mal v 0 Lac v / 800; 1200 resp. [Note reversal of + > −].

B. 4 Lac v / 500. 0 Malac / 150

C. 2 Lac v / 400 0 Malac − / 600.

D 3 Lac v ? / 2000

E. 8 Lac v / 2200

F. 2 Lac v / 1500 EMS: No −

		L+	L-
D	3.	4 M+	10 L-?
	1	10 M+	
	2		
E	1	10 M+	10 M-
	2	7 M+	5 M-
	3	10 M+	10 M-
	4	10 M+	10 M-
	5	10 M+	10 M-
	6	9 M+	6 M-
	7	5 M+	10 M-
	8	10 M+	10 M-
F	1	7 M+	10 M-
	2	10 M+	5 M-

No Recombination

August 28, 1949.

a) Large series of lac- and Mal- mutants obtained from W1069 and W1077 ca 8/25. (W1140 = LyMe-Trypo-Lac-; W1141 = Hist-dbVal-Mal-).

b) N28. Mix W1140, W1141 in Penassay tubes.

N30. Wash and plate on T(0); EMS Lac

P31. 1 colony/15 plates.

Streaked out on T(0) agar. 4 s.c. picked and streaked on lac; Mal EMB. Non-coliforms dominate, but contamination with a Lac+ Mal+ seen. Purify and test nutrition:

These derivatives of W1045 show no signs of recombination.

Hfr crosses.

August 28 1949

Mix heavily in Penassay:

A) W1059 + W814

B) W1059 + W1084

A). Malac ~~For~~ 5 x 100. 13% Malac − 3/500 Malac ±.
Test on other sugars, ~~=~~ TI.

Lac 25 x 100. >1% Lac ±.

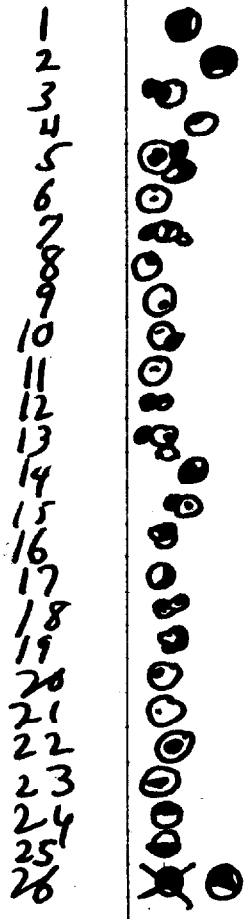
Mal+ Xyl+

Mal+ "tested on lac" 33 L− (2 L+) (1 or 2 midf.) were (B).
Mal− 10 L− ~~50~~ L+

B) Malac 10 x 400 = 1000 No Malac− !
Mal 5 x 100 = 500. 6 Mal ± ?
Lac 25 x 150

Mal+ : 56 Lac−. (from Malac platings, all Mal− are Lac+).
33 Lac− 2 Lac+
Mal+ : 89 Lac− 2 Lac+

A) Loc x colonies



602A count

337+ : 102−

L+ : 60 : All Xyl−, All Gal−(?), MHl−, 14 Gal−,

L− :

Maltose scoring unlfinite because of fading on 1% Mal EMB. Use 1.5% hereafter to avoid this difficulty

M	?	X	MHl	Bal
+		+	+	+
+		+	+	+
+		+	+	+
+		+	+	+
−		−	−	−
−		−	−	−
−		−	−	−
−		−	−	−
−		−	−	−
−		−	−	−
−		−	−	−
−		−	−	−
+		+	+	+
+		+	+	+
+		+	+	+
−		−	−	−
+		+	+	+
+		+	+	+
+		+	+	+
−		−	−	−
+		+	+	+
−		−	−	−
−		−	−	−
+		+	+	+
+		+	+	+
+		+	+	+
−		−	−	−
−		−	−	−
+		+	+	+

M	?	X	MHl	Bal
−		−	−	−
−		−	−	−
+		+	+	+
−		−	−	−
+		+	+	+
+		+	+	+
−		−	−	−
−		+	−	−
−		−	−	−
+		+	+	+
+		+	+	+
+		+	+	+
−		−	−	−

24M−
25M+

Random isolations show 77% L+ X− etc.−
11% L− M+ 11% L− M−

September 2, 1949. xyl tests

	Lac +	Lac −
1.	−	−
2.	−	−
3.	−	+
4.	−	+
5	−	−
6	−	+
7	−	+
8	−	+
9	−	+
10	−	+
11	−	+
12	−	−
13	−	+
14	−	−
15	−	+
16	+	1 −
17	−	−
18	−	−
19	−	−
20	−	+
21	+, −	+
22	−	+
23	−	+
24	−	−
25	−	+
26	−	−

of 25 Lac v, 10 were Xyl − Lac −
15 Xyl + Lac − // Xyl − Lac +.

		Rec.	Par.	
Among Lac −	Seg. Cols.	10	15	35
	Whole pop.	24	25	
		34	40	

Hfr selection.

Sept 2, 1949.

602A = mixture of Lac-Mal- recombinant colonies (5).
Grow overnight in ✱ Penassay.

A) Assay cultures

	$\times 10^7 ml^{-1}$	$\bar{m}$
602A	2,5	3
K-12	42,64	53
1033	20,27	23
58-161	40,56	48
Y10	35,33	34.

Sept. 2.

B) Inoculate .001 ml 602A + following: into Penassay tubes.

A	K12 1ml	46+/59-	31+/27-
B	W1033 .5ml + Y10 .5ml	213+/0-	136+/0-
C	58-161 .5ml + Y10 .5ml	71+/2-	156+/3-

Plated out $\underline{c_9}$ 10^{-7} Sept 5, 1949.

~~Lac Mal~~

B. 17 Lac u. Test +, - on Maltose. (5 each)

1-16 parentals only.

#17. 4 Lac+Mal-, 1 Lac+Mal+, 5 Lac-Mal+

Hetastoli Maltose - uv mus.

September 7, 1949.

Irradiate W466 and W477 on EMB Mal 7 secs. uv.
25 plates each. ca 200/

W466 12 mutants purified. #3 is glucose -
All but #3 and #10 are maltose - slow. T.O.

#3	Mal -	Glu -	
#10	Mal -	Glu +	1187?

W477 16 mutants purified.

#3, 7, 13 are glucose -. #2 is "thin".
#s. 1, 2, 8, 11, 14 are maltose slows
#12 forms minute colonies.

1178-1188

#3	Glu -
4	
5	
6	
7	Glu -
9	
10	
12	
13	Glu -

Check for 30° fermentation of Glu - — None were temp. sens.

W466 Mal −

60.ia

September 15 ff. 1949.

as 604. W466. 20 Mal EMB plates; 200/ = 4000.

1-8 are slow or nearly − fermenters of maltose. Test on Glu

#9, 10 are Mal −. Streak out. (Glu+); W1208-1209

#11 as streaked from 1st isolation had mostly Mal−(slow) colonies, but 3 colonies sectoring in maltose. [These may be suggested to be $\frac{\text{Mal+}}{\text{Mal−}}$ $\frac{\text{Lethal}}{=}$]. No Mal+ were seen. Streak out on Mal EMB.

N18 When streaked out, Mal$\underline{v}$ colonies above gave mixtures of pure + and − and no apparent Mal$\underline{v}$. One possible Mal$\underline{v}$ (more bluish conglomerated) was noted. Streak out: These colonies are very difficult to interpret, mainly because there were no pure + colonies on the original plate. Conceivably, there had been induced an unstable intermediate allele which usually shifted to Mal− but rarely (i.e., with ~3 colonies) reverted to Mal+.

Test cross for Mal$_1$ –

September 13, 1949

(cont'd)
W1178 – 1183 × W1014
W1187 × W814
(W1186)
} Mal EMS (B_1).

Mal+/Total progeny.

1187	20% +	
1178	0/100	Mal$_1$
1179	30%	
1180	1/3 (ca 20 colo.)	
1181	80% +	
1182	30% +	
1183	0/100	Mal$_1$

677 SR × W478
= W1177

cultures purified

612

M. Doudoroff

culture	from	Malt	Streptomycin	Lactose
1	malt Synth	+	−	−
2	"	+	−	+
3	"	+	−	−
4	"	+	−	−
5	"	+	−	−
6	"	+	+	−
7	"	+	−	+
8	"	+	−	+
9	"	+	−	+
10	"	+	−	+
11	"	+	−	+
12	"	+	−	+
13	"	+	−	−
14	"	+	−	+
15	"	+	−	+
16	from Malt Synth + B'	+	−	+
17	"	+	+	−
18	"	+	−	+
19	"	+	−	+
20	"	+	−	+
21	"	+	−	−
22	"	−	+	+
23	"	−	+	+
24	"	−	+	−
25	"	−	+	−
26	"	−	+	−
27	"	−	+	−
28	malt. Synth	−	+	+
29	"	−	+	+
30	"	−	+	−

		Lac+	Lac−	Total
Strept resistant	malt+	0	2	2
	malt−	4	5	9
Strep sensitive	malt+	13	6	19
	malt−	0	0	0

		Total organisms tested		Tested for lactose	lac+	lac−
Strep sensitive.	Malt+	30		26	16	10
	Malt−		0	0	0	0
Strep. resist.	Malt+	8		5	1	4
	Malt−		44	36	6	23
		38	44	31	36. 17	6 14 23

612a
H.D.

Cross - 677 SR (1177) X 478.
4 persistent diploids selected, Segregated

Original diploid	Segregant	Lactose	Synth	Streptomyc Resistance
1	1	−	−	+
	2	−	−	+
	3	−	−	+
	4	−	−	+
	5	−	+	+
	6	+	−	+
	7	+	−	+
	8	+	−	+
	9	+	−	+
2	10	−	+	+
	11	−	+	+
	12	−	+	+
	13	−	+	+
	14	−	+	+
	15	+	−	+
	16	+	−	+
	17	+	−[2]	+
	18	+	−	+
4	19	−	−	+
	20	−	−	+
	21	−	−	+
	22	+	−[3]	+
	23	+	−	+
	24	+	−	+
	25	+	−[3]	+
	26	+	−[3]	+
	27	−	−	+
	28	+	−	+
6	29	−	−	+
	30	−	−	+
	31	−	−	+
	32	−	−	+
	33	+	−	+
	34	+	−	+
	35	+	−	+
	36	+	−	+

Dark!

Diploids grew on streptomycin EMB, showed segregation into lac+ and lac−, all growing on streptomycin. Diploids could be reisolated from streptomycin plates.

[2] some colonies grew

??

Hence: Streptomycin resistance is dominant, ~~sensitivity~~ recovered in all segregants for lactose fermentation & nutrient deficiencies. ~~sensitivity~~ is lost in cross. Hemizygous?

612b
M. Rockoff

677 SR × 478

cultures not purified

	Streptomycin Synthetic glucose	Lactose Synthetic	maltose
Prototrophs 1	+	+	−
2	+	−	+
3	−	+	+
4	+	−	−
5	−	−	− ?
6	+	−	−
7	+	+	−
8	+	−	+
9	−	−	+
10	+	−	−
11	− (+ colonies)	+	+
12	+	+	− ?
13	−	+	+
14	+	+	−
15	−	+	+
16	+	−	+
17	−	−	+
18	−	−	+
19	+	−	−
20	+	−	−
21	−	+	+
22	+	+	+
23	−	+	+
24	+	−	−

	Streptomycin Synth. glucose	Lactose Synth	Malt.
Prototrophs 25	+	−	−
26	+	−	−
27	+	−	−
28	+	+	−
29	+	−	−
30	+	−	−
31	+	−	−
32	+	−	−
33	+	−	−
34	+	−	−
35	+	−	−
36	+	−	−
37	+	+	−
38	+	−	−
39	+	−	−
40	+	−	−

		Lac +	Lac −	Total
Strep. sensitive	mal +	3	4	7
	mal −		1	1
Strep. resistant	mal +	1	2	3
	mal −	6	21	27

		Maltose
Prototrophs 1	+	+
2	+	+
3	−	+
4	−	+
5	− (+ colonies)	+
6	+	+
7	−	+
8	−	+
9	+	−
10	+	−
11	+	−
12	+	−
13	+	−
14	+	−
15	+	−
16	+	−

		Lactose Synthetic	Maltose
Prototrophs 1	+	−	+
2	+	no gr.	+
3	−	" "	+
4	−	+	+
5	− (+ colonies)	no gr.	+
6	+	" "	+
7	−	+	+
8	−	−	+
9	+	−	−
10	+	no gr.	−
11	+	"	−
12	+	"	−
13	+	"	−
14	+	−	−
15	+	no gr.	−
16	+	−	−

		Malt +	Malt −
Strep sensitive	malt +	4	
	malt −	0	
Strep resistant	malt +	3	
	malt −	8	

W 108 mutations occurring spontaneously on nutrient agar.

Strain	Deviated ca + on:	Gluc	Galact	malt.	Lact.	Xylose	Mannitol	Treh
1	Glucose	+	S	[illegible]	S-			
2	"	+	S	-	G-			
3		+	S	-	G-			
4		+	S	-	S-			
5		+	S	-	S-	S	—	+
6	maltose.	-	+	+	[illegible]			
7		- (S-)	+	+	+	+	+-	- [illegible]
8		-	+	+	+			
9	Galact	-	+	S	S-			
10		-	+	S	S"	+	+ ?	- [illegible]
11		-	+	S	S"			
12	Lactose	- S-	+	S-	+ (not very strong)	+	- (S-)	- (S-)
13		- "	+	S-	+	+	- "	- "
14		- "	+	S-	+	+	- "	- "

D. D. 9-15-49 613

Fermentation tests on Shapiro's cultures.

	Sucrose	Rhamnose	Arab	Inositol	Xyl	Mtl	Gal	Mal	Glu	Sorbitol
W1115 (1)	+		+	−	+	+	+	+	+	
W1116 (2)	−+							+		
W1113 (3)	±							+		
W1114 (4)	−+							+		
W1045 (5)	±							+		
W1176 (6)	±							+		
Sh 7	±							+		
8	±							+		
9	±							+		
10	+							+		
11	±							+		
12	±							+		
13	±							+		
14	±							+		
15	±+							+		
16	−							+		
17	+							+		
18	+							+		
19	±							+		
20	+±	All slow +						+		
21	±									
22	−									
23	−									
24	±									
25	±+									
26	−									
27	±									
28	±									
29	±									
30	+−									
31	±									
32	±									
33	±									
34	−									
35	+									
36	+									
37	−+	−								
38	−+									
39	−+									
40	−+									
W1222										

−+ are mutabile

Het h-crosses.

Sept. 16, 1949.

A.	58-161 ×	W1178	*6 tests;	all Lac++; 96 tests.	1 Lac v	2?
B.	58-161 ×	W1183	52 tests.	2? Lac v; 60 tests	6 Lac v	2?
C.	W478 (Mal+) ×	W1178 (Mal−)	100 tests.	4 Lac v		
D.	W478 ×	W1183	52 tests.	1 Lac v		

A		#2, 3 (?)			
B	1 het	Lac v	Mal++	614-B1	
C.	1-4	Lac v	Mal++	614-C: 1-4	[#1 and 2 thrown off frequent lac-pseudotypes I.
D.	1	Lac v	Mal++	614 D1	
B.	1-6	Lac v ×	7, 8 Lac v?	5, 6, 7 are Mal +, − 8 is Mal + Lac++.	

1-4 are Mal−

* M.O. Misinoculated.

See 618

Het ou[redacted]es.

September 17, 1949.

		Rev EMS Lac ;	EMS Mal.
A. W1178	x W828		52 tests to 1?? Lac v
B W1178	x W836		100 tests. 4-5 Lac v 6-7?
C. W1178	x W760 very infertile	~~6 tests 1 Lac v~~	6 tests. 2 Lac v.

In 615 B, both on Mal EMB and Lac EMB, — colonies seem to grow better than + ! Streaked from Mal EMB:

A Mal −

B: 1-4 Mal − − ; 5: Mal+ (v??) ; 6 M+, − 7 Mal − (Recheck on resolution)
All Lac v. Isolated Lac v of 5, 6 were pure Mal+, Mal − resp.

C 1-2 Mal −

See 618

September 17, 1949

D. W1189 × W1195 [Val$^{A\cdot C}$ Arg^{1} Mal− × Leu Thr Lac^{2}−]

E. W1189 × W1205 [Val^{AA} Arg^{1} Mal− × Thr His^{3} Lac−]

F. W1195 × W1191 [Leu^{CC} Thr^{2} Lac− × Thr His^{4} Mal−].

Wash cultures from 42. Conc. 5x. Use .1 ml/plate

Controls:

1 (W1189) 4 colonies / 4 plates Lac+ Mal−

2 (W1195) 0 " / " "

3 (W1205) 0 " / " " Lac− Mal+

4 (W1191) 8, 12, 16, 11 / 4 plates ca. 12/plate !

D. 2 colonies / 9 T(0) plates 2 Lac+ / 4 EMS Lac plates

E. 7 colonies / 9 T(0) plates 1 Lac+ / 2 "

F. 10, 6, 2 / T(0) plates

W1189 and W1191 appear to be exceptionally unstable. Their nutrition should be carefully checked.

Tests on "cross" prototrophs

P19

	Mal	Lac
D: 1-4	+	+
E 1, 6	+	−
2-5, 7	+	+
F 1-16	+	+

These prototrophs are clearly either contaminants or recombinants, probably former. Parents had been checked on EMB and found pure −.

Tests on "inversion" prototrophs

A20

1	Mal+ Lac+	} must be contaminated !!
4	Mal+ Lac+	

1	0, 2, 2	1113	Thr Hist. x	Val Arg.
2	1, 2, 2	1113 x 1114	"	Val-Leu; Arg.
3	2, 1, 4	1113 x 1115	"	Leuc Trypt.
4	0, 0, 0	1113 x 1114	Val Arg	Hist Leuc
5	0, 2, 1 sm sm	1113 x 1115	"	Thr Hist
6	1, 2, 0	1114 x 1114	Val Leu Arg	Hist Leuc
7	2, 0, 0	1114 x 1115	"	Thr Hist
8	7, 4, 8	"	"	Leuc Trypt.
9	0, 0, 0	1115 x 1115	Thr Hist	"
10	>15.	1113 1115	Val Arg	Thr Hist

Prototrophs occur amidst rather heavy syntrophism!

Pick colonies from #8, #10 + streak on T(0).
Each of 12 tested from #8 and #10 grew out as single colonies on T(0), and were further picked to EMB Lac, Mal, Xyl on which they agreed with their parent in being ++.

September 25, 1949.

Collect following heterozygotes: 4 = 614 5 = 615.

5A = 1178 × 828
5B = 1178 × 836
5C = 1178 × 760

4A = 58-161 × W1178 4B = 58-161 × W1183
4C = W478 × W1178 4D = W478 "

mEMS lac

614 A. 1 Lac+ Mal+ } Resol!
2 Lac+ Mal+ } Resol!
3 Lac+ Mal+ } Resol!

0
0
0

614B 0 1? 2 3 Lac v Mal+ or v?! Mal+.
1 Lac slow? Mal −
2 Lac slow? Mal −
3 ~~Lac +~~ Lac v Mal −
4 ~~Lac v~~ " "
5 Lac+ "
6 Lac+ "

C 1 Lac v Mal v?
2 " Mal +
3 " Mal v?
4 " Mal +

D Lac v Mal+

615 B 5 (4 hits) #1, 4 Lac v ; All Mal+ but #1 shows mottling on Mal. but Mal+

6 (4 hits) All Lac v Mal − (segregating blue + white) Use for Rev

6_0 (1 hit - broad streak) Many Lac v Almost completely Mal −.

615 B5 = Lac v Mal+ See 615 for data on other Lac v.

615 B6 = Lac v Mal −

Mal$_1$ - x-ses of W466 mutants

Sept. 23, 1945.

W466 Mal$_x$ - x W677.

A) W1208 x W677.
2 Mal+ / 600 Mal -.

B) W1209 x W677
5 Mal+ / 1600 Mal -

Streaks out + protoplasts:

A) Pure Mal+

B) 3 Mal $\underline{v}$, 4 Mal ++.

4 plates Mal E MS

In view of rarity of Mal+ in W^{Mal+}466 x W677, these low frequencies do not necessarily speak for close linkage.

Resolate [to use for Lac reversion studies].

Coli new crosses.

September 28, 1949.

Mixtures grown together 48 hours. Plate ca. .5 ml = 0 / plate T(0). Inc. 48 hours.

A	W1189	0	0	0		Lac+M- Val Leu Sh 3
D	W1191	0	0	0	0	0 Lac+M- Th His Sh 1
E	W1195	0	(1)	0	(1)	Const? L-M+ Leu Try Sh 1
F	W1205	(5)	(1)	(5)	0	L-M+ Th His Sh 3
G	W1189-1195	2	3	5	4	
H	W1189-1205	3	1	2	0	
I	W1195-1191	0	0	0	0	

Prototrophs grew more poorly on T(0) agar than those below.

Pick, dilute and test on Lac; Mal:

E	2	Lac- Mal+	
F	10	All Lac- Mal+	
G	12	All Mal+; 8 Lac- 4 Lac+.	
H	6	Lac+ Mal+	IIII
		Lac- Mal+	II

These results strongly suggest recombination between W1189 and either W1195 or W1205. However, there is a remarkable instability of the individual parents. The Lac+ Mal+ prototrophs are, however, unique.

Repurify and retest parents!

October 7, 1949.

A) Reversions of parents. Plate ca 10^9/plate ± or without single components.

Purified cultures

Some tests not recorded owing to subsequent contamination.

W1205:

T(0): 11, 11, 13 37 tested all Lac − Mal+

Hist 32, 33 18 tested on T(0) 7 prototrophs!

Th. Crowded ± contaminants.

1195

T(0) 0, 0

Leuc. 7, 4

B) Crosses.

A 1189 × 1195 B 1189 × 1205 C 1195-1191 No prototrophs

all Mal+ on EMSMal

7 tested: all Mal+

4 Lac (3 Lac+) 17 Lac (8 Lac+)

25 Mal+

Are these Lac+ Mal+ recombinants?

October 14. 1949.

1 ml (10 x) per plate. Grown in Penassay overnight and washed. Spread on T(0); mist; thereafter.

O	8, 11
H	448, 380.
T	15, 14

Test T colonies for prototrophy
25 : all prototrophic!

Oct. 17, 1949.

9AM. Reinoculate from overnight Permassay cultures .1 ml / 10.
Grow to 8PM+
A W1177^{SR} B W1217^{42R} C W1177 + W1217
Use nutrient agar + 100u/ml SR + 130 γ/ml Azide (= M/500)
1) Inoculate 2 ml culture / 50 ml nutrient agar med. Pour 1 ml/plate
2) " 5 ml " ....

100 colonies picked from C, streaked on EMB Lac. 34 Lac+ : 66 Lac−

A1 Eventually produces numerous minute colonies, especially when unevenly dispersed. 13 countable colonies / 4 plates.
= 13/2×10^9.

A2 " " very minute 1 " " / 5 plates.

14 small Lac− Xyl+ str.

B1 4 plates. No colonies. Clear plates!

B2 5 plates 1 colony. Clear plates. Pick this colony and streak out on the same plate, as well as EMB Lac.

C1 Many minute colonies. 98, 121, 98, 91 (add 1 one picked)
= 102 / plate average
= 500 / 2×10^9

Lac+ 34. 28 were Mal− Xyl− Mtl−
Xyl+ Mtl+ Mal− #2
Xyl+ Mtl− Mal− #4
Xyl+ Mtl+ Mal+ 1

Lac – 66.

A) 1 Xyl+ Mtl+ ~~Xyl~~ Mal+
2 Xyl+ Mtl+ Mal –
1 Xyl – Mtl – Mal +

B) 1

1 Xyl+ Mtl- Mal-

	Total:	Lac m	Xyl	Mtl	Mal	T5	Nutr. sec. infect.
1	61	–	–	–	–	(16R) 45S	.....
2	1	–	+	–	–	R	B_1
3	2	–	+	+	–	1S; 1R	M-
4	2	–	+	+	+	1S; 1R	1M; $1B_1$
5	1	–	–	–	+	S	M-
6	28	+	–	–	–	S	
7	3	+	+	+	–	2 S	M; +
8	4	+	+	–	–	S	2+; $1B_1$; 1L
(9)	1	+	+	+	+	S	M-

Linkages.

Xyl+ / Lac – : Xyl+ / Lac+	5 / 61 : 6 / 28
Xyl+ / Mtl+ : Xyl+ / Mtl –	6 / 8 : 3 / 92
Mal+ / Xyl+ : Mal+ / Xyl –	3 / 9 : 1 / 91

Test 10 type 1 for nutr., phage samples of all other types

Complete phage tests
nutritional

6305

10/25-6/1949.

10 Lac- .... - tested (all T_5^s)

1 +
2 B_1
* 3 M
4 +
5 TB_1
6 TB_1
7 TB_1
8 n.g.
9 +
10 +

* useful?

Test Lac- .... V_1^R for mutation

1
2 B_1
3 B_1
4 TLB_1
5 TLB_1 2
6 TLB_1 1
7 B_1
8 B_1
9 TLB_1 4
10 T
11 TLB_1 5
12 T
13 TLB_1 3
14 TLB_1 6
15 ~~#~~ TB_1 ~~#~~

40 -
2 T -
4 TB_1 -

111-3 coli mm crosses.

Oct 17, 1949

A 1195
B 1205
C 1222
D 1222 × 1195 (grown together overnight)
E 1222 × 1205 " "

10/21/49:

A	4 T(0)	3 EMS.	2 colonies.
B	3 "	3 "	14 cols.
C	3 "	4 "	0 cols.
D	8 "	10 "	1? Lac+ colony as EMS
			10 colonies
E	5 "	5 "	5 T/O)
			2 EMS Lac

Ferm. tests:

A 2 Lac− Gal+ Mal+ grew poorly on EMS Lac

B 9 Lac− Gal+ Mal+.

C — No growth.

		Gal	Lac	Mal	
D	1	−	−	+	×
	2	−	−	+	×
	3	+	−	+	
	4	+	−	+	×
	5	+	−	+	
	6	−	−	+	×
	7	−	−	+	×
	8	+	+ −	+	×
	9	−	−	+	×
	10	+ −	+ −	+ −	
E	1	+ −	−	+ −	×
	2	+ −	−	+	×
	3	+ −	−	+	× ±
	4	+	−	+	
	5	+	−	+	
	6	+	−	+	
	7	+	−	+	
Parentals	{	+	−	+	
	.	−	+	−	

× grew poorly on EMS Lac

October 24, 1945

Streak on galactose

D

1 5 colonies from streak. all Lac+ Gal+ Mal+ (R)
2
3
4
5
6
7
8 Lac+ Mal− Gal−
Lac− Mal+ Gal+ (P)
9
10 Lac+ Mal− Gal−
Lac− Mal+ Gal+ (P)

Gal+ P Lac− Mal+
Gal− P " − "
Gal+ P " − "
Gal+ " − "
Gal+ " − "
Gal− P " − "
Gal− P " − "
Gal− P

E

1 Gal+ Lac− Mal+
2 "
3 "
4 Gal+ Lac− Mal+
5 Gal+ " Mal+
6 Gal+ " Mal+
7 Gal+ " Mal+

Gal+
Gal+

11.

No definite recombinants in E. In D, # 1, 2, 6, 7, 9 are Gal− Lac− Mal+ which is a new combination
These are partly mucoid and plaqued!

Remember 631: 1–5

Time of prototroph formation.

10/25/49.

58-161 x W677. Spread .1 ml on T(B) plates 11 AM. At stated times, respread with .1 ml saline. Count at 48 hours:

		Time	Numbers of prototrophs / plate	M (m.)
0	11 AM	0	199, 278, 209 Means: 132, 236	211
1	12^10 PM	1^10	170, 202, 207, 213	198
2	2^30	3^30	228, 158	193
3	3^20	4^20	346, 113 (sic!), 380, 307	286
4	4^40	5^40	265 110 240 181	199
5	5^30	6^20	133, 409, 143	262

October 26, 1949.

First prepare inocula overnight. Then mix.1 ml inocula, grow together 36 h.

Wash and plate on T(0).

A. 1117 B. 1135 C. 1197 D. 1222 E. 1222+466 F. 1222 + 477 G 1222+1117
0,0,0 1?0,0,0, 1?,0,0 0,0,1 6,1,3,2,3 1,0 0———

H. 1222+ 1197 I. 1222 + 1135.
000000 0000000
W-1222 is Val- ArgGlut- Mal-Gal-Lac+ Other stocks are Lac-.

Yields (per plate, 3 days) See above

Note prototrophs on E (W-466[K-12?] x W-1222. Streak out on EMB Lac to purify and test:

	Lac	Gal	Mal	Sucr	T5		
W-466	-	+	+	-	S		
W-1222	+	-	-	+s	R		
1	+	—	—	+	R	P	
2	+	—	+	+	S		x
3	+	—	+	+	S		x
4	+	—	—	+	R	P	
5	+	—	—	+	S		x
6	+	—	—	+	R	P	
7	+	—	—	+	R	P	
8	+	—	—	++	R	P?	
9	+	—	—	+	R	P	
10	+	—	—	+	S		x
11	+	—	—	+	R	P	
12	+	—	—	+	R	P	
13	+	+	+	++	R		x
14	+	—	—	+	R	P	

Numerous recombinations! (at least 5)

Try 1113, etc. as x 677, etc.

October 25 "B" 1949.

W 1217 x W-677 on Lac EMS. Good yield. Pick 100 Lac+ and streak out on Lac EMB for Lac# v. 14 Lacv found. [All were lambda+ /518]

Reisolate to EMS Lac. 635-1 and 635-8 streaked out, and Lac- and + segregants isolated. Test on NA + Az. : (NA controls all +)

	635 -1	-8
5 Lac-	1r 4s	2r 3s
5 Lac+	5 r 0s	4r 1s
4 Lacv	all r (segregants!)	all r.

Both these cultures, therefore, are segrgating for Az r/s.

Note totals:

Lac- 3:7 Lac+ 8:1 indicating linkage of Az to Lac, with frequent crossovers.

Retest diploids on synthetic medium.

Oct. 25 1949 "C"

58-161 x 677. EMS Mal. Look for Mal+/- sectored prototrophs.

7 found. Streak out on EMS Mal to seprate components.

from severak hundred. Atest.

	Mal		Lac		Xyl		Mtl		TS	
1	+	−	+	+	−	−	−	−	S	S
2	+	−	−	−	+	−	+	−	S	S
3	+	−	+	+	−	−	−	−	S	S
4	+	+	−	−	+	+	+	+	R	R
5	+	−	−	−	−	−	−	−	S	S
6	+	−	+	−	+	−	+	−	S	S
7	+	−	+	−	+	−	+	−	S	S

No. 4 is an error. No. 6-7 not especially concluded.

In others, possibility of Mal- recessive must be considered.

Nov. 7, 1949.

A. 1235 x 1239

B. 1243 x 1239

C. 1238 x 1236

D. 1238 x 1242

EMS Lac B_1 Crosses by NZ

A) heavy - background 20 small +/pl

B) about 200 large +/pl -indisting from bckg

C) Heavy background 200+ several -, many intermediates

D) Heavy bckg no ± many large+

100 from each to EMB Lac to look for Lac v

Sectoring on EMS Lac also observed.

A [1] Lac v Mal -

B [1] Lac v Mal+

Streak out single colonies of Lac v from B for auth. tests: (T, L)

1	A1	+ only	
2	A1	+,	
3	A1	+, -	
4			

	Lac -	Lac +
1	(T) L	T
2	(T) L	T
3	(T) PROTOTROPH	T
4	(T) L	T
5	(T) L	T
6	(T) L	T v
7	L	T
8	L	T
9	L	T
10	L	T

No3 crossovers.

Restreak #3 Lac -

Symmetry test Bxcrosses. 638

November 10, 1949.

Repeat 637

A 1235 x 1239 dilute, use .1 ml = suspension/plate

B 1243 x 1239 EMS Lac Add B, to susp.

C 1235 } Turbid!

D 1243 } Controls Turbid!

E 1239 } clear.

Most plates with background much too heavy! Pick 120 Lac+ from B and look for Lac^v. 2 ?? Restreak on EMS Lac; Mal.

None Mal^v.

Drug resistance mutants for colicins

November 13, 1949

Grow K-12, W1113 ~~and W1115~~ in Penassay aer. overn.

W1113/Sr and K12/Az found. No K-12/Sr noted with this run. Ca ~~10~~ 2×10^9 ca. tested.

Rep as W1247-1254.

Mg effects on recombination

November 13, 1949.

5 P13 inoculate 1:5.

8+ P13 Wash W1217 and W1177. Minimal and plates as:

1) T(0)

2 Davis minimal

3 " " + .1ml $MgSO_4$ Molar. Spread

4. " " Drop in center of plate for diffusion effect.

K12 x W1113
with drug resistance

Nov. 23, 1949

From overn. cultures, take 1ml inocula 1244 and 1247 into Permassay 10AM.

A) 7^30 PM. Plate 2ml into 4 plates Nutr. Agar (+ A2 + Streptomycin) to select possible recombinants

B 12^30 PM 11/24. 5ml/50ml agar in 4 plates

C. do. 2ml " "

K-12 Mutant Rem (Sam Tove)

11/25/49.

Pilot run to search for liver extract requiring mutants.
K-12 subjected to penicillin selection in Y2 broth, then plated on same + liver extract. Colonies tested on T(0); EMBlac; Y2+L.E.
No L.Ex differentials found, but 6/103 tested failed to grow on T(0) on first test. Retest from EMBlac.

[1 Lac- also noted. Streak out and test purified culture on glucose on EMBlac plating. Glu+]

4/6 grew on T(0) this test. → AS; HC; Y-Ls.

1 gave a few colonies on T(0) Restreak.

1 did not grow on T(0). Rundown.

K-12 × W1113. 644

Nov 20ff. 1944

		Yields	colo.	
A. W466 × 1229	3		0	
B. 478 × 1118	6		10-20/	Picks at random
C. × 1153	6		2	
D × 1156	7		24+	Mostly Suc−
E × 1159	6		#10/	Picks 4
F × 1160	6		4	

In streakings, a "lytic?" effect of Suc+ on Suc− was noted. On verification, W1156 was found to lyse W478, indicating a lysogenic phage! active on K-12, even though λ+.

Test purified (streaked on EMB Suc.) isolates on Suc, Lac, T5, T6:

B: 12 - all Suc+ Lac+ $T6^R$ $T5^R$

C 2 " " "

E 4 " " "

F 4 " " "

D 24: 23 Suc− Lac+ T_6^S $T_5^{S (=P)}$ No unselected recombinations

1 Suc+ Lac+ T_6^R T_5^R observed.

For these crosses, mutually resistant or mutually desinfected parents should be used! But try W1156 × W677.

Dominance at the S2 locus.

12/3/49.

W1246 x W1178. 10 plates. Only 5 ~~colonies~~ prototrophs

#lac+3 is lac v. Test segregants on SR.

all Mal −

#1 is $V1^R$; others are V_1^S

#3 appears partially Streptomycin sensitive, showing slow growth

In cross B, lac- 2, 3 are S^R; others are S^S

Streak out A3 from EMS Mal on EMB lac and EMB lac+SR 100.

UV effects on diploid

12/3/43 ff.

4 Mal-colo. from H213 10^{-6}/3s. UV streaked on Mtl.

1,2 Mtl+ 3,4 Mtl−

10^{-1}/30s. UV.

1 Mtl+
2,3 Mtl−
4. Mtl ± or ±.

→ Residuals possible Mtl± colonies. = 646 − 1

None. Just Mtl+; Mtl−.

Irradiate H213.

12/6/49.

Spread 10^{-6} and 10^{-7} ml H213 (from EMS tac) suspension on EMB Mal. Expose some to UV, 3 seconds.

	UV.	+	−	V	?	
10^{-7}	0	1	2	10		1
		0	4	11		2
		1	9	9		3
		0	6	9		4
TOTAL		2	21	39	/62	
	%					
10^{-6}	3	2	10	2		11
		5	5	3		12
		3	6	9		13
		5	4	4		14
		1	7	2		15
10^{-7}	3	1				
		1	1	1		
		0	0	2		
TOTAL		18	33	23		
10^{-6}	0		41	156		
		8	[illegible]	[illegible]		21
		3	[illegible]	70		22
			23			
		6	35	93		23
		[illegible]	25	76		24
		2	23	96		25.
		22	147	491		

Re examine 7-0.

1	1	3	10
2	0	2	13
3	1	9	9
4	0	5	11
	2	19	43

χ^2:

2	21	39	62
18	33	23	74
20	54	62	136

6/66.

10^{-1} 8 sec.

	+	−	V
31	6	16	26
32	2	16	22
33	2	22	16
34	0	14	10
35	5	15	10
36	7	11	15
37	1	6	13
38	4	12	19
39	5	13	39
	32	125	170

Some clonis ● counted as +.

Unirradiated:

		+	−	v	Tot.
A		24	166	534	724
	%	3.3	22.9	73.8	
B	Irradiated 3 secs. ca. 10^{-1} surv.	18	33	23	74
		24	44	31	
C	Irradiated 8 secs. ca 2×10^{-7}	32	125	170	327
		10	38	52	

W1205 v W814

649

~~#80~~ do from EMStac

130 +

20 –

No Lac v.

12/5/49

W1156 x W1257 "B".

Student EMB Lab.

C W1257 x W1229 (11/15/49)

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Wednesday
Dec 14

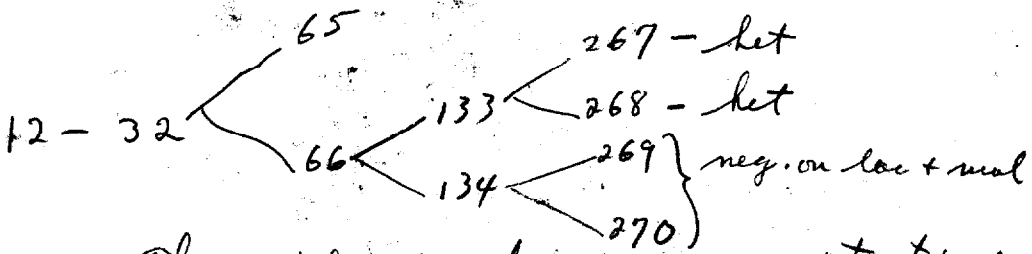
Dear Josh.

I have 4 series, 10, 11, 12 & 13 with H206-1., a strain I have maintained from the original culture you sent.

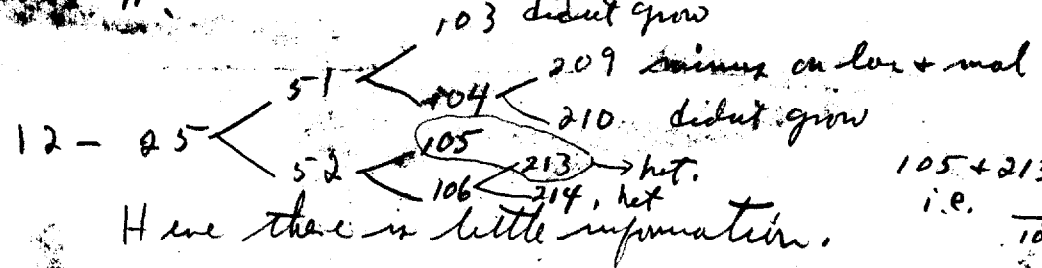
I have not had time to give the complete story but results so far are somewhat disappointing. I am sending a few cultures and the portions of the pedigrees pertinent.

Series 10 + 11, with about 100 cultures in each gave no segregants. I thought I had a couple of + segregants (have used + 113 lactose mainly, maltose to a certain extent) but in every case could isolate a het colony.

In series 12, the only true segregants so far occurred. These are; 12-269, 12-270 and 12-209.



This indicates the same type of situation as before, i.e. no complementary types.



105 + 213 could not be separated
i.e. 105 213 214

Here there is little information.

12-27 was a filamentous seed & could not further separate. It was largely minus lac, plus mal but there were a few hets also.

In addition 12-198 and 12-196 looked to be all positive on lactose but again I could find some small het colonies. These were in every case smaller colonies with a positive center & were not clearly mosaics. But on streaking they proved to be 13-316 and 13-163 behaved similarly, i.e. < 1% hets, and negative on 30 other items not positives

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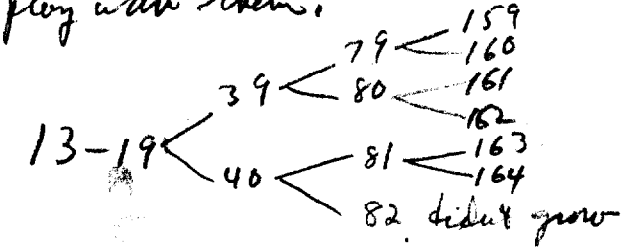
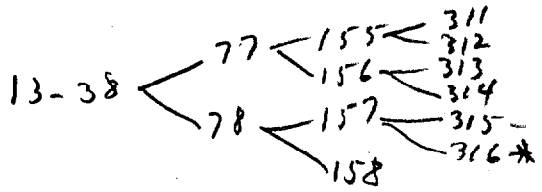
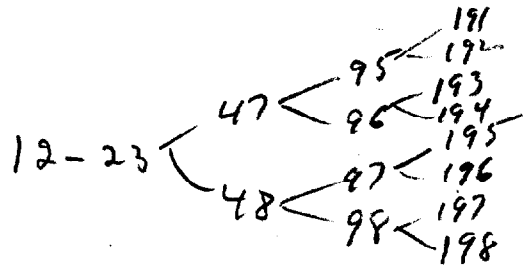
on lactose. It strikes me as curious that so many cells could be hets, segregate to + positives very early and end up with a microcolony composed a few het cells and almost no negatives. I wonder if all the positive cells are alike in other segregating characteristics. On replating 12-196 showed a few negatives but 12-198 showed no negatives among some 3 or 4 thousand colonies. Similarly 13-316 and 13-163 showed a few negatives on replating. Probably in all these cases, a segregation occurred in the first division of the cell but the very unequal + to − ratio probably indicates also that complementary types are not formed at the time of segregation. I'm sending some of these in case you want to play with them.



159, 160, 161, 162, 164 were typical het cultures
163 as above indicates.



158, 311, 312, 314 were typical hets
313 mostly + 's few means + negatives.
316 mostly +, <1% hets + negatives.



all typical hets but 196 + 198

I have observed some unusual forms of cells in these cultures. Haven't yet worked your latest H206, will do so next week. Sorry to be so shabby but must leave for Washington

Sincerely
Max

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Dear Jack. 4 Jan 1950

Sorry to be so hurried in getting this off but at the moment life is a rat race. So I'll try to give the pertinent information & get this off.

1- Source for the 1231-pedigree was your new culture of H206. This culture behaves differently in the moist chamber. Fewer filaments, fewer cells not growing, no Y forms & mentioned.

2- The number duplicate earlier pedigrees. I started from 1 when I started on H206 + there are #5, 6 & 7. This is not good but not fatal. I renumbered the H206 pet dont know all my records here so will leave as is.

3- Some of the cultures gave blank plates. I think they are indicated on the pedigrees. They were inoculated & sent along for you to check.

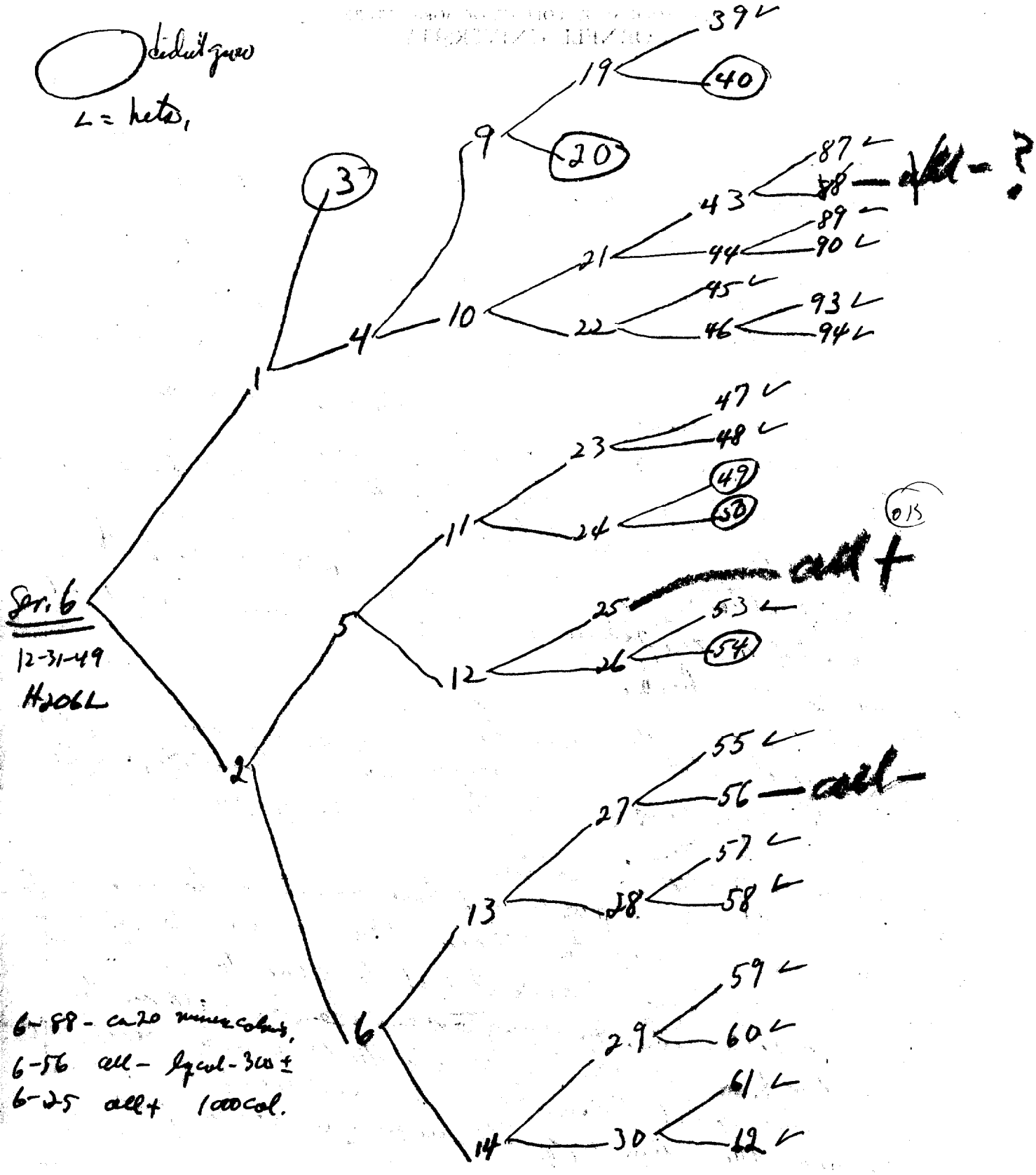
4- I picked 6 + colonies from the plate of 5-223 since they were nearly half the total colonies observed + on the chance they might be all alike and complementary to 5-223. You can do what you wish with them.

5- Cultures were tested on lactose.

6- I have seen the NIH people and have permission to do some of this down here. I'm going to borrow the weekend to get my equipment. Initially, I think it best to send the cultures to you for classification. I'm going to send them in both in the vials at present. If they are too old to be easily classified I think I could do it down here but I don't want to kill the goose laying the golden eggs by moving in here too fast.

So in a couple of weeks I hope to have some more cultures for you. Is it necessary to send them airmail? I inoculate into 4 ml of broth. They can't grow too much to make classification difficult I don't believe. Probably you'll have to send each lot overnight - they are amazingly expensive.

Very truly yours Allen


deduit germ
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all -
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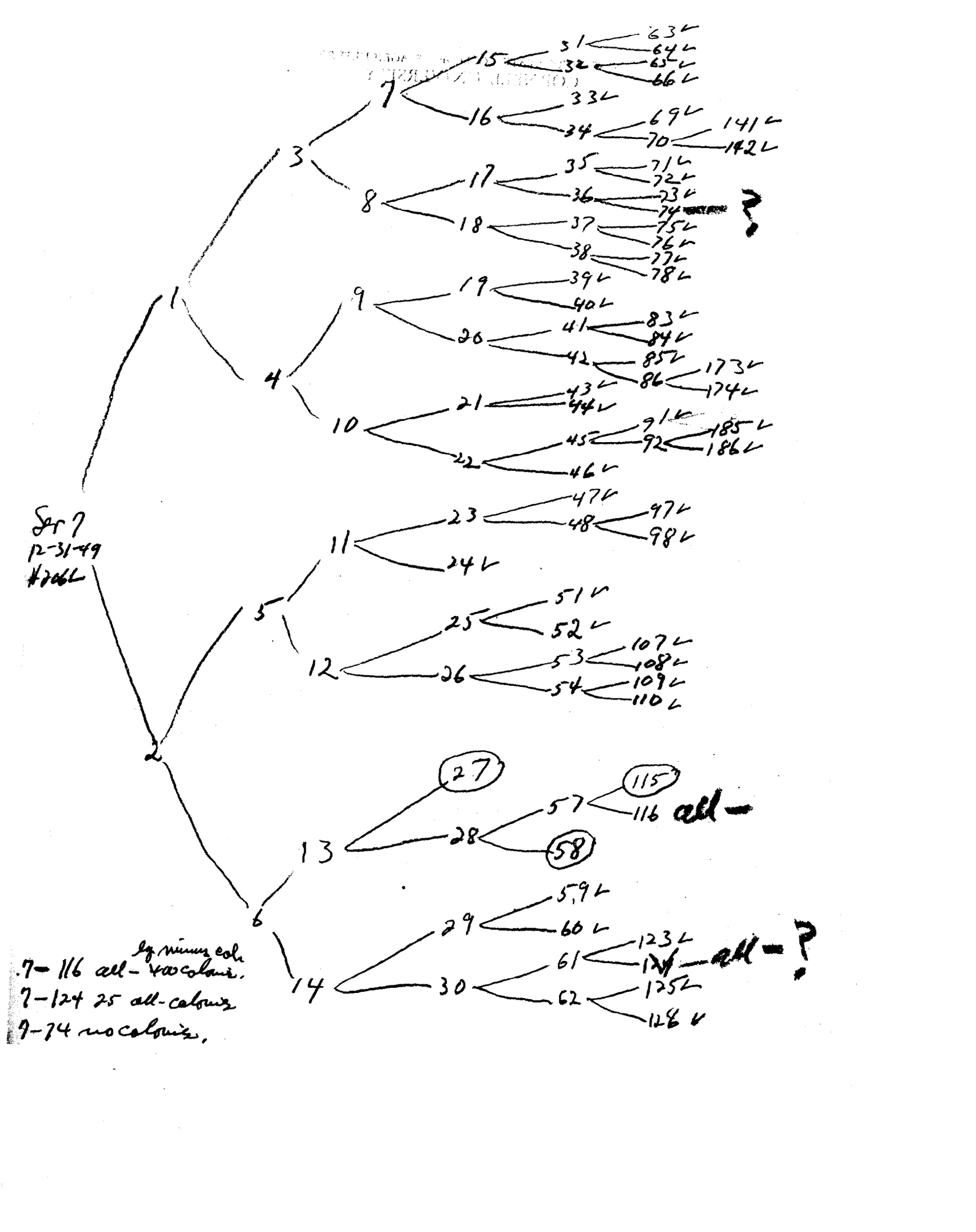
6-88 - ca20 minor colors,
6-56 all - lgcol - 300 ±
6-25 all + 1000 col.


Set 7
12-31-49
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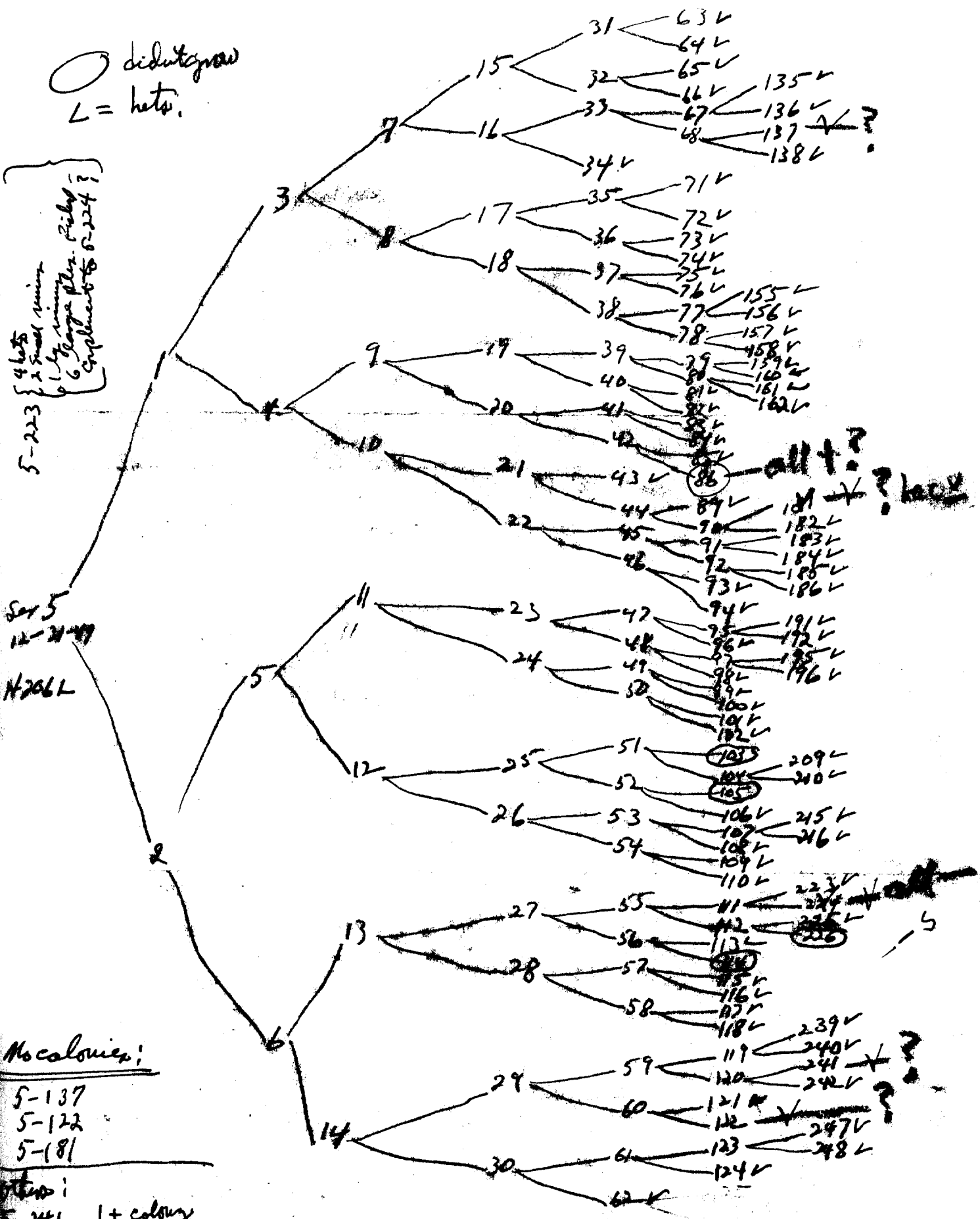
126
lg minus colr
7-116 all-two colours.
7-124 25 all-colours
7-74 no colours.


○ didntgrow
L = hets.
5-223
4 hets
2 small minim
1 big minim
6 large plus. Pinky
explained 6-224?
Sep 5
12-21-99
N206L
Mocolonies:
5-137
5-122
5-181
5-241 1+ colony
5-224 good all- colonies, small
5-86 1 big all+ colonies.
all +?
lacy
all
?
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Irradiation of H-168

December 15, 1949.

H-168 Reisolated from EMS lac. Suspend in saline

Unfortunately, these particular cells appear to be almost entirely segregated.

A. 10^{-7}

B. 10^{-6}; 3 seconds UV.

C. 10^{-1} 8 seconds UV.

On EMB Xyl and Lac.

A

156 –	1 v.		587	10
153 –	2 v	1?+	1294	0
126 –	3 v			
152 –	4 v			
587	10 v.			

B

164	all Xyl –
135	
263	
149	
215	
190	
178 / 1294	

C

Rather heavy and uneven. Very low level of + or v. ca 1/1000

Note, although not counted, lac plates gave parallel results: majority of true lac +, some lac –, Lac v. Ballpark Lac + or –

Irradiate H-168

December 15, 1949.

As above. Cells from EMS Xyl.

A). EMB Xyl.

		—	+	V
1		16	1	21
2		10	0	19
3		12	1	18
		48	2	58

probably contaminated!

EMB Lac

4		11	4	40
5		7	0	41
6		2	1	58
7	x	3	5	31
8		8	5	49
		31	15	199

B Xyl.

11		3	0	0
12		39	2	12
13		10	0	0

Lac

14		28	23	23
15		31	22	26

C Xyl crowded but predom. Xyl −!

Survival much higher on EMB than on Xyl!

12/17/49.

Recount 651a after 12 hours further incubation.

Xyl A

	−	+	v	
1	10	1	30	
2	12	0	26	
3	11	3	~~7~~ 23	
	33	4	79	/116

Xyl B

11	4	0	0	
12	43	5	18	
13	13	0	1	
	71	5	19	/95

% comparison:

A	28	4	68
B	75	5	20

	Pure	Var.
	32	68
	80	20

Lac ~~A 4 11 8~~

Scoring of Lac v vs. Lac − very difficult. Record only −, +.

		−	+, a v.
A	4	11	57
	5	4	50
	6	2	57
	8	7	55
		24	219

B. 14

Many more clear cut Lac+!

		±	−	+	v
A. Mtl.	9	2	8	2	49
	9a.	2a	10	3	55

Picks + to confirm balanced duplicates

A. 8 sec. Xyl → Lac 12L−; 3v; 30+

B 3sec Mtl → Lac 4+ 4−

C 3sec Xyl → Lac 2+; 3−

D 3sec ~~Mtl~~ Lac → Xyl 10+; 136− Xyl+ Recherked.

E 0 Xyl → Lac, Xyl (4+)

F 0 Mtl → Lac, Xyl, Mtl (4+)

G 0 Lac → Lac, Xyl bursh (8+)

Tests on possible diploids from UV-irradiated 4168. 651bc

Dec 21, 1949.

A. 20 Lac+ streaked out on lactose and xylose.

1–16 essentially pure Lac+ Xyl+ ($A_1 - A_3$; v)

A_4 (1,3 mixed Lac+, −; mostly Xyl+).

A_5 (6). mixed Lac+, −; Xyl+.

A_6. (Lac v?) — held for development of v.

B. 2 Xyl+ Lac+. B1-2

C. Lac+; 1 Xyl+, − v? Lac + +−v? B3
2 +, −, v? + B4
3 Xyl+ + + B5
4 Xyl − + +, v?

D. Lac+ Xyl+ #12 Lac+ Xyl− ~~D1-11~~ D1-11

E

	Xyl	Lac
1	+−	+
2	−+	+
3	+	+
4	−	+

Exceptional recombinant?? B6

F

	Xyl	Lac	Mtl
1	+	−	+
2	−	−	+
3	−	+	+
4	+	−	+

G. Bunches. (v. dilute) #1,3 −+ Other 6 — . (Xyl)
+− " + (Lac)

Tests on presumptive balanced diploids

651bd

December 23, 1949.

A (from uv 8secs., Xyl + Lac+): Test on Mtl T1.

	Mtl	T1	Nutr (TO)	Mtl	T1	Nutr (TO)
1	−	P	−	+	P	
2	−	P	−	−	P	+
3	+	P		−	R	
4	+	P		+	P	
5	+	P		+	R	
6	+	P		+	P	
7	+	P		−	R	+
8	+	P		−	R	
9	−	P		+	P	+ * 651bd 1
10	−	R		+	P	

		Mtl	T1	Nutr (TO)		Mtl	T1	TO
B − D	01	+	P	−	4	−	P	−
3sec uv	2	−	R	+	5	+	P	−
Lac + Xyl +	3	+	P	−	6	+	P	−
	4	+	P	−	7	+	P	−
	5	+	P	−	8	+	P	−
D	1	+	R	−	9	+	P	−
	2	+	P	−	10	+	P	−
	3	+	R	−	11	−	R	−
No uv.	B6	+	P	+ *				651bd 2

No V_1^S as would indicate V_1^P/V_1^R, and very few prototrophs. i.e., no stable diploids. Recheck * (put on slants.

New sp. heterozygotes

12/14/49. W67 x W677

20 plates. ca 60-100/plate. 1500 cols.
EMSlac

No Lac+ formed.

W67 x 1272

Repeat 1/12/50. 500 cols.

No lac+.

1/15/50 300 no lac+

1/18/50 H-226 500 — 1? lac+ ✓

This is proven lac V.

1/23/50.

W67 x W1272. ca 6 10 all —, 1+. S.O. (652-2)

"A" pure lac+.

[Save for ~~tests as stable diploid~~ See 669.]

W45 x W1272 "B" 5 plates. 87- 17+. Streak out +. all +

H-226: segregating for lac, Mal, Mtl, and probably for Stl, Ar., Gal. should also be segregating for V_1, not for V_6.

1/25/50.

67 x 1272 ca 1000. 3+ colonies.

Streak out on EMB, EMSlac; Mal.

	Lac	Mal
"1"	L	+, −, ± (V)
"2"		
"3"		+, − no V sens.

See 652a.

1/30/50.

67 x 1272

Spont diploids

January 30, 1950, et al...

8 lac+ from 1/25/50 W67 x W1272. Each gave both types

11. Mostly lac−, several lac v Mostly ~~lac−~~, Mal+; Mal−. some ○ others ● · Restreak for type! Another Mal v. Restreak.

12. Lac v (?) primarily lac+. Predominantly Mal−. Few + (contam.)

13. distinctively lac v. Mostly ~~lac~~ Mal+, some Mal−. Restreak coll. lac v.

---

11. Mal v? : rely Mal+, − on restreak! Some − on Mal EMS.

12. Definitely lac v - like H226. On Mal EMS, all Mal−.

1/31 13. 8 lac v streaked: all Mal +. (Mal − probably contam. or admix.)

"W67 x W1272" ca 1/8 lac+! (probably some error). 140 lac+ picked and streaked on EMB lac. All fast+. (58-161 x 1272?)

2/1 Thru out!

See 673

Conclusion:

11: lac v Mal v. H228

12. lac v Mal− H229

13. lac v, probably Mal+ H230

Results some Zelle 1-cell isolates from H206.

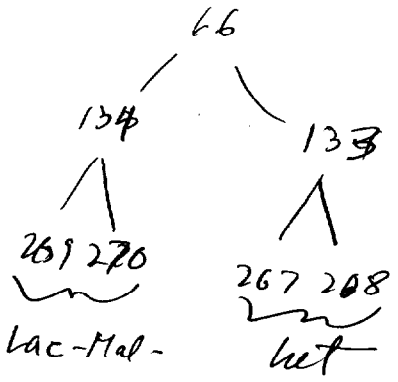
Jan 5, 1950

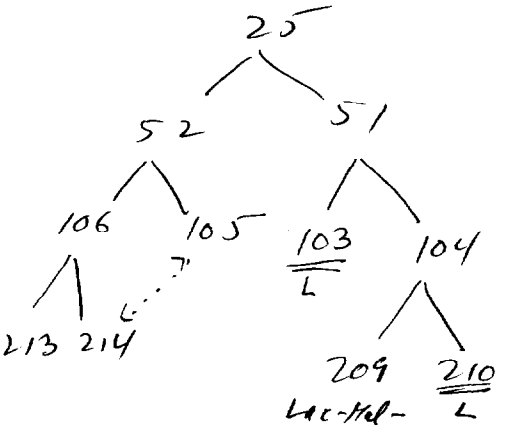
Record for pedigree and summary of H206

		Mel	Bal	Lac
1	12-267	+, −, v?		+
2	12-268	+		+
3	12-269	−		− very tiny
4	12-270	−		− very tiny
5	12-209	−		−
6	12-214	+ − v?		+ − v
7	12-198	all +		+
8	~~12~~ H206 stock.	v		v

This is in accord with Zelle's letter except that the identification of v colonies here is somewhat uncertain.

12-198 here appears to be pure +. Restreak!





4206 ≡ 544D4.

W477 × W980.
TL13, Lac-0, K × 478 Gal-Mal-. Try T5also.

Zyke's single cell

Series 15:

	Jan 5, 1950.	Lac	Mal	
1	5-83	−+	−+	
2	84	−+	−+	
3	85	−+	−+	
4	86	+	+	
5	122	−+	−+	−+ look for V
6	137	−+	−+	−+ " "
7	181	−+V	−+	lac V
8	+5-223	+	+	
9	223	−+	−+	
10	224	−tiny	−tiny (contam?)	
11	225	−+	−+	
12	15-123	+	+	
13	239	−+	+−	
14	240	−+	+−	
15	241	−+	+−	−+ look for V
16	242	−+	+−	segp V
17	+5-223	+	+	
18	"	+	+	
19	"	+	+	
20	"	+	+	

Sorbitol Linkage — Preliminary.

1/11/50. W1262

~~50~~ W478 × W677. on EMS Lac
" Stl.

Pick Stl+ only (ca 5%). "Purify" by spotting on EMS Lac or Sal.
Replica after 16 hours, and test directly on EMS:

	Stl	Lac	Sal	Mal	Xyl	Mtl
1	+	+	+	+	+	+
2	+	−	−	+	+	+
3	+	−	+	−	−	−
4	+	−	−	−	−	−
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	−	−	−	+	+
8	+	−	−	−	−	−
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+ (−)	−	−	−	−	−
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+	+	+	+
16	+	+	+	+	+	+
17	+	−	+	−	−	−
18	+	+	+	−	−	−
19	+	−	−	−	−	−
20	+	+	+	+	+	+
21	+	+	+	+	+	+
22	+	+	−	−	−	−
23	+	+	+	+	+	+
48	+	−	+	+	+	+
49	+	−	+	−	−	−
50	+	+	−	−	−	−
51	+	+	+	−	−	−
24	+	+	+	+	−	+
26	+	+	−	−	+	−
26	+	−	−	+	+	+
27	+	−	+	+	−	−
28	+	+	+	+	−	−
29	+	−	+	+	+	+
30	+	−	+	+	+	+
31	+	−	+	−	−	−
32	+	/	+	−	−	−
33	+	/	+	+	+	+
34	+	+	+	+	+	+
35	+	−	+	+	+	+
36	+	/	+	−	mixed	−
37	+	+	−	−	+	−
38	+	+	+	+	+	+
39	+	−	+	+	+	+
40	+	−	−	+	+	+
41	+	−	−	−	−	−
42	+	−	+	+	+	+
43	+	/	+	+	+	+ −
44	+	±	+	+	+	−
45	+	−	+	+	+	+
46	+	−	−	−	−	−
47	+	+	+	−	+	+
		/	−	+	+	+
52	+	+	−	−	−	−
53	+	+	+	+	+	−

Summary – Preliminary linkage tests.

Stl.

4/12/50. 53 tests: Sorbitol +. Following +:

	Lac	Gal	Mal	Xyl	Mtl
#	27	38	33	34	31
%	51	71	62	64	58
Standard %	ca 30		ca 15-20	ca 10	ca 10

Distinct linkage to Gal is apparent.

Other linkages: (in this Stl + prototroph sub-samples):

Lac-Gal.		Mtl-Xyl.	Mal-Xyl	Mal-Gal
++	22	30	30	29
+-	5	1	3	3
-+	17	4	4	9
--	11	18	16	12

An unusually high concordance of Mal-Gal-Xyl is noted here. It is clearly profitable to study the full sequence: Lac V6 Gal Stl Mtl Mal Xyl ...

Characterization of W1258 (Cavalli 123)

Jan 4, 1950 ft. dr D(0):

(1) Standard additions series:

Y. Extr.	+++
HC	+
0	≡

(2) Amino acid Rundown.

HC	+
AA	+
AA + Vits	±
Vits	—
HC + V	+

No vitamin Requirement ?

Add vits. in succeeding tests.

Vits + single AA groups	—
HC + V + A	±

(3) AA mission series + Vits.

AA+	++ (30 hours).
− A12	—
− A3	—
− A4	++
− A5	—
− A6	++

Characteristic Requirements from A12, A3, A5. Adding vitamins and A4 + A6.

AA
AA + V.
A12 + A3 + A5
" + V.
A4 + A6 + Vits.
− A12 series
− A3 series
− A6 series

Threonine
Methionine
Isoleucine indicated

After 24 hours, Methionine, Threonine, Isoleucine ~~was indicated~~ ; AA mix gave poor growth

48 hours −M, −A3 ±, −A5 ±, but −A12 + M ± ?? Isoleucine ??

K-12 × Cavalli 123

January 7, 1950. (+ pur.)

W-1178 × W-1258 on EMS Lac. Picks 100 + colonies. 20 ± or − " .

[W-1177 × W-1258 " . Heavy background and plaguing noted. See infra. Many pototrophs, but do not pick.]

Ca 99% Lac+ 1% Lac−

Streak out Lac+ for V. None V. Test ē Mal, T1.

Lac+.	Mal+ V_1^R 1	Mal+ V_1^S 98	Mal− V_1^R 0	Mal− V_1^S 1	Σ 100
Lac−	3	5	1	0	9
Lac ±	1	8±	2 mixed.		

At least #1 of the Lac ± is Lac V after 30 hours.

	Lac	Mal	
1	V, +, −	+, V??	Reisolate on EMS Lac
2	+, −, V??	−	
3	+, ±	−	
4	+, ±	+	as 656-1
5	+, ±	+	-2
6	+, ±	+	
7	+, ±	+	
8	+, ±	+	
9	+, ±	+	
10	+, ±	+	
11	+, −	+	

#1 — definitely Lac V. Attempt reisolation on EMS Lac. However, many Lac− pototrophs noted, and Lac V colonies on EMS virtually preclude selective isolation.

Jan 6-7, 1950.

In cross of W677 × W1258, heavy plaquing of background was noted. In view of this, tests for λ sensitivity were made in soft agar plates.

	λ	W518	W677	W1258	T1	T6
W1258	L	–	L	–	L	L
W677	–	–	–	–	–	L

To obtain resistant mutants, plate W1258 with λ at various dilutions. Plates in).

1/10/50. Inoculate Nutr Br + 4% extr. (NB4) with 5 ml young culture W1258 + ca 10^8 λ. Aerate 5-6 hours. Cool overnight and filter, just streaking out the turbid supernatant. Control was much denser.

① titrate filtered λ stock

② Test 1258/λ for resistance; lysogenicity

Map stk - 657

Jan. 4ff. 1950.

"A"

W1262 x SB-161 in EMS Loc

Pick back and Loc — to stk EMB.

	stk -	stk +
L+	21	1
L-	30	2

Linkage of or # - linkage to M is indicated. Repeat crosses, plating in EMS-stk.

Putter testes summary:

	stk -	stk +
L-	105	9
L+	75	4

Stl heterozygotes.

Jan 10, 1950

W478 x W1262. Pick Lac+ from EMS lac and streak for Rutiels on EMS lac; EMB....

Lac ✓.

	Lac	Mtl	Stl	Xyl.	Mal
#1	+ −	+ −	+ −	+ −	−
2	+ −	+ −	+ −	v	−
3	v	−	−	v	−
4	+ −	−	−+	v	−
5	v	−	−	v	+
6	v	−	−	−+	−
7	v	+ −	+ −	v+	+
8	+ −	slow*	−	+	+
9	v	v	v very weak	v	= H225
10	v	−	−	v	−
11	v	−	−	v	−
12	v	−	−	v	−
13	v	−	−	−	−

* Idea suppression Mtl+

Select for further study.

+

Stl+ (not v)

H168 Irradiation

January 16, 1950.

Scrape H-168 from EMSlac; disperse in water, spread on EMS lac and EMB lac and irradiate as indicated.

A). Undiluted .1ml samples. 8 and 15 secs. UV. EMB lac

#	UV	lac+	lac-	lac v
1	8	57	41	104
2	8	81	30	33
3	15	38	34	95
		176	105	232
		281		232
Omitting #2		95	75	199
			170	199

? Anacc. count? Too crowded; only a part of area ctd.

-log$_5$ Kill/hour	UV	lac+	lac-	lac v	% lac v
0	0	14	9	131	85
1	2	56	55	38	26
5-6	8-15	176	105	232	48

B). 10^{-6} ml samples. 3 secs. UV.

4	14	23	13
5	20	16	11
6	22	16	14
	56	55	38
		111	38

C) 10^{-7} ml. No UV.

7	4	5	35
8	5	1	43
9	5	3	53
	14	9	131
		23	131

January 16, 1950.

Similar samples (659) irradiated on EMS Lac to test for occurrence of balanced lethals here.

		+	−
C: (No UV). Counts:		44	0
		21	0
B	3 secs UV.	11	1 ?
		14	0
		18	0
A	8 secs.	31	1 sect
		83	3
	15 secs.	18	1 sect
		52	1?

Pick and streak ~~out~~ 20+ colonies in each category (and all −).
For 1st test. Compare cross streaks vs. T1 on EMS Lac; EMB Lac. Haploids and stable diploids will be same on both media (PaR; S); segregating diploids will be distinct. Save suspensions. 20 from each tested.
All were T_1^P except B10, T_1^R on both media. All were also clearly mixed ± on EMB Lac. Restreak a few impossible exceptions

Recessive lethals can account for only a part of the killing.

H225 segregants

1/18/50

Streak out individual lac V segregants on EMB Lac. Pick 1 Lac- and 1 Lac+ each for further classification.

Lac	Gal	Xyl	MH	TS		Lac	Gal	Xyl	MH	TS	
−	+	+	+	−	−	−	−	R	S	−	+	+	+	−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−						+		R	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−	+					−	−	S	S	−	+			−	−	+	−	R	S
−						−		R		−	+			+	−	+	−	R	S
−						−		R		−	+			−	−	−	−	R	S
−	+					−	−	S	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S

all apparently sorbitol+, arabinose +

Precludes use for linkage tests. Recheck segregation.

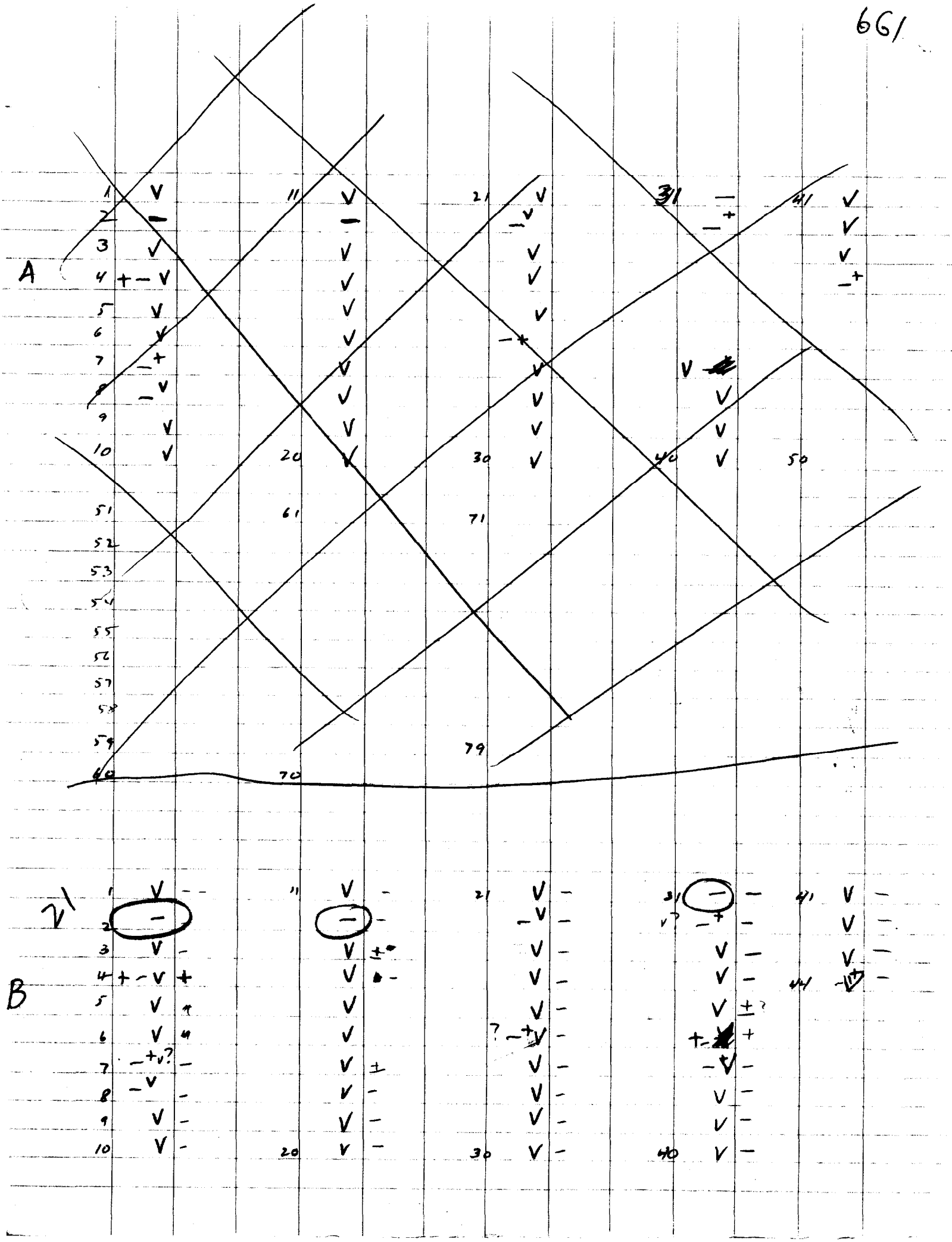
Zelle's single cell isolations

66

1	—	o	—	11	V	—		21	V	—		31	V	+	
2	V	—			V	—			V	±			V	+	
3	V	±			V	o ±			V	—			V	±	
4	+ ——				sec'd cont. V	±			V	—			V	±	
5	V	+			V	—			- V	—		+	—	V	+
A 6	V	+			V	—			— + V	—				V	—
7	+ - V	+		2	— +	—			V	—			4	V	—
V 8	+ - V	+		3	4 + —	±			V	—			4	V	—
9	+ - V	+			V	—			V	—			—	+ V	—
10	+ - V	+		20	V	—		30	V	—		(40)	—		o
41	V	—		51	V	±		61	V	—		71	V	—	
2	V	—			V	—			V	—			V	—	
3	V	—			V	—			V	—			- V	—	
4	V	—			V	—			V	—			V	±	
5	— V	—		2	— + no V	±			V	±			V	—	
6	— V	—			— V	—			V	—			— + V	—	
7	— V	—			V	—			—	o			—	o	
8	— V	—			V	—			—	o —			—	o —	
9	V	—			— + V	—			—	o —		79	V	—	
50	V	—		60	V	±		70	V	—			V	—	

* * 41 gave only very tiny colonies o poor growth on EMS

A: 1-10 show a special propensity for + colonies.


A
B
1
11
21
31
41
51
61
71
79
70
20
30
40
50

1	V	−	11	V	−	21	V	−	31	V	−
2	+− V	+		V	−		V	−		−V	−
3	V	−		−	−		−+ V	−		V	−
4	V	−		V	−		−	−		V	−
5	V	−		+−	+		−	−		−(V)	−
6	V	−		V	−		Nodule −	⊖		V	−
7	V	±		V	−		−	−		V	−
8	V	−		V	−		−+ (V)	−		V	−
9	V	−		V	−		V	−		+− V	−
10	V	−	20	V	−	30	V	−	40	V	±
41	V	−	51	V	±	61	V	+			
42	V	−		V	−		V	−			
43	−+V	−		V	−		V	±			
44	V	−		−+V	−		V	−			
45	−+ V	−		V	−		V	−			
46	−+ V	−		V	−		V	−			
47	V	−		V	−	67	V	−			
48	V	−		V	±	68	V	−			
49	−+V	−		V	−						
50	V	±	60	V	−						

Almost all plates predominantly − unless noted

° pongrowth on EMS lac

Zelle isolates

January 18, 1950.

A). Check possible spurious variegated (from EMS Lac to EMB Lac)

A17, A18, A55, — B7, 32, 36

C15, A B

A from EMS
B from ori. tube

A17	1	lacV	−V
A18	2	lacV	+, −
A55	3	lacV	+−V?
B7	4	−V?	−V?] only doubtful.
B32	5	lacV	−V
B36	6	lacV	+, −
C15	7	lacV	+−V

C)

Segregants

A1	1
A40	2
A67	3
A68	4
A69	5
A77	6
A78	7
B2	8
B12	9
B31	10
C13	11
C24	12
C26	13
C27	14

1 Lac+ each from 20 - [115-118] isolated and tested:
each was Lac+Mal+ V_1^R 20-115: TLB1, Lac+Mal+ V_1^R

2 from 5-223 Lac+Mal+ V_1^R 5-223+ : TLB1, Lac+Mal+ V_1^R

---

(12)

133 < 267 / 268
134 < 269 / 270

Tests on M206 segregants isolated by M.R. Zelle

January 20, 1950.

		Lac	Mal	TS		Nutr.	
1	5-224	−	−	S		M	5-223 + colonies L+M+V_1^R.
2	6-25	+	+	R		TLB_1	
~~3~~	~~6-88~~	~~−~~	~~−~~	~~S~~		Lac v isolated impure? (Lac+, Mal+)	
4	7-116	−	+	R		TLB_1	
~~5~~	~~7-124~~	~~−~~	~~−~~	~~S~~		Lac v isolated impure (Lac+).	
6	12-269	−	−	S		M	slow growth
7	12-270	−	−	S		M	
8	12-209	−	−	S		M	
~~9~~	~~22-85~~	~~−~~	~~−~~	~~S~~		Lac v isolated from EMS lac impure?	Rare + seen
10	22-103	−	+	S		+	
11	22-104	−	+	S		+	
12	22-106	−	+	S		+	
13	20-113	−	−	S		M	
14	-198	−	+	R		T.. ✓ TLB_1	Retest!
15	-392	−	+	R		TLB_1	
16	-393	−	+	R	clone 48	TLB_1	
17	-394	−	+	R		TLB_1	
18	-783	−	+	R		TLB_1	
19	-784	−	+	R		TLB_1 ✓ TLB_1	Retest B_1
20	21-98	−	+	S		B_1	
21	-60	−	+	S		B_1	
~~22~~	~~25~~	~~−~~	~~−~~	~~S~~		Lac v isolated from EMS lac	impure (Lac+, Mal+, V_1^R) Rare + seen in streaks.

Restreak possible impure cultures on EMS Lac.

3/21

A page must be missing here but data seem to be from the cross W478 x W1272, with Lac U omitted! See 655.

662
b.

See later 662

Lac+ (not ±) from streak plates, picked to water and checked on other EMB.

	T6	TS	Sal	AR	MTL		Xyl	STL	MAL	
1		S	+	+	+		+	+	+	41
2		S	−	+	+		+	+	−	
3		S	+	−	−		+	−	−	
4		R	−	−	−		−	−	−	
5		R	+	+	+		+	+	+	
6	S	S	−	−	−		−	−	−	
7		R	−	−	−		−	−	−	
8		S	+	+	+		+	+	+	
9		S	−	−	±	−?	+	−	+	
10		S	+	+	+		+	+	+	
11		S	−	−	−		+	−	−	51
12		S	+	+	±	⊕	+	+	+	
13		S	−	−	−		−	−	−	
14		S	−	−	−		−	−	−	
15		S	−	−	−		−	−	−	
16		S	−	−	−		−	−	−	
17		S	−	−	−		−	−	−	
18		S	+	+	+		+	+	+	
19		S	−	−	−		−	−	−	
20		S	−	−	−		−	−	−	
21		S	+	+	+		+	+	+	61
22		S	−	−	−		−	−	−	
23		R	−	−	−		−	−	−	
24		S	⊖	−	−		+	−	−	
25		S	+	+	+		+	+	+	
26		S	+	+	+		+	+	+	
27	S	S	−	−	±	⊕	+	−	−	
28		S	−	−	−		−	−	−	
29		R	+	+	+		+	+	+	
30		S	−	−	−		−	−	−	
31		S	−	−	−		−	−	−	71
32		S	−	−	−		−	−	−	
33		S	−	−	−		−	+	−	
34		S	+	+	+		+	+	+	
35		S	+	+	+		+	+	+	
36		S	+	+	+		+	+	+	
37		S	+	+	+		+	+	+	
38		S	+	+	+		+	+	+	
39	S	S	−	−	−		−	−	−	
40		S	+	+	+		+	+	+	80

	T6	TS	Sal	AR	MTL		Xyl	STL	MAL	
1		S	−	−	±	⊕	+	−	+	
2		S	+	+	±		+	+	+	
3		S	+	+	±		+	+	+	
4		S	+	+	+		+	+	+	
5		S	−	−	+		−	−	−	
6	S	S	+	+	−		+	+	+	
7		S	+	+	+		+	+	+	
8		S	+	+	−		+	+	+	
9		R	−	−	−		−	−	−	
10		S	−	−	−		−	−	−	
11		S	−	−	−		−	−	−	
12		S	−	−	−		−	−	−	
13		S	−	−	+		−	−	−	
14		S	+	+	+		+	+	+	
15		S	−	−	−		−	−	−	
16		S	+	+	−		+	+	+	
17		S	+	+	+		+	+	+	
18		S	−	−	+		+	−	−	
19		S	−	−	+		+	−	+	
20	S	S	−	−	±		+	−	−	
21		S	−	+	+		+	+	+	
22		S	−	+	+		+	+	+	
23		S	+	−	−		+	−	−	
24		S	+	+	+		+	+	+	
25		S	−	+	+		+	+	+	
26		S	−	+	+		+	+	+	
27			−		+		+	+		
28		S	−	−	−		✱	−	−	
29		S	+	+	+		+	+	+	
30		S	−	+	+		+	+	+	
31		S	+	−	−		✱	−	−	
32		S	+	+	+		+	+	+	
33		S	−	−	−		−	−	✱	−
34		S	+	−	−		−	−	−	
35		S	+	−	−		+	−	−	
36		S	+	−	±		+	−	−	
37		S	+	+	±		+	+	+	
38		S	−	+	−		−	−	−	
39		S	+	−	−		±	−	−	
40		S	+		−		−	−	−	

Repeat ?

661:

		MHL
42	+	
43	+	
12	+	
9	very slow +	score as − or +?
41	very slow +	score as − or +?
27	very slow +	score as − or +?
60	" "	source of modifier?
76	" "	source of modifier?

Gal 24 intermediate + score as −

Kyl 79 Pure +.

	T6	T5	Gal	Ar	Mtl		Xyl	Stl	Mal
81		S	+	+	+	+	+	+	+
82	S	R	−	−	−	−	−	−	−
83	S	S	−	−	−	+	−	−	+
84		S	−	−	−	−	−	−	−
85		S	−	−	−	−	−	−	−
86		S	−	−	−	−	−	−	−
87		S	−	−	−	−	+	−	−
88		S	+	+	+	+	+	+	+
89		S	+	+	−	−	−	−	−
90		S	+	+	+	+	+	+	+

	T6	T5	Gal	Ar	Mtl	Xyl	Stl	Mal
91		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		R	−	−	−	−	−	−
96		S	−	−	−	−	−	−

All are V_6^R unless S is indicated. Scoring with T5 was unsatisfactory

Summaries of lac+ data.

Parental combinations were +++++ $V_6^R V_1^R$ V_6 is known to be linked to lac.

Of 96 lac+ the following parental couplings (single) were observed:

V_6^R	89	All parental:	33
Xyl	57	Ar	44 (+2 ?)
Mtl	49	Mal	45
Gal	46	V_1	88
Stl	44		

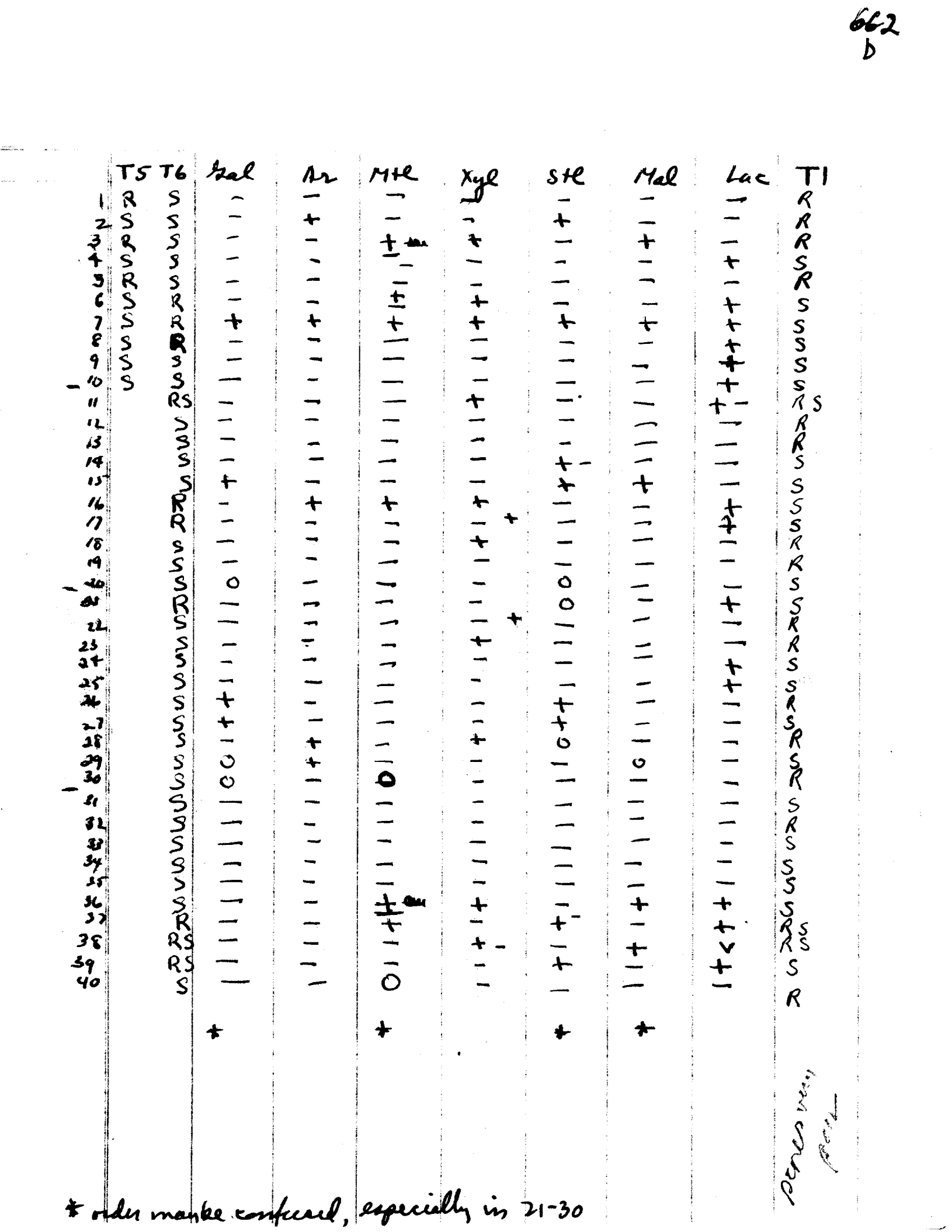
Mal, V_1 should also be scored

NOTE: All 8 exceptions were V_6^R and either all − or all +.

	A+ BCD+	A− BCD+
A = Xyl	||	
A = Mtl	||	|||
A = Gal	||||	𝍸 ||
A = Stl	|	

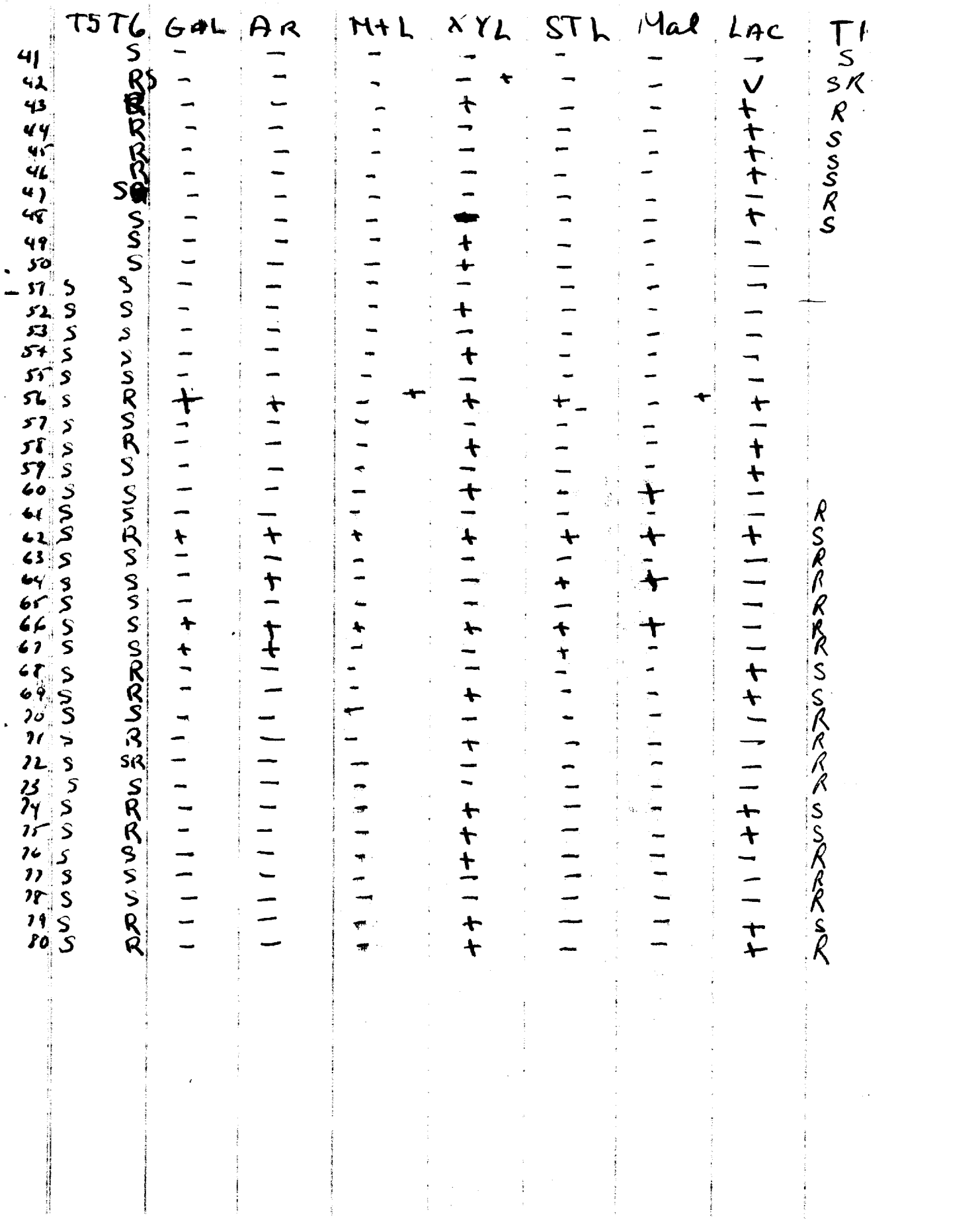
Stl Linkage: discordances with: Ar Linkage

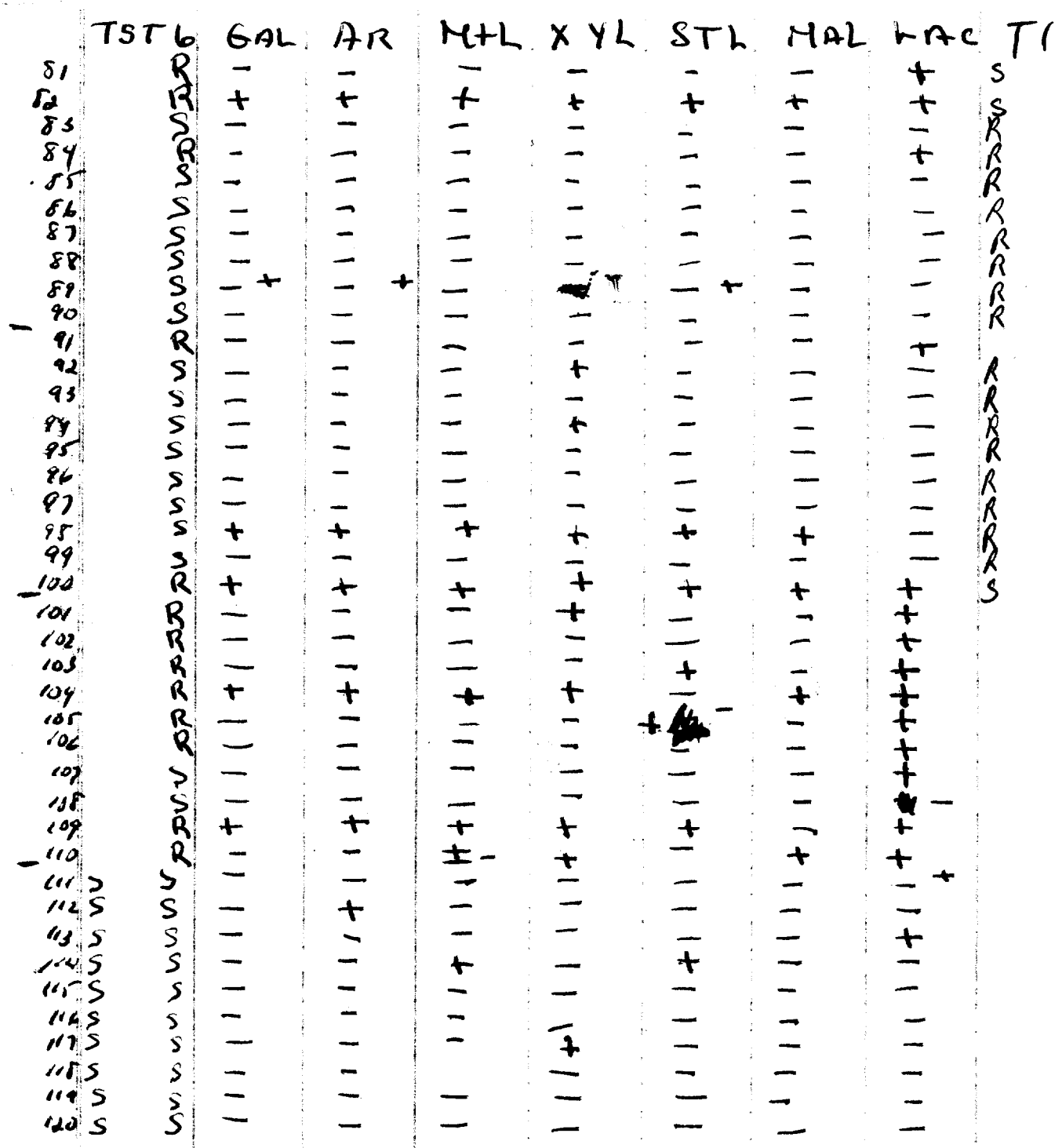
Mal	6
Gal	16
Ar	3 *
Xyl	15
Mtl	13



* odds marks confused, especially in 21-30

662
D




TST
GAL
AR
MTL
XYL
STL
MAL
HAC
TI

	TI	LAC	MAL	STL	XYL	MTL	AR	GAL	STG
121	S	+	-	-	-	-	-	-	R
122	R	-	+	-	+	-	-	-	R
123	S	+	-	-	-	-	-	-	S
124	S	+	+	+	+	+	+	+	S
125	O	+	-	O	O	O	O	O	R
126	R	-	+	-	-	-	-	-	R
127	R	+	-	-	+	+	+	+	S
128	S	-	+	+	-	-	-	-	S
129	R	+	-	-	+	+	+	+	S
130	S	-	+	+	-	-	-	-	S
131	S	+	-	-	+	±	+	+	V
132	S	-	+	+	-	-	-	-	R
133	S	+	-	-	+	+	+	+	S
134	R	-	+	+	-	-	-	-	R
135	S	+	-	-	+	+	+	+	S
136	S	-	+	+	-	-	-	-	R
137	S	+	-	-	+	+	+	+	S
138	S	-	+	+	-	-	-	-	R
139	S	+	-	-	+	+	+	+	S
140	S	-	+	+	-	-	-	-	R
141	S	+	-	-	+	+	+	+	S
142	S	-	+	+	-	-	-	-	R
143	S	+	-	-	+	+	+	+	S
144	S	-	+	+	-	-	-	-	R
145	S	+	-	-	+	+	+	+	S
146	S	-	+	+	-	-	-	-	R
147	S	+	-	-	+	+	+	+	S
148	S	-	+	+	-	-	-	-	R
149	S	+	-	-	+	+	+	+	S
150	S	-	+	+	-	-	-	-	R
151	S	+	-	-	+	+	+	+	S
152	S	-	+	+	-	-	-	-	R
153	S	+	-	-	+	+	+	+	S
154	S	-	+	+	-	-	-	-	R
155	S	+	-	-	+	+	+	+	S
156	S	-	+	+	-	-	-	-	R
157	S	+	-	-	+	+	+	+	S
158	S	-	+	+	-	-	-	-	R
159	S	+	-	-	+	+	+	+	S
160	S	-	+	+	-	-	-	-	R

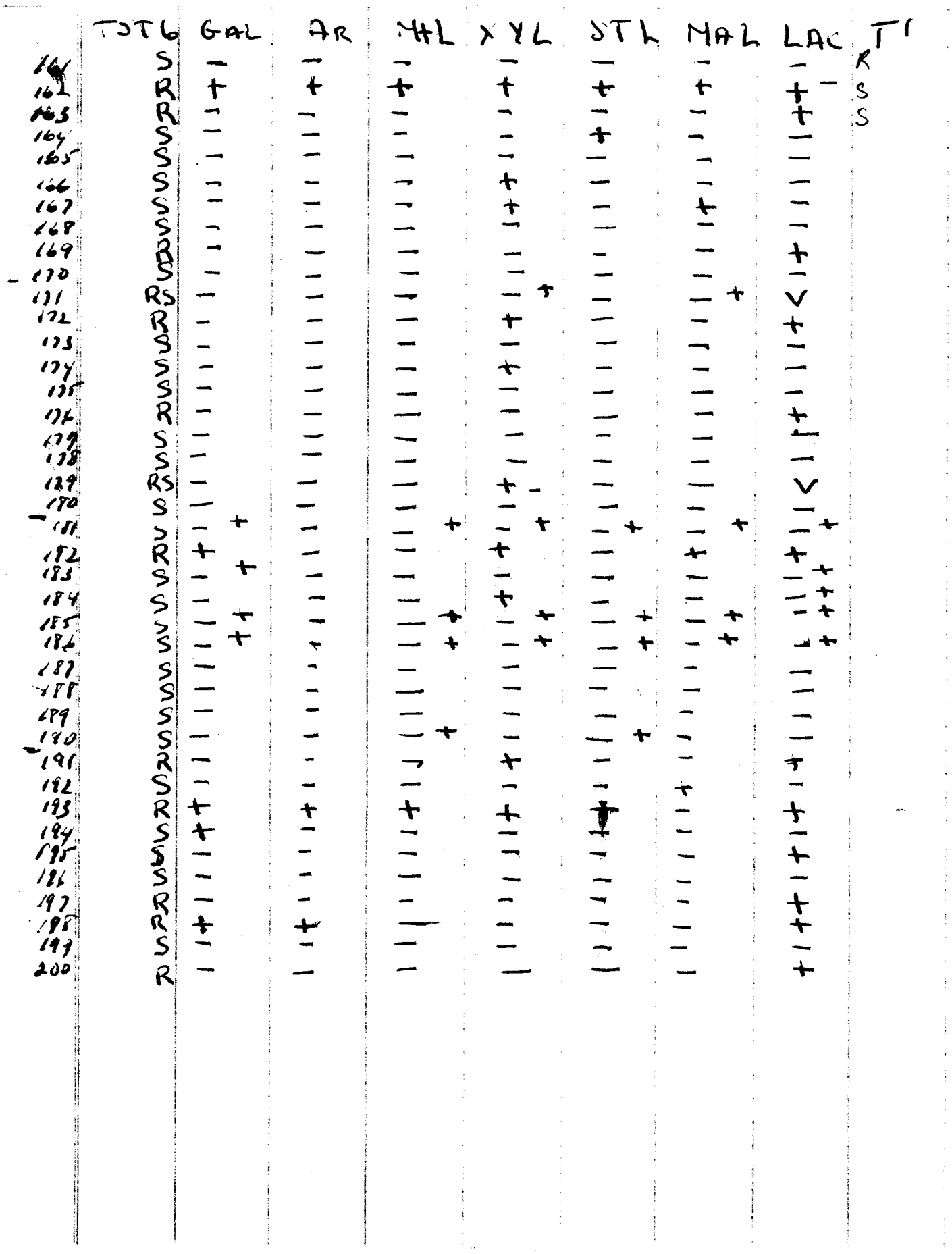
		No.
#	11	1
	38	2
	39	3
	42	4
	84	5
	122	6
	145	7
	162	8
	171	9
	179	10

are possible lac V.

strain out on EMB & EMS lac

as 662-b No...

—


TJTG
GAL
AR
MHL
XYL
STH
MAL
LAC
TI
K
S
S

Linkage relations of Stl etc. 662c

Jan. 17, 1950 ff.

~~W1278~~ W1262 × W1269 on T(o); EMS Lac; EMS Stl.

1) From EMS lac, test 100 lac+ for lacV. (struck out on EMS Lac).

8 lacV found. Reisolate to lacEMS, and check for segregation on other sugars.

	Lac	Xyl	Mtl	Stl	Gal	Mal
1		—	—	—	V	—
2		~~m~~ V⁻	—	—	+	—
3		—	—	—	— v?	—
4		—	—	—	— v?	—
5		V+	~~—~~ v?	—	— v?	—
6		V⁻	v?	—	V⁻	—
7		—	—	—	V	—
8		—	—	—	V	+

1/23 Additional 100 Lac+ picked and tested:

8 possible lacV: Retest 4 confirmed (not certainly).

Check on all sugars as 9–12 662c.

		Lac	Save as
"b"	1	V	13
	2	V	14
	3	+	
	4	+	
	5	+	
	6	—	
	7	+	
	8	+	
	9	V	15
	10	V	16

Irradiation of H-168.

January 20, 1950.

All previous irradiations have been done with Hanovia high pressure Hg lamp. To prepare for further experiments, calibrate killing with G.E. sunlamp.

P20. Suspend H168 from EMS bac. to ca 10^9. Dilute in saline to 10^{-5}.
~~Spread~~ Irradiate 5 ml in Petri dish (10 ml) under lamp and spread on EMS bac.
Suspension apparently viable: no colonies from controls!

P23. Repeat with H225.
By mistake, plated 10^{-6} and 10^{-7} ml with 0 irradiation and 10^{-7} only with various U.V. doses.

UV	Dil.	Count		V	+	−	
0	10^{-6}	~~~					
0	10^{-6}	~~~					
0	10^{-7}	35	45	~~V~~	1	5	79 : 10
0	10^{-7}	54			1	3	
5 s.	10^{-7}	18	15		5	5	3 : 26
5 s.	10^{-7}	11			7	9	
10 s	"	0					
10 s.	"	0					
20		0					

↓

For future work, increase distance of lamp to 20 cm and multiply dosages by 4x.

Irradiation of H-225.

January 23, 1950.

BE UV lamp at 20 cms. Diluted coli shaken 0, 20, 30 secs.

	UV	Dil.
A.	0	10^{-7}
B.	0	10^{-6}
C.	20	10^{-6}
D.	30	10^{-6}

Survival = ca 11/411 = ca 2½ %.

	V	+	−	Σ
A	71	8	13	92
	78	7	12	97
	88	9	17	114
	95	8	5	108
	332	32	47	411
C.			1	
			0	
		1	4	
		3	2	
	0	4	7	11.

D. Blank.

Initial TS and TI scores are thoroughly unsatisfactory!

Recheck ~ control stories + mimes before repeating

Jan 20, 1950.

"A"

"B" W1155 × W1258 } Backgrounds rather heavy. A few + colonies observed.
"C" W1268 × W1262 }

D W1268 × W1178.
L+M+V_1^S L-M-V_1^R

B. Pick + colonies which stand out slightly over background. Spot suspensions on T(0) agar to purify. Streak these out on EMB Sucr. All Sucr – V_1^S V_6^S but grow well on EMB Sucr with W1258!

C. Pick colonies (rather small) from T(0) directly to EMS Lac. V.G.!

D. (Colonies rather larger than C). Background very noticeable on EMS; OK on T(0).

Pick ~~#~~ colonies from T(0) and streak out on EMS

100 tests: 5 lac – [may contain Lac+] 95 lac+.

Pick purified colonies and test on Mal EMB vs. T1.

95 Lac+: All V_1^S. 94 Mal+ 1 Mal–

5 lac– 5 Mal+ : $3V_1^S$ $2V_1^R$

∴ All lac+ are V_1^S; Some lac– are V_1^S; some V_1^R.

January 24, 1950.

Prepare suspensions from EMS Lac. Prepare rather dilute susp.

	UV	Dil.
A.	0	10^{-7}
B.	0	10^{-6}
C.	5	10^{-6}
D.	10	10^{-6}

All plates too dense for accurate count (over compensation for "dilute" suspensions.

Total counts (¼ plate)

A 90

D 153

∴ 10 sec ≐ ca 85% killing.

Relative counts

		v	+	—	
A.		39	2	8	ca 80% v.
		41	6?	5	< 15% —
		80	8 <	13	
	C	6	2	6	

Impression ca 50% —.

D.		28 ~~30~~	24	50

Duplex plotting

January 23, 1950.

"C". W1269 (478/6) × W1272 (1262/"6") on a variety of EMS media:

EMSB$_1$: Mal + observed but not clearly scoreable. Yields very high. Mal± not readily detected at 48h. s = sectored.

P25. : Xyl clear, small colonies. A few possible Xyl± noted and marked. Reincubate for further development.

P26: Lac 6 plates. No ± colonies. 92+ 128−

−B$_1$ Xyl 5 plates ca 100/plate. No ± seen, but colonies too small for accurate + − scoring.

Mal 5 plates. Many small +. 2? [1,2] Mal±. 91+ 61 − [2 plates].

Lac 6 plates. heavy background. No Lacs seen. ca 400+.

+B$_1$ Xyl 4 plates. 6 ✱ fairly clear Xyl v. [3−B].
ca 136 scoreable +. 42+ : 50 − (1 plate)

Mal Too crowded to estimate precisely 7 plates. Some Mal± probably are coincident colonies.

14 possible Mal± picked and streaked on EMB Mal.

Mals, +, − comps-scoreable Lac.

Last Mal →	+	−	
1	+	+	
2	−	−	
3	−	−	
4	+	−	*
5	+	−	*
6	+	+	
7	−	−	
8	−	−	
9	+	+	
10	−	−	
11	−	−	
12	+	+	

Xyl →	+	−
1	+	+
2	−	−
3	−	−
4	−	−
5	−	−

Very definite correlation for Lac of +/− components. (Two exceptions *)

667a

	Mal		Xyl		Lac		Gal		Mtl		Sbl		Ar		T5	
	+	−	−	−	+	+	−	−	−	−	−	−	−	−	S	S
	+	−	+	+	−	−	−	−	+	+	−	−	−	−	R	R
	+	−	+	+	−	−	−	−	−	−	−	−	−	−	B	B
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	S	S
n.g.	+	+	+	+	+	+	−	−	−	−	−	−	−	−	S	S
	+	−	+	+	+	−	−	−	−	+	−	+	−	−	S	R
	+	−	+	−	+	+	−	−	"±"	−	−	−	−	−	S	S
	+	−	−	−	−	−	−	−	−	−	−	−	−	+	S	S
	+	−	−	−	−	−	−	+	−	−	−	+	+	−	S	S
	+	−	+	−	+	+	+	−	±	−	−	−	+	−	S	S
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	R	R
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	M	R
	+	−	+	+	+	+	−	−	+	+	+	+	+	+	S	S
	+	−	+	−	−	−	−	−	−	−	−	−	−	−	S	S
	+	−	−	−	−	−	−	−	±	±	−	−	−	−	R	R
	−	−	+	+	+	+	−	−	−	−	−	−	−	−	S	R
	+	−	+	−	+	−	+	−	±	−	−	−	+	−	M	R
	+	−	+	−	−	−	−	+	−	−	−	+	−	+	S	S
	−	−	+	+	−	−	−	−	±	±	−	−	−	−	S	S
	−	+	−	−			−	−	−	−	−	−	−	−	S	S

Mod. plaque!

Segregation of M226

January 26, 1950.

Streaked out 8 Lac v of M226, on EMB Mal, Lac.

a) test reduced Mal (+ or −) on Lac

b) " " Lac v on Mal. [No Lac+ noted].

A number of peculiar variegated colonies were noted on Mal EMB. Picks and number & description. Streaks out on Mal, Lac.

c) 1.  2.  3.  4. 

A27. a) Mal−. 72 tested on Lac All apparently pure Lac−.

a Mal+ 84 tested. 80 definitely mixed. 4 possibly Lac v or containing Lac+. (These may have been Mal v miscounted as +). Streak these out as a [1-4].

a Mal v 24; tested 22 Lac v 2 apparently pure Lac−; a [5-6].

~~c [1-4].~~

Also, a considerable number of "pure" Mal+ and− colonies unpooled, and streaked out on EMB Lac. Among several hundred Lac− colonies there were two identifiable Lac v or+, in addition to numerous papillae in the thick streak. Attempt recisolation of Lac v. a [7-8].

b]. 52 Lac v tested on Mal: all + or contain +.

c].

All these tests were made by picking media directly from colony to agar. ∴ + cannot be distinguished from v.

January 27, 1950.

C]. 1. Lac — virtually all −, rare ⊽, papillate.
Mal — only + and − seen.

2. Lac — rare-occ. ⊽, heavily papillate
Mal — Mostly or all pure +, some −.

3. Lac — all − colonies, but papillate
Mal — only +, −

4. Lac — occ. ⊽ colonies.
Mal — only +, − noted.

Pick Lac ⊽ and pool as far as possible. Streak out on lactose for resolution of Lac ⊻

January 30, 1950

a) 1. Mal+ with occ. Mal−, and simple sectors. lac− with occasional lac v.

2. Do.

3. Mixed Mal+, v, − } ~~Mal v, M~~

4. Mal v, Mal+. } lac v; lac−.

5 Pure lac− } largely segregated (Mal+, −) → +, − only.

6 Pure Lac− } maybe some Mal v. [Attempt reisolation].

7 Mal−, some +, v?

8. Mal+, some −; v? } lac v, −. Reisolate lac v and test for Mal purity.

c) 1–4 Lac−, Lac v. Test isolated lac v for Mal. purity.

---

a) Pick lac v from 1, 2 and 7, 8 above. Streak on Mal.

1–3 = a1
4–9 = a2
10 = a7
11–16 = a8.

	Mal
1	+, −, v
2	+ −
3	+ −
4	+ −
5	+ −
6	+ −
7	+ −
8	−

	Mal
9	+ −
10	+ −
11	+ −
12	+ −
13	+ −
14	+
15	+ −
16	+ −

Reisolate lac v from these

These may be Mal pure. 668d. (1) & (2)

---

c: 8 each.

1 all impure (Mal+, −).
2 " "
3
4

} All were Mal v, not Mal+.

Segregation of H226

1/31/50.

EMS Lac

1. Lac$\underline{v}$; Lac− (Mal−) H227.

2. Lac$\underline{v}$; Lac− mostly Mal+, occ. Mal−, simple Mal$\underline{v}$
Repeats 4 Lac$\underline{v}$; all some Mal+; Mal$\underline{v}$.

---

pool H227 on EMS Lac, EMS Mal.

---

131. Picks 200 Lac$\underline{v}$ from 671 A2 Bursts very sparse on EMS Mal.
1-100 No Mal−. Mostly Mal+, −. 2 maybe pure Mal+ : retitrate as 668 d: 3-4. 3: No Lac$\underline{v}$ (exc. papilla); 4: Mal+/− ; Lac$\underline{v}$
101-200 No Mal−. Mostly Mal+ & detectable −.
201-240 All Mal+/−

Irradiation of H225 and H226

668+

N.G. Too dilute

January 25, 1950.

Resuspend H-225 from EMS lac in H_2O, 10 ml, to get. Lens. ca 120
Dilute 1/100 for irradiation. 5 ml sample in Petri dish (10 cm).
Remove 1 ml to 10 ml for each interval. 20 cm. distance.
0, 5, 10, 20 sec. This leaves suspension at 10^{-3}, or as estimated 5×10^5
A, B, C, D. before irradiation.

Dilutions:

	1	.1 ml
	2	.03
	3	.01
	4	.003
E only	5	.001
	6	.0003
F 10^{-6}	7	.0001

H226 O.D. 090
E 0
F 20

A. 4	5, 4	∴ Initial count (00 = 120)
5	1, 0	
6	0, 0	was 3×10^{-6} ml = 5 colonies = ca 10^6/ml. (viable)
7	0, 0	
B. 6	0, 1	
C 3	12	∴ count = 12/10^5 = ca 10^6/ml.
4	7, 5	
5	0, 1	no killing detected.
6	0	
01	0	
D. 3	0	

E	10^{-6}	4, 4, 3
	10^{-7}	0, 1, 1
F	10^{-4}	0, 0, 0

Test lac+ [Lac$_1$ x Lac$_4$] as diploid
Irradiate Seligmann's lac+.

January 27, 1950.

Suspend "pure" colony of 652-2 in H_2O. Spread drops on EMBlac.
Expose to high pressure UV 6, 7, 8, 9 secs.
Ca 1000 survivors in total. No Lac- or Lac$^{\pm}$ noted

Conclude that 652-2 is probably not a stable diploid; but a typical recombination of lac$_1$- with lac$_4$-.

2/4/50. #2922A received from E. Seligmann, Beth Israel Hospital, New York, N.Y.

streaked out on EMBlac: all +. Suspend + colonies in water, spread on EMBlac and subject to 8 secs UV (high pressure lamps).
4 plates; ca 500 colonies. All lac+.

Effect of medium on segregation.

January 27, 1950.

EMS BMTLB, apparently does not permit *lac v* colonies of H16B. Check effect of various supplements.

In this case, *lac v* was observed on BMTLB.

Irradiation of H225-226

January 29, 1950

a) H226 susp. in 10 ml O.D. 275 ca 2×10^6

b) H225 " " " O.D. 380. ca 3×10^6

Count in Petroff-Hausser. ca 3×10^7

Approved dilutions. dilute ~~H20~~ to estimated 10^5 each a) .5/10 b) .33/10

			uv	record $\log_{10}$ est. number
1x	A=	226	0	
2x	B=		5	
50x	C		20	
1x	D	225	0	
2x	E		5	
50x	F		20	

January 30, 1950.

A.	W226	No UV.					
		"2".	+ = V	−	Σ	% V	Counted readily under lens.
			457	73	530		
	*		432	96	528		
			656	84	740		
			593	102	695		
			2138	355	2493	86	
		"1"	388	46	434		
B.	W226	5 secs.					
	2	(+ maybe count.)	25	382	407		
			27	401	428		
			52	783	835	06	
	!	"1 uncon".	24	←	536		
C		2				0	
			0	2		2	
			1	2		3	
			0	3		3	
			0	8		8	

No full or stable + noted. Reincubate to search for same.

Note 2 counts. Two sets of "2" were made.
The first set was uncountable, probably ca 10 × 434
The second is given. Platings appear very erratic!

D	ouv	+	−	V	Σ	%v
	1	10	21	110	141	
		22	21	128	171	
		32	42	238	312	76
			74			
E	ss. uv.					
	1	24	37	21	82	≈ 36
		27	57	34	118	
		51	94	55	200	22
			145			
F	2	7	15	2	24	
		11	22	2	35	
		6	7	1	14	
	"7"	9	11	2	22	
		33	55	7	95	
	"20"	2	1	1		
		1	2	0		

No absolute increase, as relative; actual count is one half that recorded

i.e., survival was $\frac{100}{312}$ = ca 32%

These counts seem more consistent than those of A–C: even shorter doses should be used (ca 3 seconds)

Analysis

2/3/50.

	A	B
a) Initial assays were	6×10^7	0×10^7
After 20 hours, refr. in water	2×10^7	5×10^6

Note drop in titer, especially with H226!

b) H225 data show uncomfortably high variance, but clearly indicate absolute increase of segregant types with 2 sec. irradiation.

c) H226 data not very useful owing to too low deletion

d.) 5 mems. at 60° already kills most cells. Use at high conc. and plate various deletions.

Irradiation of H226 - H225. 672 A

February 2, 1950.

a) P1. Assay suspns.

		O.D.^{4200}	Titer
A	H225	340	6×10^7
B	H226	290	6×10^7

Note error in calculation!!

For critical, low dose irradiations, dilute to 3×10^{-5} (estimated 1800/ml), irradiate, and plate .1 ml samples. Use 2 and 5 secs. UV. 20cm. 2 ml/10cm Petri dish

C. H226 Take 3 ml sample, undiluted, and give ~~~~ doses at 10 sec intervals. Plate .1 ml for each. Start at 40 sec. 27 survivors at 40 sec.

D. 60° exposure. (in oven) Use 3×10^{-4} dilution H226, 3 ml in small test tube. Sample at 5 min intervals (.1 ml).

2/3 3PM A 0

	+	−	V
1	8	19	36
2	8	13	36
3	7	24	50
~~4~~	~~23~~	~~56~~	~~42~~
4	8	8	42
5	10	7	31
~~22~~ 22	41	71	145

% .

Av/plate.

~~28~~	~~19~~	~~41~~
8	14	29

A 2.

survival
3PM
(somewhat early)

		+	−	V	Σ	% surv.
A0		8	19	36	63	
		8	13	36	57	
		7	24	50	81	
		8	8	42	58	
		10	7	31	48	
	Σ	41	71	195	~~307~~	
	Av.	8	14	39	~~31~~ 61	
		13	23	64		100
A2	1	13	27	19	59	
	2	12	29	7	48	
	3	11	14	5	30	
	4	13	28	20	61	
	5	6	36	17	59	
	Σ	55	134	68	257	
	Aver.	11	27	13	51	
		22	53	25		84
A5		13	16	11	40	
		17	25	15	57	
		5	8	3	16	
	Σ	35	49	29	123	
	Av	12	16	18	41	
		29	39	32		67

These results indicate an increase in the absolute number of segregant types with very low doses, with appreciable killing.

		+ = V	−	
D.	0	16	7	23
	5m.	0	0	
10 ↓	0.			

Note: assay should be 10 × B0 (av. 5.5)

Lac series.

	+ = V	−	
B 0	4	0	
	2	4	
	6	1	
	7	0	
	0	2	
	2	4	
	22	11	33
	3.67	1.82	5.5

Mel.

	−	+	V	
	0	1	3	
	0	3	2	
	0	2	1	
		6	6	/ 12
		2	2	4.0

B2	~~5~~	~~B~~	
	0	7	
	0	10	
	2	4	
	1	4	
	1	6	
	4	31	35
	0.8	6.2	7.0

		2	3	
	1	3	0	
	2	3	3	
	0	9	1	
	3	17	7	/ 27
	.75	4.25	1.75	6.75

B5	0	5	
	0	4	
	0	11	
	0	4	
	0	3	
	0	27	5.4

	2	3	0	
	0	2	1	
	1	3	1 ●	
	1	0	0	
	4	8	2	/ 14
	1	2	.5	3.5

This series highly unsatisfactory owing to killing!

40 sec.	0	26
50 sec	0	0

Same original was ca. $\frac{5.5}{3} \times 10^5 = 2 \times 10^5$ / .1 cc

The 40 sec survival was ca 10^{-4}.

Spontaneous heterozygotes
continued from 652.

February 2, 1950

A) W67 x W1272. [Streaks out puritine on EMBlac OK]

EMBlac ca 2500 prototrophs

2 lac+. Streaks on EMB, EMBlac; EMBMal. 2 additional A5.

1	Lac v?	Mal −	maybe lac+ or very stable lac v. = 673 − 1
2	lac −	Mal −	
3	Lac −	Mal −	
4	Lac v	Mal −	clear Lac v. = 673 − 2

very weak + !

B 2/5/50.

W67 x W1177 EMBlac 21 x 60/ = ca 1200 prototrophs.

8 lac+ colonies streaks out on EMBlac; EMBlac; EMBMal.

	Adj. to (−)	Lac	Mal
1.		v	− +
2		+	−
3		v	!
4		?	+
5		?	−
6		v	v?? mod.
7		v	−
8		v	+

PROVISIONAL

Keep on D(Lac) agar.

Evidence concerning diploid intermediate in segregation of M226.

2/3/50.

A Picked well isolated lac - colonies from pre-assay plates B5 - etc. of 672. Some should be Mal+/-. 40 tested by sparse brushes. Compare with material from 675.

39 +, apparently pure; 1 —.

B. Do. from c40 28 colonies brushed. 22 were pure Mal+
4 " " " Mal—
2 were predom. Mal-, ; Mal+. Picked and restreak → Mal EMB was badly contaminated.

Save 677 plates!

From 677 Ax and Bx, picks isolated ~~Mal~~ lac - colonies and brush on Mal EMB.

41 pure Mal-

18 "pure" Mal+

M mixed Mal+/-: ? Restreak on Mal EMB; lac EMS to isolate.

76 total.

Restreaked cultures:

	Mal	lac EMS	EMB
15	+ —	pr. — +	—
16	+ —	pr. +	
17	+ —	pr. +	

Mal+ - check lac EMS and transfer to lac EMB.

3 are lac +
2 are pure lac -

	Mal	lac EMS → EMB
1	++	
2	++ —	⊖
3	++	
4	+, —	○ ~
5	+ —	○ ~
6	++	
7	+ -	○ 4
8	++	— 7
9	++	
10	++	
11	+ +	
12	+ —	○ 5
13	+ —	— 6
14	++	

∴ 7 of these colonies are apparently lac — pure, but Mal+/— !

Heat and uv killing of H226.

Febr. 3, 1950.

Make up from EMS Lac to optical density of 420_{420}. H226 only.

Assume titre ca. 10^8 (but also work on basis of 10^9; 10^7)

A. (assume 10^8). Dilute to 2×10^{-5}. Use. 1 ml samples/plate before and after 2 secs. irradiation. A and AX

B (assume 10^9) Dilute to 2×10^{-6}, etc.

C. (assume 10^7) Dilute to 2×10^{-4}.

D. Heat 2ml original culture 5 mins., 60° and plate out at various dilutions

irradiate A, B, C 2 secs. UV 20 cm.

P4: Viable count too low! Ca 10/plate at 2×10^{-4} = 5×10^5.
D5 gave 9 colonies, indicating no killing by the heat treatment!

Apparent proportion of Lac – rather high in current suspensions.
Start a fresh restreaking

	+	V	−		+	V	−
C:	1	1	4		1	2	0
	2	9	4		5	1	1
Lac	0	7	4	Mal	0	0	0
	0	5	4				
	1	6	5				
wipings from each of ↑	4	28	21 /53				
	0	0	1				
		2	9		4	2	1
Cx		0	10		5	7	1
		2	8		12	3	1
		0	7				
		2	11		2	0	1
wp.		06	2	45751			

		+	V	−
D	4	7	123	88
	5	0	6	3

These ratios are comparable to C, which is consistent with the lack of killing.

---

streaks or apparent lac+.
1-3 from C
4-9 from D

all are clearly lac V. lac+ cumip to spreading habit.

Preparation of diploid inocula

February 4, 1950

D(@) liquid + 0.1% lactose

1-4 single colonies from old EMS stac

5,6 from broad streaks. Incubate 5 aeration on shaking.

Streak out initially and after growth. Estimate % lac⁻.

	Initial	20 h.
1	25	85
2	<10	90
3	40	90
4	80	>90
5	50	>90
6	40	>90

This appears to be a satisfactory method for preparing diploid inocula!

Use (4) for irradiation study in hopes that high proportion of lac⁻ is maintained

Irradiation of H226

~~676~~ 677

February 5, 1952.

Use liquid culture 676-4, ca 20 hours. O.D. 400

Assume titer of ~~[redacted]~~ 10^9, and work for 10^2 colonies/plate.

For safety, also plate at 10^{-6} and 10^{-5}.

Dilute to ~~[redacted]~~ 10^{-4}. Irradiate 3 ml 2 secs 20 cm low pressure uv.

A) Take .1 ml samples Crowded.

B) Dilute to ~~[redacted]~~ 10^{-5} .1 ml samples

C) 10^{-6} .1 ml samples.

D) Expose* 1 ml original sample to ~~[redacted]~~ 57°C. 10 mins.
(.7 ml) Dilute 1:10 in cold H_2O and plate out at various dilutions
~~All~~ plates sterile.

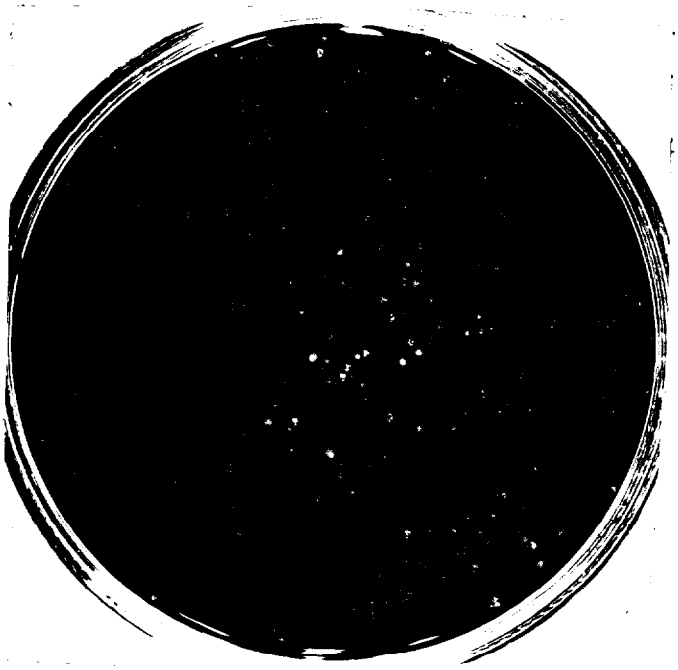
E) Plate original sample at all dilutions from 8 – 3.

	V	—	
10^{-8}	4	0	
10^{-7}	48	4	
10^{-6}	305	24	
	357	28	385.

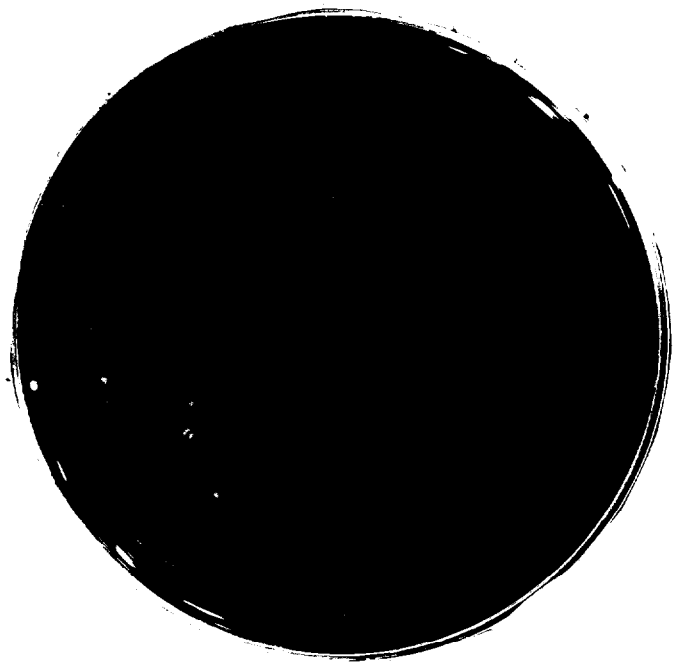
5×10^8 = titer

% ~~[redacted]~~.

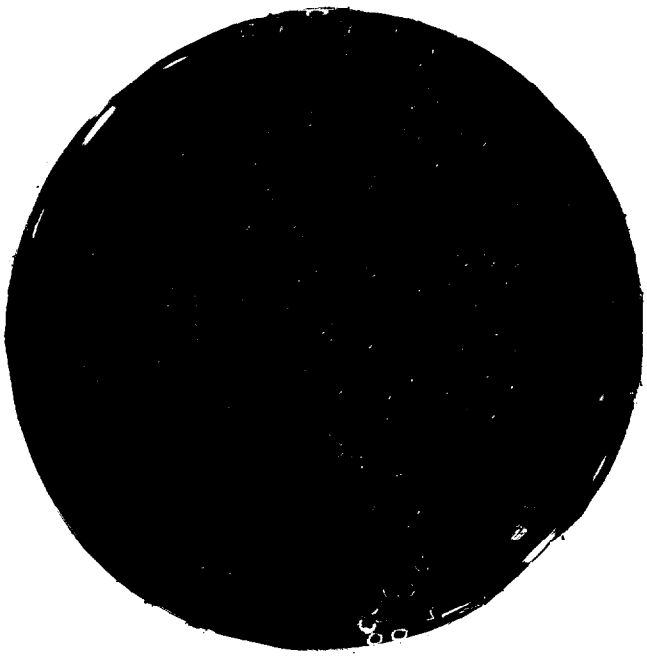
A control



A uv



B - uv



B control

Count B series.

B (no UV)

lac

	V	—	Σ
1	170	16	
2	174	10	
3	200	16	
4	197	14	
wip from 4	2	0	
	743	56	
per ml	1857	140	1997

B_x (UV 2 sec.)

(wip)

6	63	
12	64	
0	2	
18	127	
per ml 90	635	725

Photograph sample plates of B and A, (o, x.)

Survival = $\frac{725}{1997}$ = 36%

Shift lac v from 93 to 12%.

c non v.

17	3	
17	1	
9	2	
12	5	
19	2	
wip: 1	0	
75	13	
per ml. 150	26	176
10	65	75

i ex

0	9
1	3
2	5
1	9
4	26

EMB Mal counts. Most Mal plates were contaminated. Some, however, were on earlier clearer batch.

B: not readily scored; however, mostly Mal+ or Mal v.

B_x: Plates contaminated, but fairly numerous pectored colonies:

Mal+ (⁵⁄₇)	Mal−	Mal v
34	50	20

The frequency of Mal v seems higher than of lac v, suggesting some diploids. See 674.

InB, a fair proportion of crescentic colonies was seen (+) (−) etc.

(on E5) a single colony was noted that, by its appearance, might be a lac+ recombinant. Streak out for check. —

Lac+ pure, Mal+, Xyl+, Mtl+. Kept on agar & Cent.

2/10/50.

A number of colonies previously scored as lac −; lift out on desk top several days, now show central papillae. Pick and streak out as (plate photographed as 677-UV B). EMB lac.

V	−	central +
8	29	23

In general, they seem to give typical lac x!

To avoid any confusion, a special uv experiment is called for!

Treatment of H226 with Mustard (HN2)

Febr. 6, 1950.

H226, suspension 676-4 (24 h. in refrig.)

A) Control: Titrate out from buffer below.

B) Add 2 ml suspension to ~~1 ml D~~ 1 ml ~~KH buffer pH 7.0~~ 10 ml D(lac) [acetate buffer]. Add 10 mg HN2. After 5 mins., dilute 1:10 in Y2 plus to inactivate HN2. Titrate. (Initial 10^{-2}) Record in terms of initial ~~dilution~~ suspension.

		V, +	—	
A)	10^{-7}	56	2	
	10^{-6}	420	22	/ 442
B	10^{-6}	46	206	/ 252

The survivors have appeared generally to be lac- .

Treatment of M226 with chemicals

February 6, 1950.

Use suspensions 676-4 and 676-4A (o.d. = 750) 676-4A is loop transfer from D(lac) to D(lac). 1/mod = 100

A) Assay 4

B) Assay 4A

C) Dilute 4 1:5 in 6% sodium desoxycholate. 37°. 5²⁰ - 7⁵⁰ P.M. Titrate

D) Dilute 4A 1:5 " " " "

E) Dilute 4A 1:10 in D([redacted]) Add 10mg HN2, hydrochloride, (Room temperature) 10 min. dilute 1:10 in Penassay

F) Dilute 4A 2:10 in 1.2% Methyl Green. } 8¹⁰ PM — 9⁵⁵ PM 1⅘ exposure

G) " 4 2:10 in 1.2% " " . } 37°.

Record dilutions subsequent to treatments. E as original, cf. B.

H. Suspension 4. Heat to 65° 5 mins..

[redacted] I. " " " 10 mins.

J. " " " 20 mins.

F, G. appear all dead!

UV; HN2; kill by a nuclear mechanism.

Heat; dock; " " a non-nuclear " . ??

A.	Dil	V	—	
Assay	6	393	45	
	7	33	2	
B				
assay	7	57	0	
	6	n.c.	26	
C	6	72	5	
doca.				
D	~~5~~	311	29	
doca.				
	6	9	25	
E	5	37	212	
Mustard			~~249~~	Reincubate!
	4	3	n.c.	v much more numerous after after incubation
F,G	1,...	sterile		
H	4	200	22	
heat		73	5	
I	2	~~9~~	~~4~~	
J	1	0	0	

STANDARD FORM NO. 64

Office Memorandum • UNITED STATES GOVERNMENT

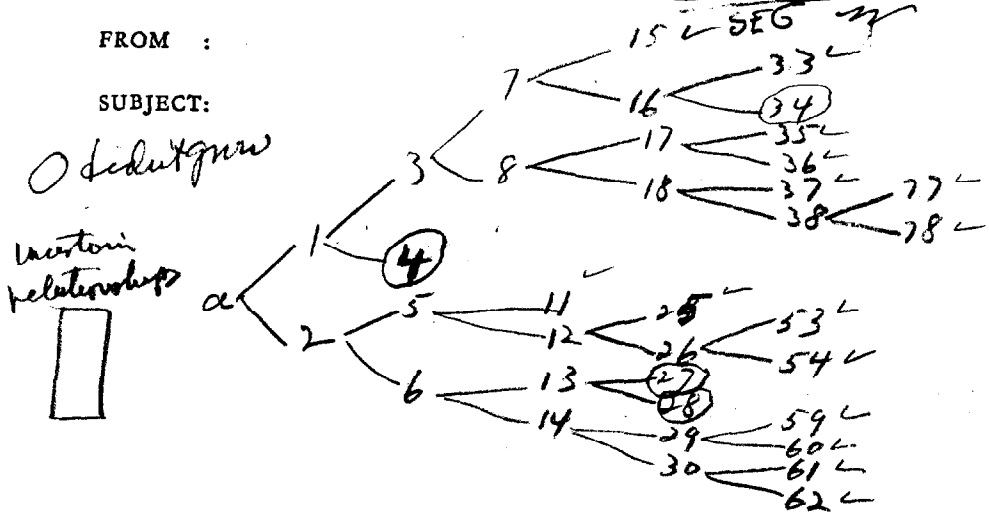
TO : # 226 DATE: 2-5-50

FROM :

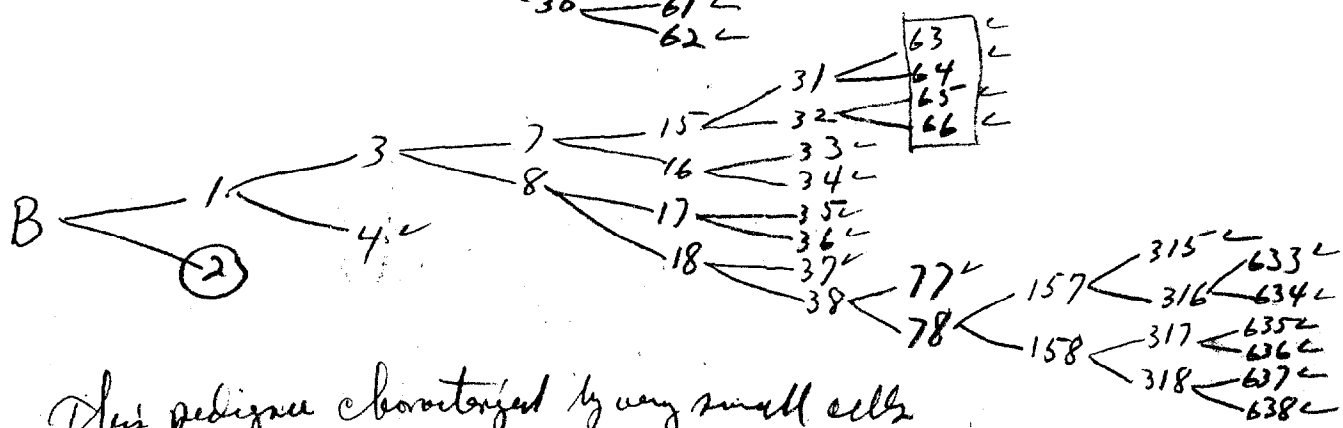
SUBJECT:

O dedutgrow

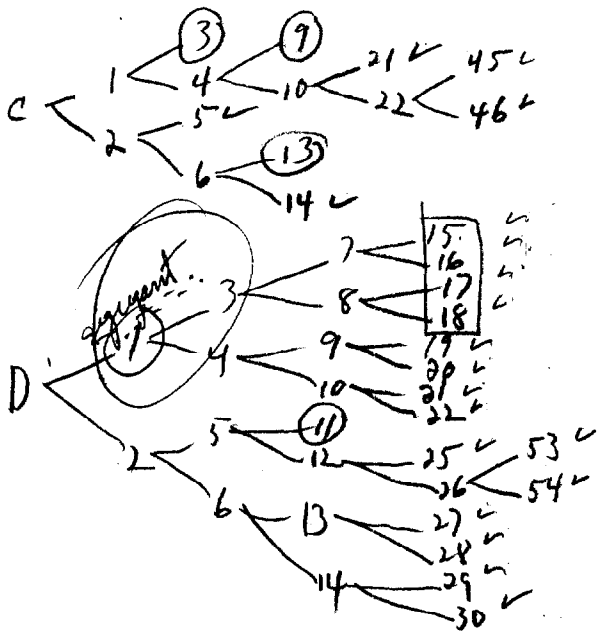
Ancestor relationships



a 35, 37, 36 mincolonies were longer, had "ramified" appearance,

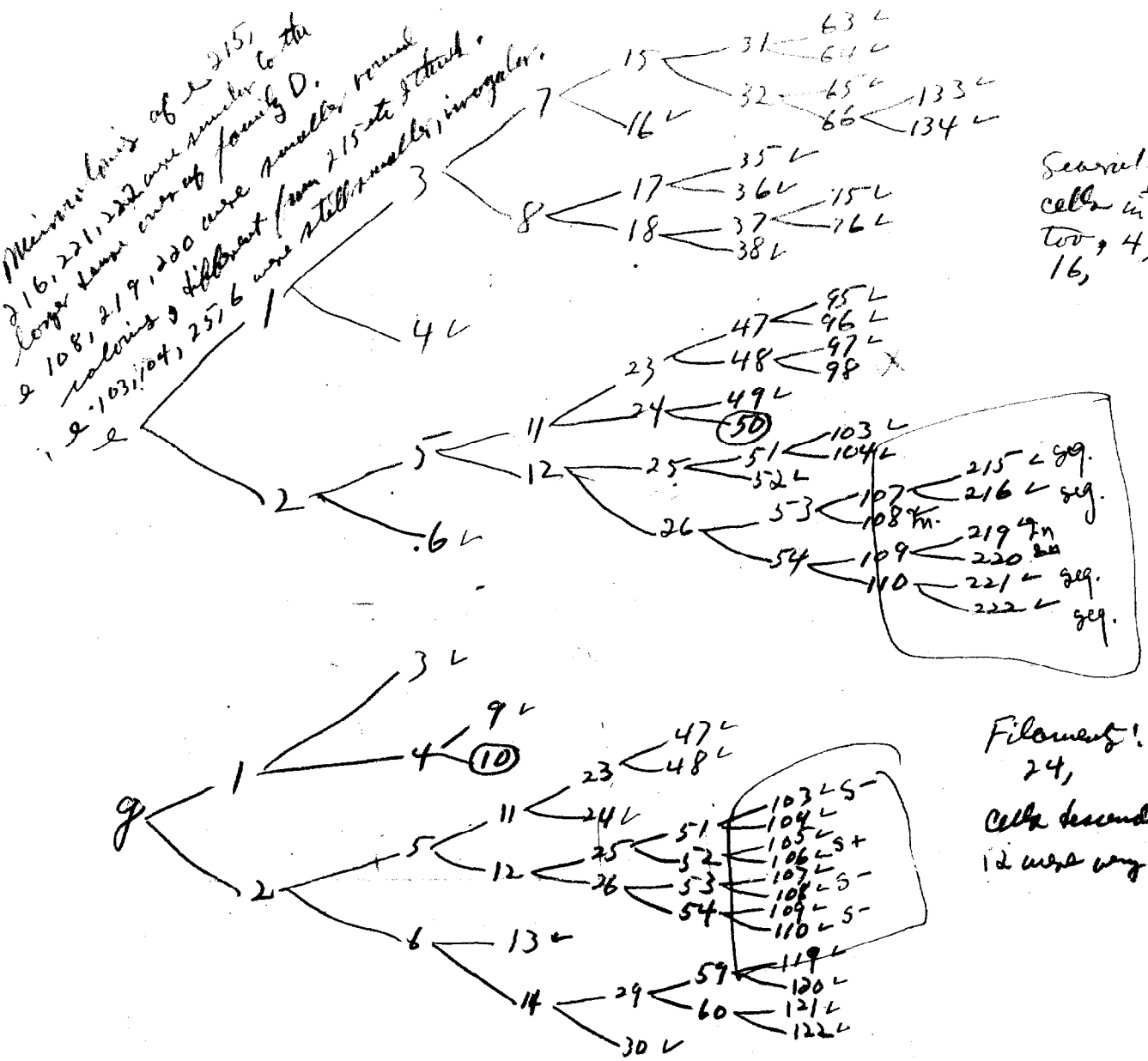


Des pedigree characterized by very small cells except #4 which was filamentous + later divided. #2 became a 8-10 μ filament—stopped there.



Most cells in this pedigree formed filaments, then divided somewhat irregularly.

Mincolonies of this family were rather faster growing, denser, harder to get into pipettes as if they were mucoid.



Microcolonies of 105 + 106 were found + smaller than 103, 104 and 107–110. 107–110 were the densest like the D family. 105 + 106 microcolonies were similar to ≥ 108, 219, 200.

Zille: single cell pedigrees on H226

February 5, 1950

		Lac	Mal	Lac EMS
A.	15 11	+	+	+
	33 15	—	—	—
	34 25	+	+	+
	34 33	+	+	+
	37 35	+	+	+
	38 36	+	+	+
	37	+	+	+
	53	+	+	+
	54	+	+	+
	59	+	+	+
	60	+	+	+
	61	+	+	+
	62	+	+	+
	77	+	+	+
	78	+	+	+

B

4
33
34
35
36
37
63
64
65
68
77
315
633
634
635
636
637
638

All lac+
Mal+
n.g. on EMS.
. . started with a segregant

14
21
45
46

	Lac	Mal	EMSlac
5	+	÷	+
C 14	+	÷	+
21	+	+	+
45	+	+	+
46	+	+	+
15	—	—	
16	—	—	
D 17	—	—	poor growth
18	—	—	
19	—	—	
20	—	—	
21	—	—	
22	—	—	
25	+	+	+
27	+	+	+
28	+	+	+
29	+	+	+
30	+	+	+
53	+	+	+
54	+	+	+
4	÷	+	
6	+	+	
16	+	+	
35	÷	+	
E 36	÷	+	
38	+	+	
49	÷	+	
52	+	+	
63	+	+	
64	+	+	
65	+	+	
75	+	+	
76 90	+	+	
95 94	+	+	
96 97	+	+	
97 [illegible]	+	+	
98 [illegible]	+	+	
103 [illegible]	+	+	
104 [illegible]	+	+	
108 [illegible]	+	+	
133 [illegible]	+	+	
134 [illegible]	+	+	
215		—	
216		—	
219		+	
220		+	
221		—	
222		—	

G

	Lac	Mal
3	+	+
9	−+	+
13	+	+
24	−+	+
30	+	+
47	+	+
48	+	+
103	−	−
104	−	−
105	−	+
106	−	+
107	−	−
108	−	−
109	−	−
110	−	−
119	+	+
120	+	+
121	+	+
122	+	+

Test segregants. H226 6802

2/8/50.

G			Vi	Lac	Mal	Gal	Ar	Xyl	Mtl	Stl	Nutr.	H226
103	1	R		−	−	+	+	−	−	+	TB,	pure +
104	2	R		−	−	+	+	−	−	+	TB,	"
105	3	R		−	+	+	+	+	+	+	B,	Gal; Ar; Stl
106	4	R		−	+	+	+	+	+	+	B,	
107	5	R		−	−	+	+	−	−	+	TB,	
108	6	R		−	−	+	+	−	−	+	TB,	
109	7	R		−	−	+	+	−	−	+	TLB,	
110	8	R		−	−	+	+	−	−	+	TB, + ?	

???
NoM−!

Chemical treatments ~~with~~ of H226

Febr. 8, 1950.

Inoculum prepared from EMS colony into D(Lac); incub. 20h.

$O.D._{4200} = 830$

A. Assay

B. Dilute 1/10 in .01% Methyl Green in D(-) 2 ½ pm — 5 ½ 5

C. Dilute 1:5 in 6% Na deoxycholate. "

Add H_2O to 1/10 express dilutions as original.

		V	—
A7.		135	12
B6	325	37	Methyl green: v. little killing no haploidizations
C6	>>600	ca 10%	Not enough killing! no haploidizations.

Treatment of H226 with chemicals.

Febr. 9, 1950

Use same H226 resp. as 681.

A) Assay

B) Dilute 1:5 in 6% Na desoxycholate 11.45 } 37°
C) Dilute 1/10 in 0.1% Methyl Green/sal 11.45 }
D) " 1/10 in ~~H2~~ saline } I am probably in error 10x dil. ?

Express as orig. conc.

	v	—
B6	230	16
C2	114	6
D "7" (8) (2 plates)	13,6 (19)	(1,2) 3

Not appreciable killing in desoxycholate! pH of 6% solution : 8.9!
1:5 bacteria/doca : 7.1
bacteria + saline (ha) :

Methyl Green kills by non-nuclear mechanism.

Chemical Treatments of H226

Preliminary data

February 10, 1950.

Add stock of H226 (36h. in 50 ml DL Lac) 1:10 to medium D(-), plus supplement added. Incubate in tank unless indicated

		Supp. vol.	Total Vol.
A.	—	0	11.
* B.	Acriflavine .05% dark	0.5	11.5
* C	" " under 4W fluorescent lamp	0.5	11.5
* D.	Pyronin Y .01%	0.1	11.1
E	NaCNO 1%	(NaCNO 5%) 2.5	13.5
F	hydroquinone 1%	(Hq 5%) 2.5	13.5
G	Formaldehyde .04% (=.1% formalin)	1	12.0

6 30

D — in H_2O to prevent ppts which is heavy in A — alone.

* 6 30

B & C agglutinated heavy ppt in D.

9 30 1 standard loopful, spread on 1 plate serially.

All but A are sterile

Repeat 2/11/50 under less drastic conditions

M226 chem. treatment

February 10, 1950.

	Same stock as 683 (refrig.)	Supp %	vol.	Total
A	–	–		1 ml.
B	Acriflavine .005 %	1	.05	11.05
C	" " (light)	1	.05	11.05
D	Pyronine Y .001 %	1	.01	11.01
E	NaCNO 0.1%	5	.2	11.2
F	Hydroquinone 0.1%	5	.2	11.2
G	Formaldehyde ~~.01%~~ .01% (=.25% formalin)	#1	.1	11.1

4PM – M.i.

Assay at 5PM. A → D Assays at 10^{-2}; 10^{-4}; 10^{-6}

E – 6 6PM.

A	7:	ca 300	90% lacv
B	6:	ca 100	mostly lacv } Many colonies ⊕
C	6:	ca 100	" lacv }
D	4:	ca 200	mostly lacv
E	6:	>500	lacv No sign. killing ??
F	Sterile		
G	6	ca 100	80% lac – !

∴ formalin has same mode of action as UV; mustard. Pyronin; acriflavin do not, but check for ~~latent~~ latent lethals. Hydroquinone is extremely bactericidal.

UV Killing Curve

2/13/50.

50 cm; 5 ml samples of H226, diluted 1:100. (H226 is 6 prom culture in flask of D(8), refrigerated 2-3 days. (see 683) Initial assay est. ca 3×10^9., After dilution, assume 3×10^7. State dilutions as of 1:100 sample.

	UV	D.I	Count (duplicate)		Survival	ρ S
A	0	5	70; 61	6.5×10^6	1.0	0
B	10	5	28	3×10^6	0.46	.34
C	20	4	144	1.4×10^6	.21	.68
D	30	4	84	$.8 \times 10^6$	.12	.92
E	40	4	25	$.25 \times 10^6$	.038	1.38
F	60	3	176	$.18 \times 10^6$	.028	1.57
G	80	1	22	.000022	3.4×10^{-6}	5.
H	100	1	5	5	$.77 \times 10^{-6}$	
I	120	1	7 - See	7	1.1×10^{-6}	
J	150	1	1 684A.	1	$.15 \times 10^{-6}$	

K Formaldehyde .05% 10 min.

L Hydroquinone .05% ca 12 min.

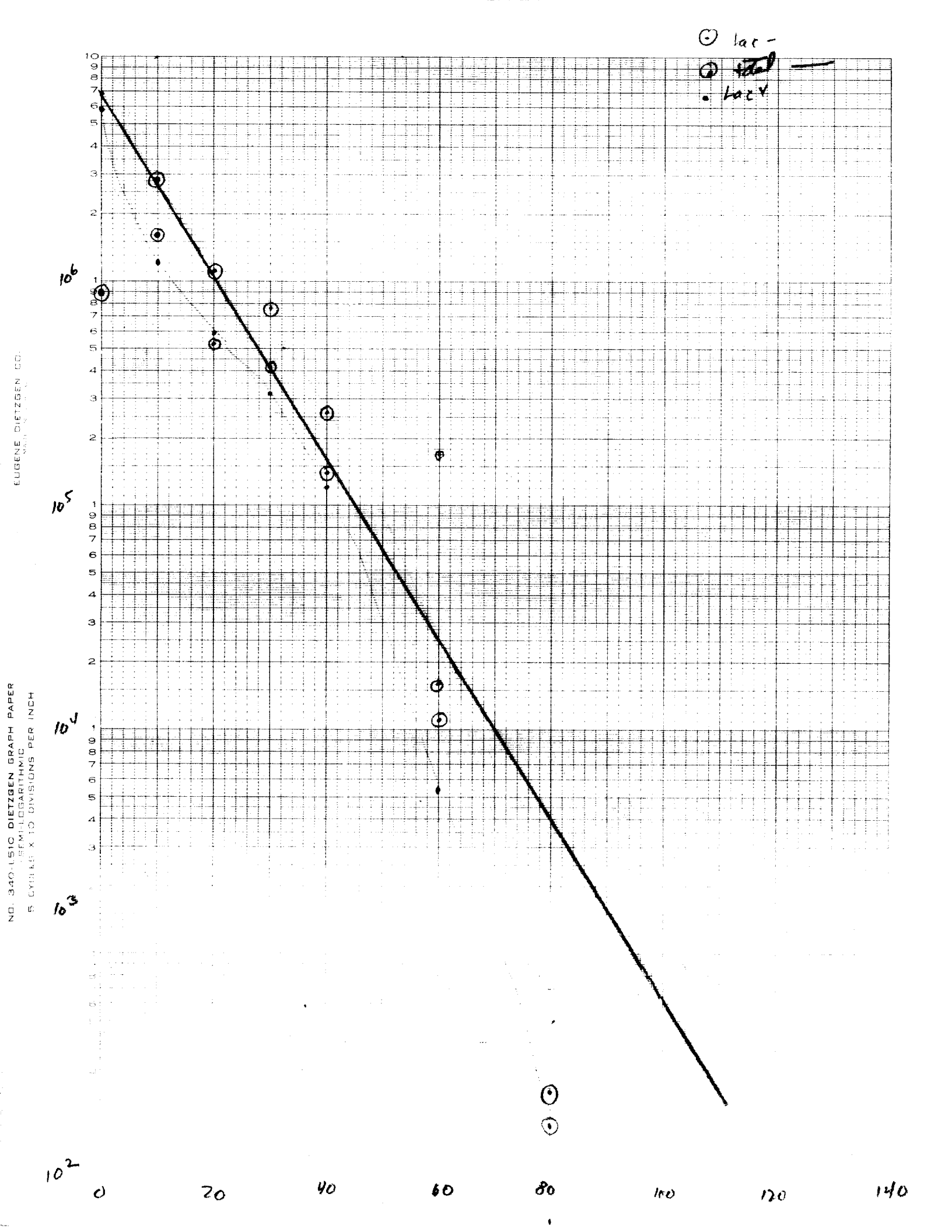
Dilution in solution of fragment

Dosage	60; 68;	8 7;		6.5×10^8	
K5	31	99	130	1.3×10^8	20% survival
L5	461	98 /	559	5.6×10^9	Negligible killing. Inconclusive

Survival

	UV	Dil	lac v	lac −	Σ	Σ	v	−	% v
A	0	5	54	10	64	68			
			64	8	72				
			118	18	136	6.8^6	5.9^6	$9.[illegible]^5$	87
B	10	5	12	16	28	2.8^6	1.2^6	1.6^6	43
C	20	4	59	51	110	1.1^6	5.9^5	5.1^5	54
D	30	4	31	42	73	7.3^5	3.1^5	4.2^5	42
E	40	4	12	14	26	2.6^5	1.2^5	1.4^5	46
F	60	~~"3"~~	~~57~~	~~112~~	~~169~~	~~1.1^5~~	~~5.7^4~~	~~1.1^5~~	
		"2"	51	105	156	1.6^4	5.1^3	1.1^4	33
G	80	1	7	17	24	240	70	170	29
H	100	1	3	1	4	40	30	10	
I	120	1	1	8	9	90			
J	150	1	0	1	1				

Note that proportion of lac v does not vanish, and may reach a minimum with low doses.



H206 – UV Killing for C.O. test

February 10, 1950.

Inoculum 24h. (age 3h) in D(Lac) (Very dense – ~ 2 x 10^9)

Plate out — Dilute 1:100; irradiate UV 20 seconds, and plate out.

A) assay 5×10^{-8}

B) Irradiate and assay.

1	10^{-7}	Sterile.
2	10^{-6}	
3	10^{-5}	

~~Repeat 2/11/1950 with same suspension.~~

2/13/50

A. assay & irradiation.

B Dilute 1:200 and irradiate 20 sec at 20cm.

4 survivors at 10^{-11}! Expressed as 1:200 sample.

assay at 10^{-5}.

too high kill

2/15/50 Same suspension

2/14/50.

A. 1ml H226 + 9ml 0% doca in D(-). 11[45] AM. — 4[25] PM.
pH 6.7 gelsmicator

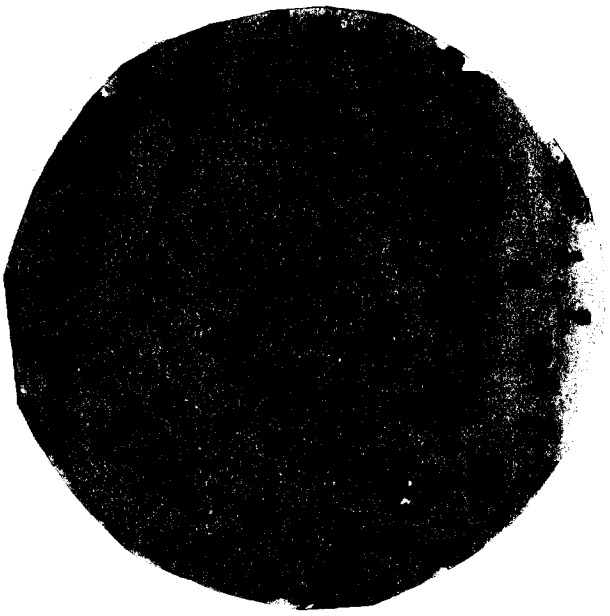
B. 1ml H226 + 2ml 0.5% hydroquinone + 7ml D(-). 4[15] PM. — SPM
= 0.1% HQ

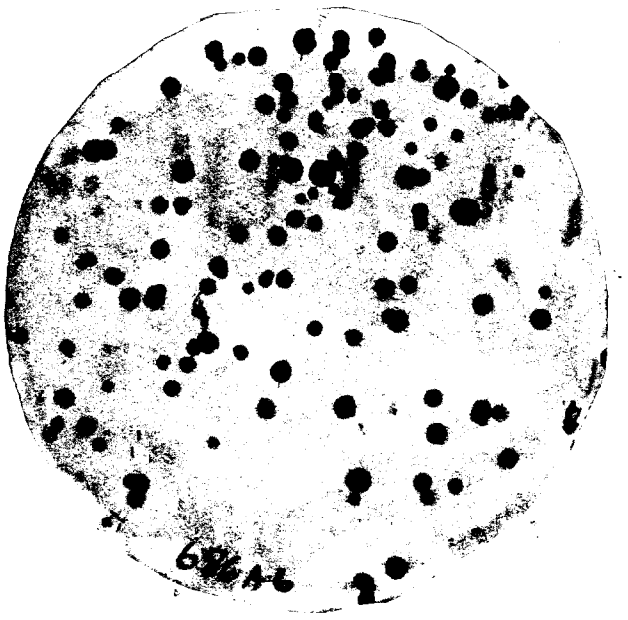
C. ~~Dilute new H226 stock 1:80 for irradiation~~ [Dil. as original stock]
Assay.

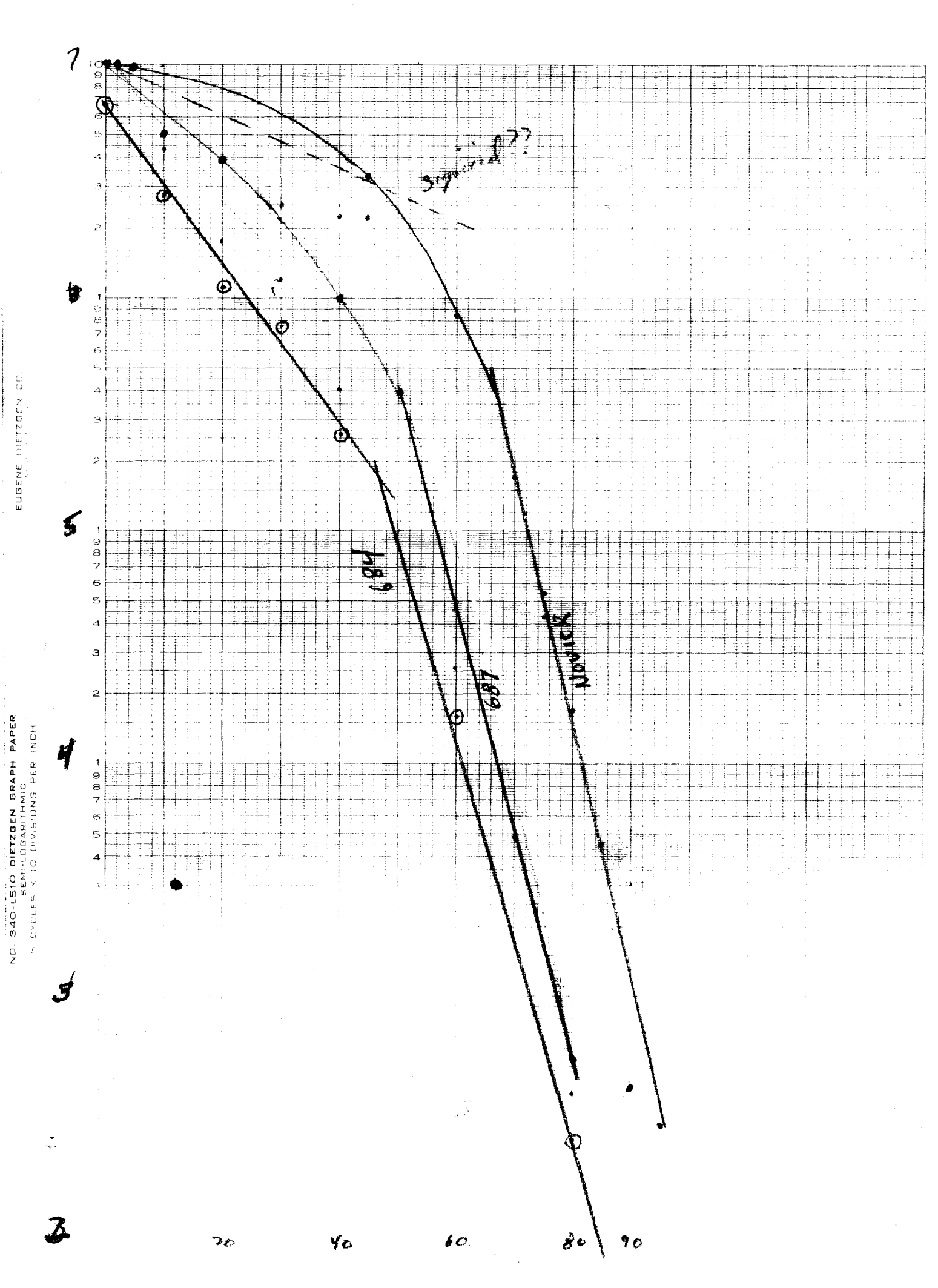
D. into 10ml H_2O, 200r/ml streptomycin 4[55] — 6[20]

		LacU	Lac-	Σ	Σ Count	
A)	6	57	80	137	1.4×10^8	~~with~~ pS
B)	−2 : sterile!	~~72~~	~~15~~	~~87~~		
c)	Assay. 7	72	15	87	8.7×10^8	
D)	(Strep). 5	230	30	260	2.6×10^7	

∴ Doca again shows only slight killing despite prolonged exposure (almost 5 hours). — Slight haploidization noted. Should be studied for balanced lethals.






7
6
5
4
3
2
20
40
60
80
90
Normal
189
189
EUGENE DIETZGEN CO.
NO. 340-1510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
CYCLES X 10 DIVISIONS PER INCH

UV Killing curve: H226

2/14/50.

Fresh (24; aer. D(lac)) shake of H226. Dilute 10^{-2} to give estimated 10^7/ml. Irradiate in open dishes, 50cm UV, as 684.

	uv-secs.	dil.		Lac V	Lac −	Count →		½	% V
A	0	5		173 {225 {199 174	22 {40 {14 12	195	1.9×10^7	1×10^7	89
B	2	5		{171; 167; 143	41; 41; 29	197	2.0×10^7	1×10^7	81
C	5	5		110	82	193	1.9×10^7	1×10^7	52
D	10	5		91 {102; 90	158; 76	208	2.1×10^7	8×10^6	44
E	20	4	Too heavy!! Count ½ pl.	(½) 270 =	(117) 510	780	7.8×10^6	3.9×10^6	35
F	30	4		123 =	385	508	5.1×10^6	?	24
G	40	3	>1800	Too heavy. ca 500	?1500?		2×10^6	1×10^6	ca 30
H	50	3	>200	184	613		8×10^5	1×10^5	30
I	60	2	>200	Too heavy.					
J	90	2	70	6; 7	98; 79	96	9.6×10^3	4.8×10^3	7
K	80	1		12; 8	128; 70	109	1.1×10^3	5.4×10^2	9
L	90	1	60	16	63	79	7.9×10^2	4.0×10^2	20

C 5 plates.

1	119	74
2	98	85
3	104	61
4	121	95
5	109	97
	551	412
	110	82

193	
183	
165	
216	
206	
963	
193	

D 2 plates

L

Served at 24h.

Aliquots plated on EMS lac

	+	−	Mean +	Mean v from 687
A	~~189~~	~~2~~		173
	189	0		
B	194	0		160
	178	2?		
C	120	6		110
	100	10		
D	106	12		91

Pick and streak out apparent − on EMB Mal.

	EMB lac	EMB Mal		Xyl	Mtl
1	−	v	*	+−	+−
2	−	v	*	−	+−
3	−	v	*	+−	+− ?
4		−			
5	−+	−+			
6	−	+			
7	−?	+			
8	−	v	*	−(+)	+ ~~−~~
9	−	v?	*	+−	+−
10	−p+	v (not −, v)	*	+−	+−
11	−	+			
12	−	+			
13	−	−			
14	−	v	*	+−	+−
15	−+	+			
16	−	v	*	+−	+−
17	−	+, − v?			
18	−	v	*	+−	+−
19	−	+−			
20	−+	+−			

Reisolate from Mal EMB to EMS lac, EMB Mal, EMB lac: *

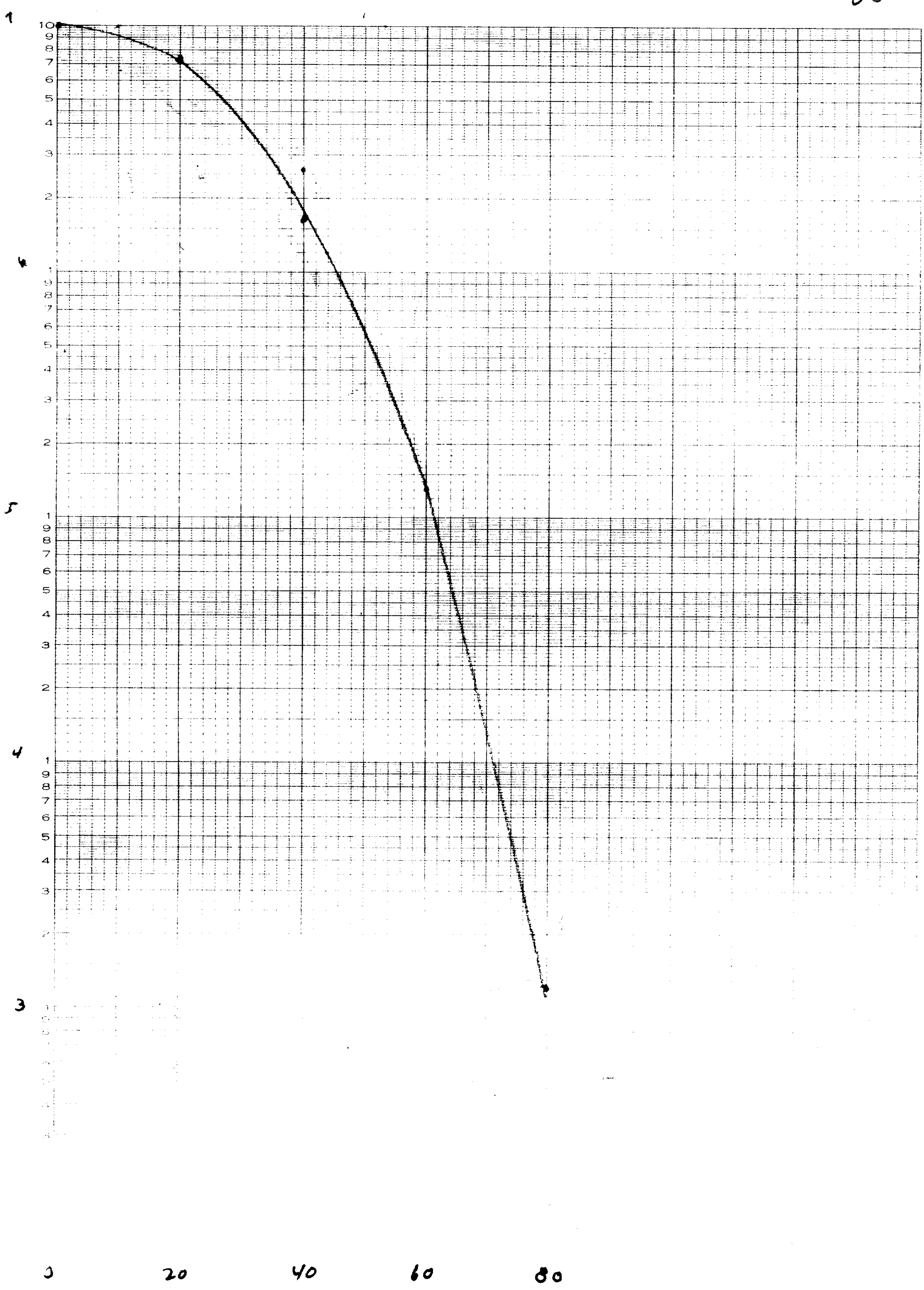
UV Killing — K-12

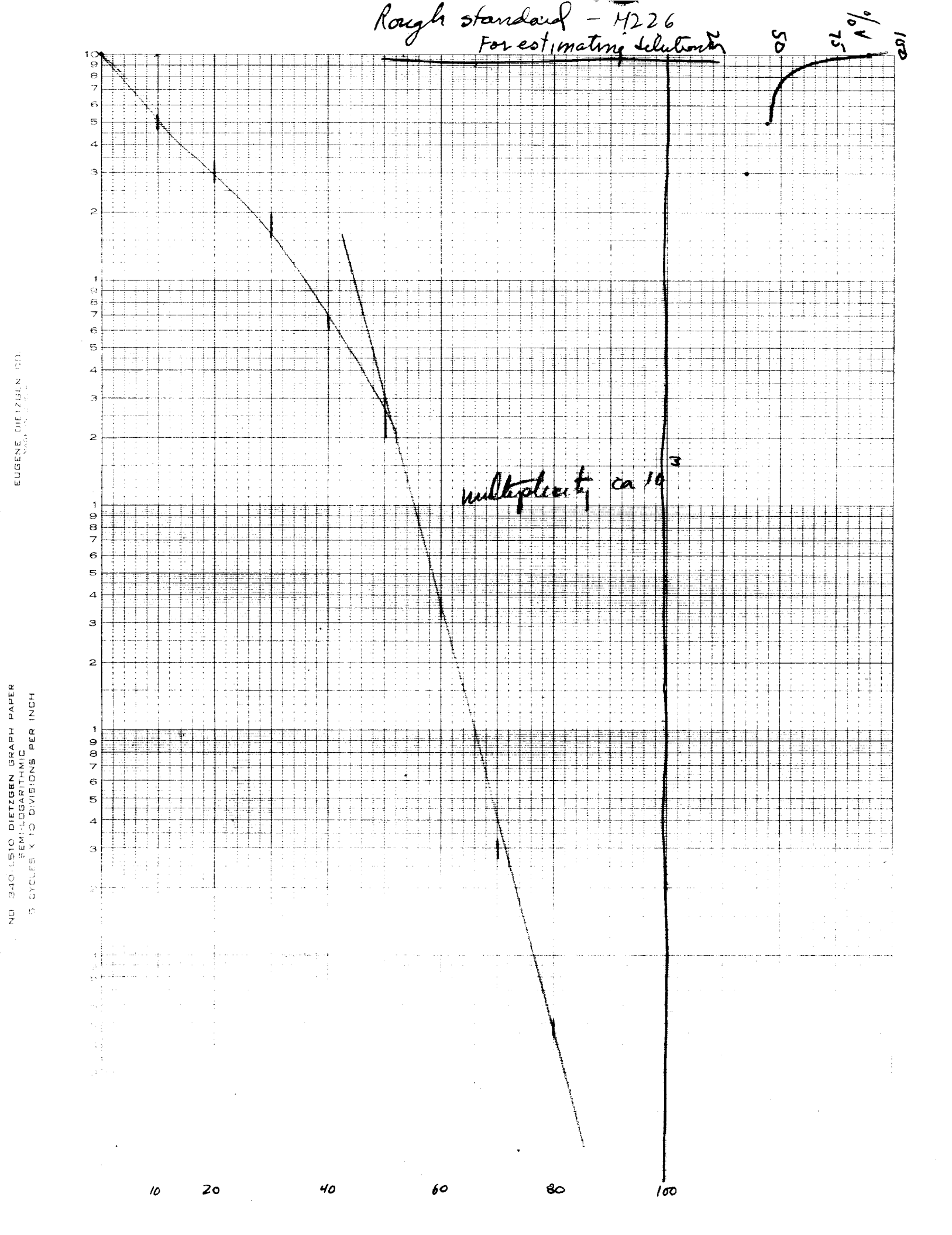
2-16-50.

Room in Dtac 24h. aer. 8 Brein-cefu ca 2-3 days.
Dilute 10^{-2} in saline for radiation.

UV sec	Dil		Count		÷ 6.1
0	5		608	6.1×10^7	1×10^7
20	5		462	4.6×10^7	75×10^6
40	4	¼ plate ×4	1600	1.6×10^7	2.6×10^6
60	3		823	8.2×10^5	1.3×10^5
80	1		730	7.3×10^3	1.2×10^3

K-12 UV 688




Rough standard – 4226
For estimating dilutions
50
25
1%
100
multiplicity ca 10 3
10
20
40
60
80
100
EUGENE DIETZGEN CO.
NO 340 L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH

Treat H226 with Acetic anhydride

2/12/50

Add 0.1 ml Ac_2O to 10 ml H_2O. Add 1.1 ml sterile 10% $CaCO_3$ suspension. Add 1 ml H226 and shake at room temperature. After 10 m., plate out on ± MB Lac (original H count not before assay.)
[Assume ≐ 3×10^8 ca 90% lac v]

All plates sterile. See 692 for effect of 0.1% Ac_2O.

2/20/50.

a) 1 ml H226 (2/19/50) 10 ml H_2O .1 ml 1% Ac_2O in 10% ale

c) " " .5 ml " "

10 mins 37°.

B6	Lac v 239	Lac − 203	442.	Count: 4.4×10^8	
A22				Survival = $\frac{4.4 \times 10^8}{\times 10^9}$	(see 624 assay)
				≥ 40% —	
C2.	0	3	Hold for delayed survival.		
A22 C5,6 sterile					
C2	14	106	Hold further		

K12 vs. H226 UV sensitivity

H226 UV; chemicals

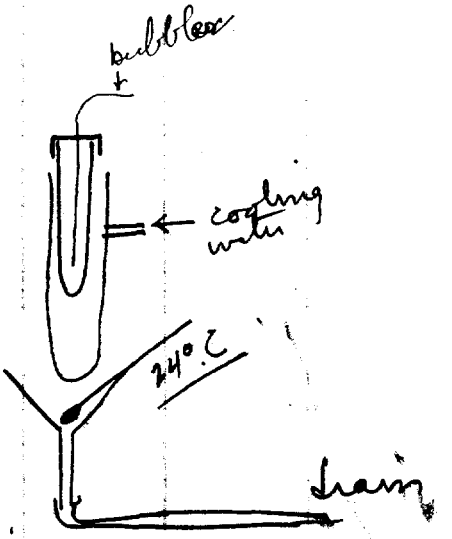
2/18/50.

Mix H226 + K12 [2/14], Dilute to 4×10^{-7}.

Take .1ml and spread on EMB Lac

A) Control

B) UV 20 secs.



C 10^{-5} H226 dil 10^{-2} for irradiation. assay.

D UV 20 1 × 10^{-5} } EMB Lac

15 ml vol. { E UV 80 secs. 10^{-1} } EMS Lac, EMS Mal

F UV 80 secs. + light. (focus Spencer projection microscope lamp on suspension, in cooling jacket, 12 cm. from lamp aperture).

4 PM → 6^{20} 100 mins.

FORMALD: 1ml susp. + 8.5 ml H_2O + .5 ml 1% CH_2O (→ .05% final) (= dil. 1)

5^{PM} – 5^{20} 20 mins. → ca 10% surv. mostly haploid

H – 6^{00} sterile 60 mins.

Note: after 40 hours, H3 had ca 10^3 colonies (∴ this agent may give a delayed recovery).

C. First count was done at ca 24 hours. "lac −" were marked for review at ca ~~24~~ 40 hours (10 A 20) 3 reproducible reversions from lac− to lac v.
This alters the means to 478/3 = 159 lac v : = ca 95%
20/3 = 7 lac− 5%.

started 10^{-5}

D. lac

	v (+)	−	Σ
1	63	106	
2	54	98	
3	64	87	
	181	291	
3	60	97	157

UV 20 secs. 2×10^{-5}

Comparative titer:

c)	159	7	166
d)	30	48	78

Killing: 47% survival
39% lac v.

Compare with D − Mal: 68/166 Mal v! Not greatly different.

E. 80 secs. 10^{-1} lac

	v (+)	−
1	15	72
2	8	72
3	16	75
4	17	106
5	19	90
	75	415
6	1	18
7	0	13

$\bar{m} = 15 : 83$,

killing 5.9×10^{-5} survivors

18% lac v.

10^{-2} } high!

UV 80s. lac + light.

10^{-4}:	29	54
10^{-5}:	3	9

Survival $= \frac{8.3}{166} = .05$ 35% v

80 (L) ≠ 45 (D) f. 387 disagreement!

Formaldehyde .105% 20 mins.

F. 6

	lac+	lac−
	22	45 / 67

Assay is 6.7×10^7
original was 1.6×10^9
Survival = $4.2 \times 10^{-2} \cong 5\%$
ca 33% Lac ±.

2/20 Repeat a, b expt.
Mix .05 ml M226 + .05 ml K-12 grown in parallel 20 h. D(lac) ass.
in 100 ml. (= 10^{-2} dil.)

			lac+	lac±	lac−
A.	10^{-5}	control	150, ~~[illegible]~~, 122	75, 59	8, 2
B	10^{-5}	20 sec uv	45, 62	9, 6	21, 23
C	10^{-4}	80 sec uv.	126, 144,	7, 7	23, 32

Control:	272 : 144	/ 416	K-12 % 65	or 1.9 : 1
uv 20	107 : 59	/ 166	" 65	1.8 : 1
uv 80	269 : 69	/ 338	" 79	3.9 : 1

i.e., ca 2-fold increase in proportion of K-12 over 4 decades of killing!

Dear Josh,

Here's another batch - I don't have too much hope for this run.

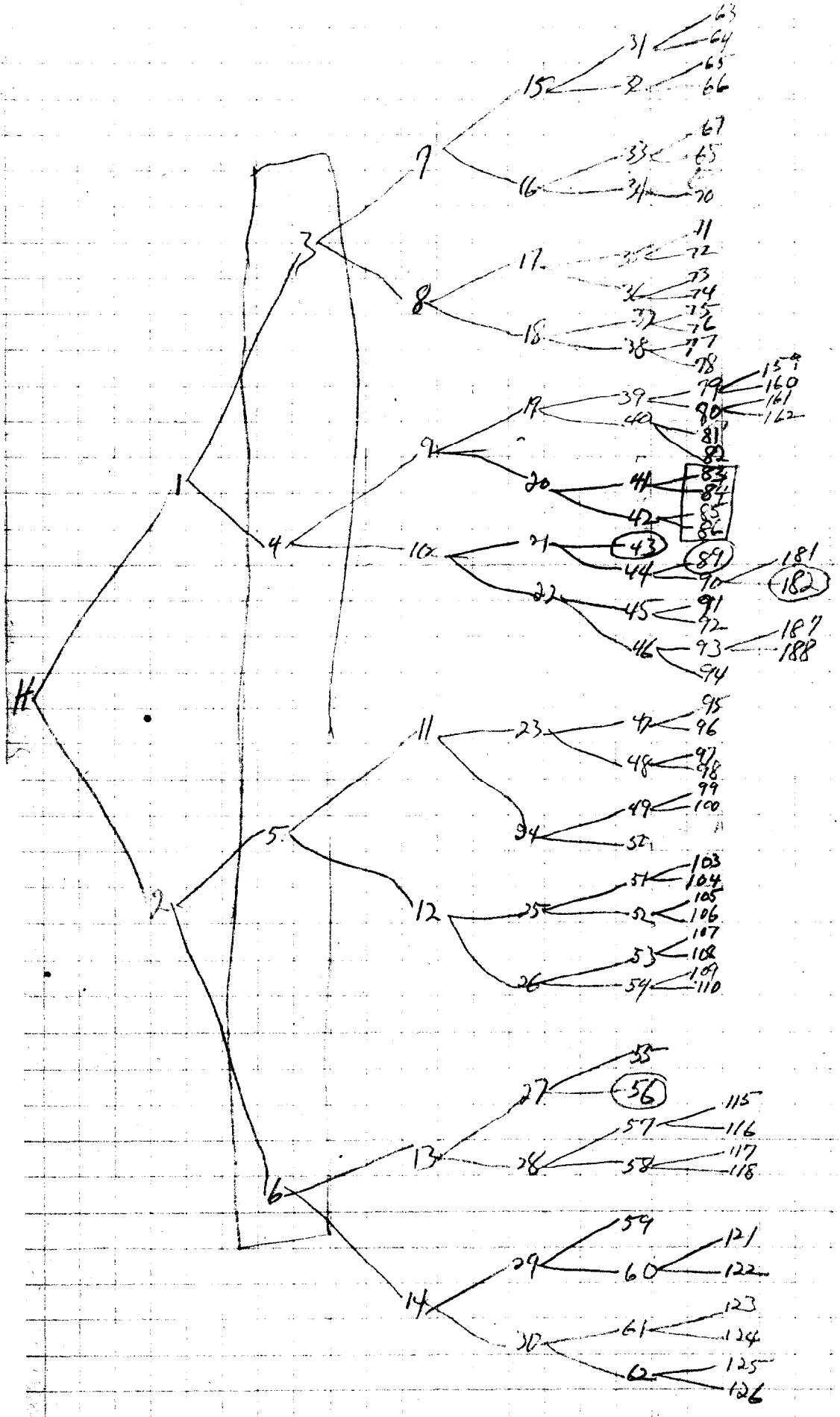
Ltr 8-5-50 pers was interesting - am anxious to hear the details.

Also anxious to learn your reaction to Oak Ridge. Please excuse me if I overstepped propriety in my last letter. Am not going to not send it but didn't get around to writing another one.

Am planning another session this Wednesday (Holiday for L. Work.) + another for the weekend. So be looking for a couple of more batches soon.

WWH

H 116 and H123 failed to grow in both tubes.

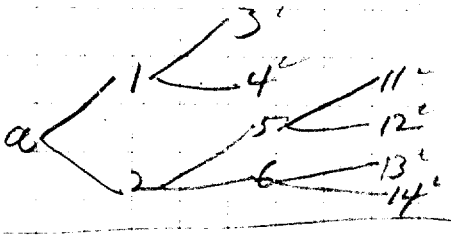


all var - Mab

2-18-50 H226 zygote

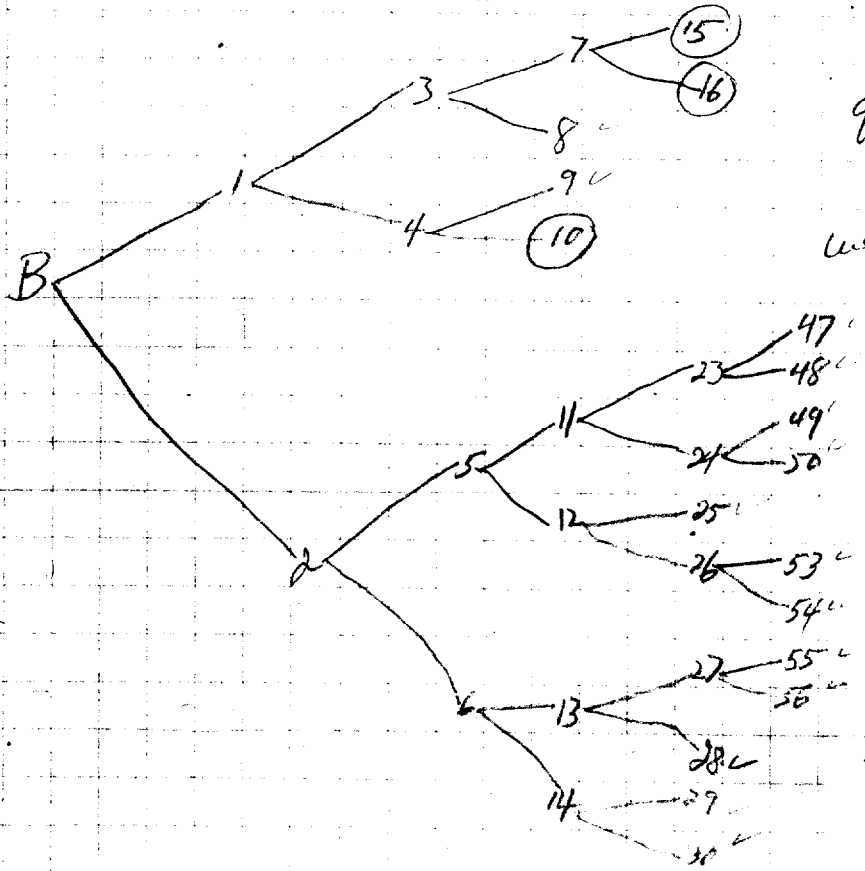
○ = dichorionic

⊓ = Unknown chorionicity



cells tended to become fibroblastic - stopped because of an engagement.

all het

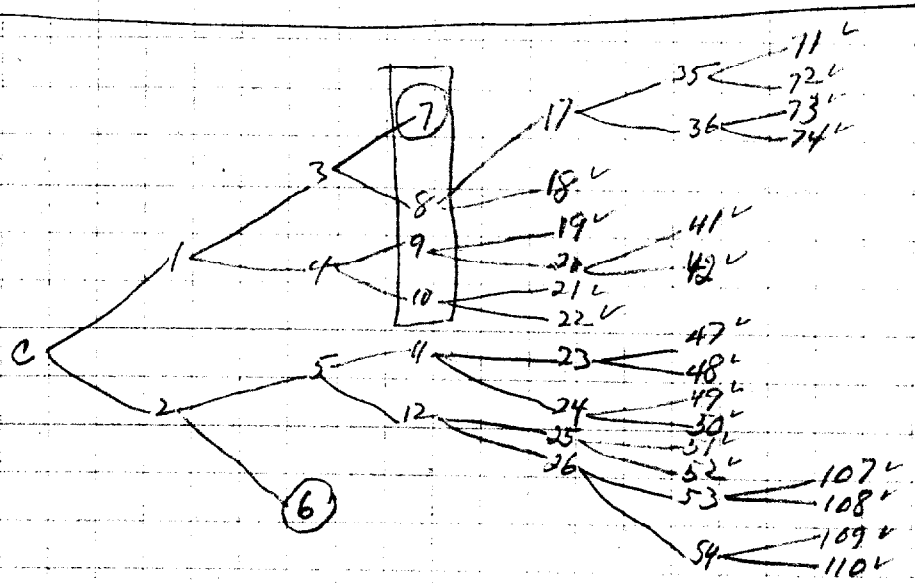


all 3 was a fetus cut, then split off small cell at each end

—— to 15, 8, 16

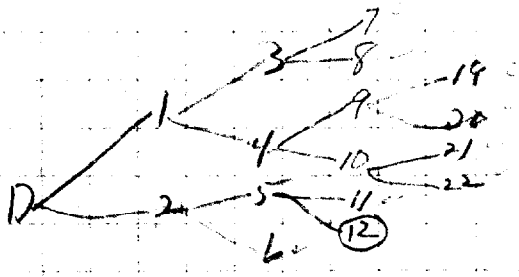
use arbitrary numbers as indicated

all het

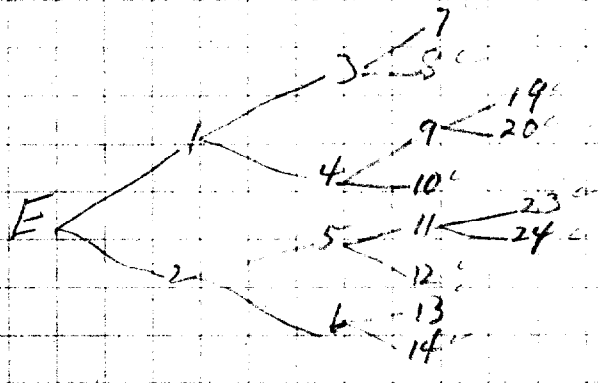


all these micromeres were [illegible] round, clear, "Lipoid" (i.e. hard to pick up in pipette) like those of clone 6 2-5-50

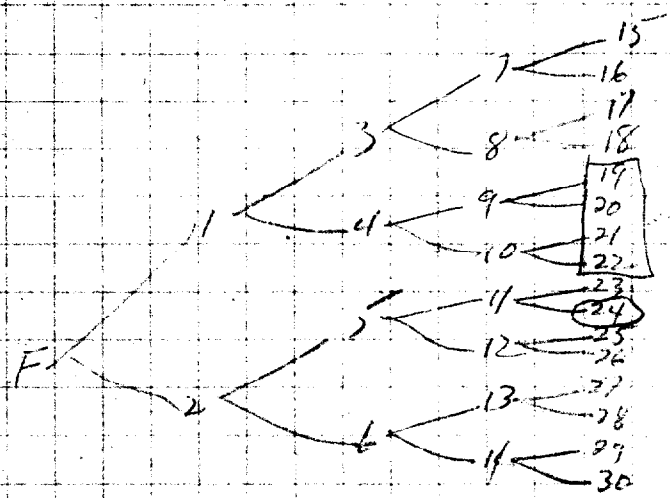
L-M



all Lac+



all Mal+

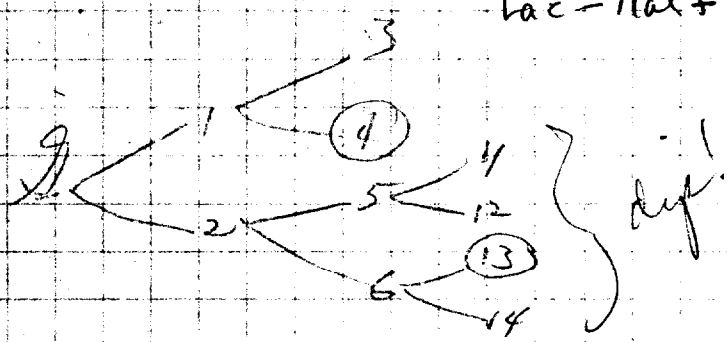


Cells in this clone were very sick from the start & would grow cells into a pregnant. Went along & took it for the Pellet set.

all Lac+ Mal+

F22 failed to grow in the broth tube due to breaking the tip off the micropipette.

Lac− Mal+



Platings of UV-M226 on EMS

690d

Febr. 20, 1950.

Platings on "EMS Mal" ~~probably~~ sterile. Medium probably ng.
EMS Lac:

Control C

+	−
154	0
136	0
146	0
436	0

in 145 + : 0 −

D

	+	−
	97	16
	126	25
	104	31
	109	34
4	436	106
	109	27

½ : 54 : 13

note absolute increase in Lac−, simulating "mutations".

E) Many mosaics, making counts difficult ca 20-25% Lac−.

Pick clean Lac− from D and streak out on EMB Mal.

690 res.

	K-12	H226		
	lac+	lacV	lac−	V+−
A)	104	29	11	40
	81	31	15	46
	97	29	19	48
	282	89	45	134

282	134	416
259	93	352

B	68	6	19	25
	109	7	24	31
	82	14	23	37
	259	27	66	93

not sign. different.
31% 35%

---

		lacV	lac−	
C Assay				
$10^{-2} \times 10^{-5}$		164	9	7
		161	7	6
		160	7	6
		475	23	20
	m	158	8	7

D av 20

defer counting EMS Lac
many lacV not yet defined!

1+ noted – strain not as possible balanced lethal type
⇒ gave lacV and lac− only.

	EMS Mal	+	V	−
		58	68	40 / 166

E

defer counting

F ca $10^3 \times$ E ca 10%

G

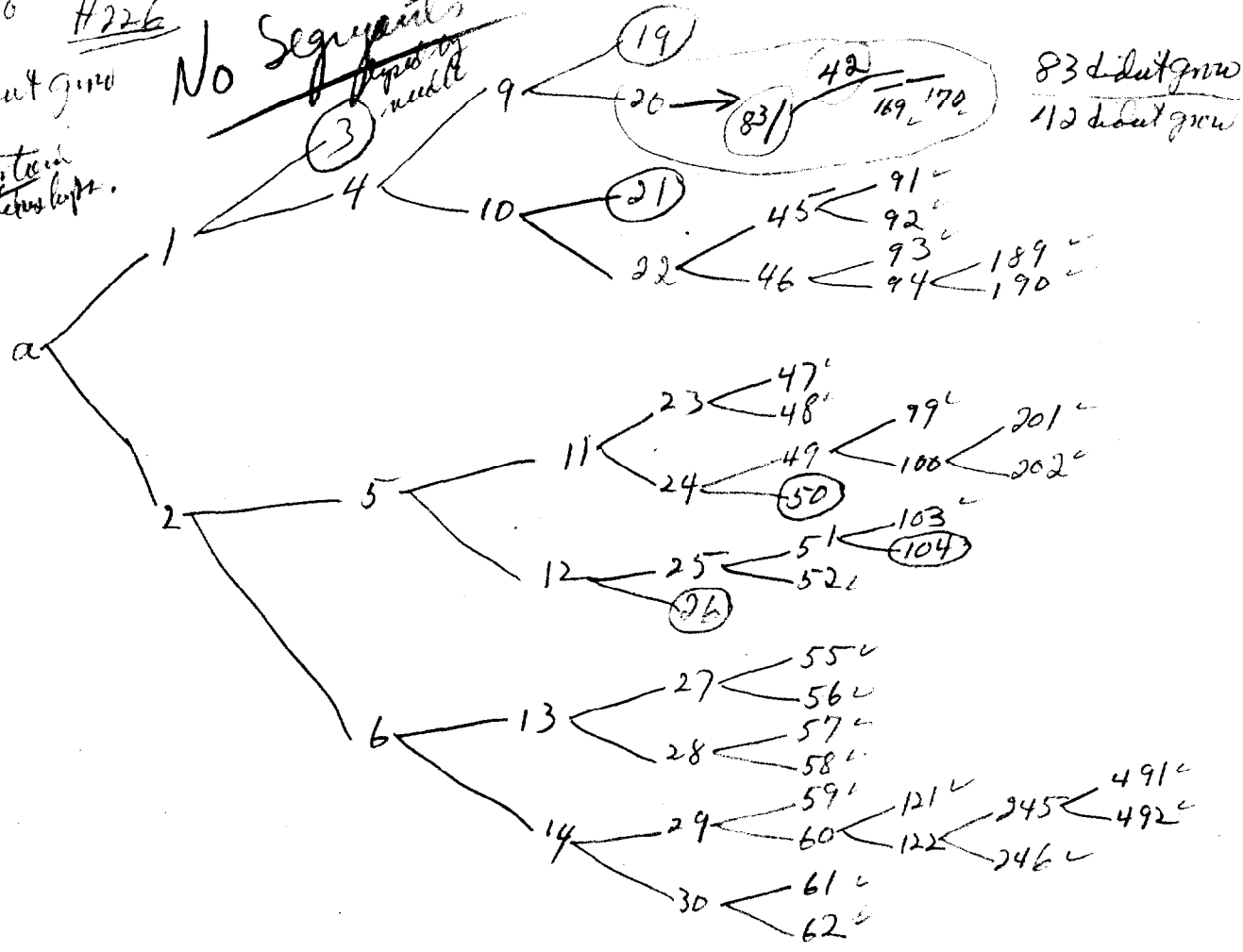
2-21-56 #226

No Segment Appeared

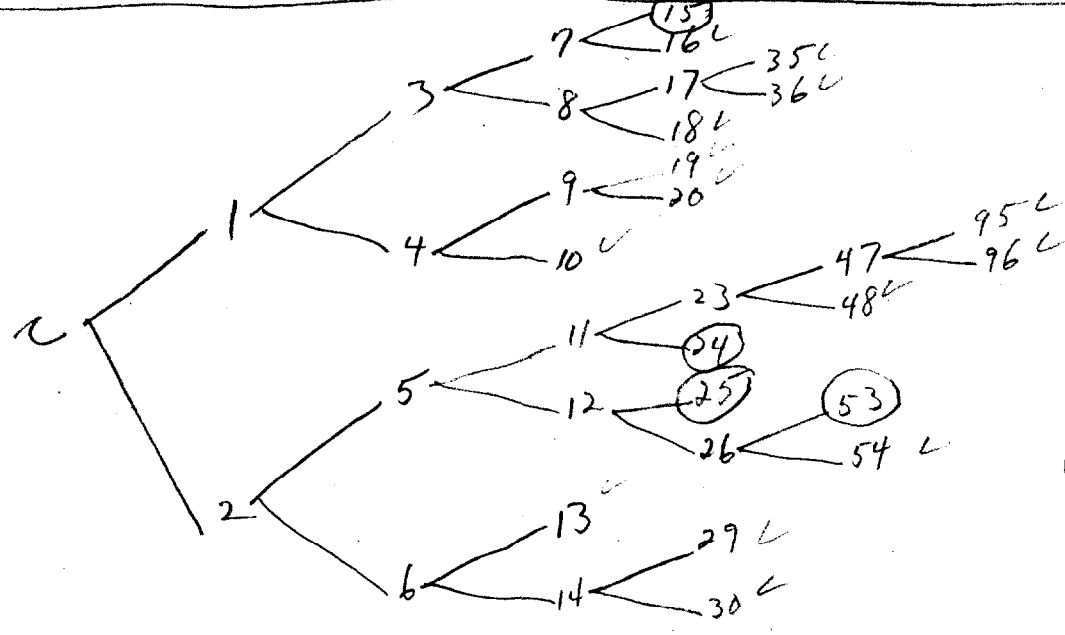
○ dichaetogrow

[ ] uncertain relationships.

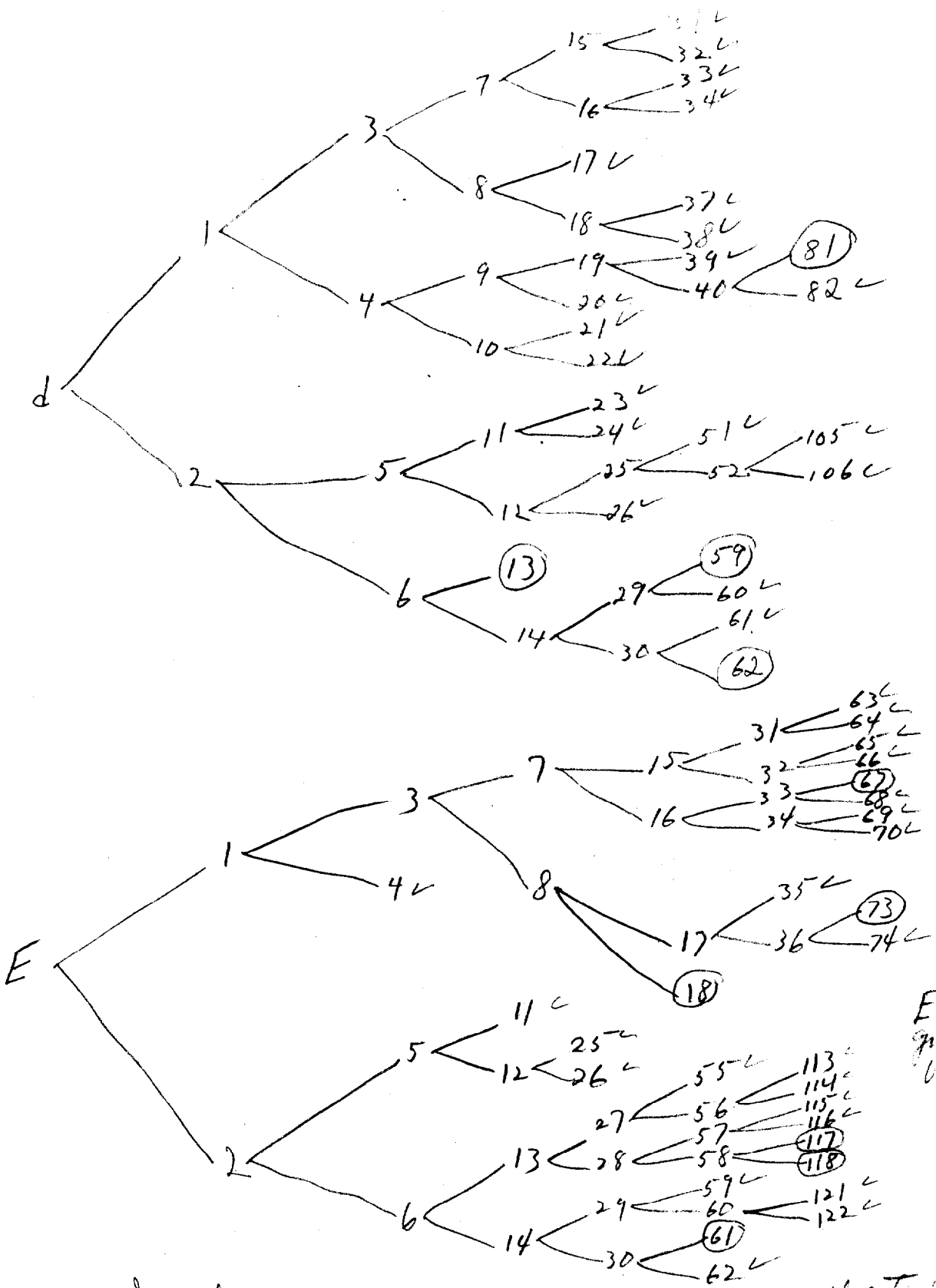
83 dichaetogrow
112 dichaetogrow



a 20 was a long filament, split off a cell at each end one of which divided. Quite arbitrarily numbered them as indicated above.



[redacted]



E65 did not grow in the broth vials.

I made an error in my records & had E2 dividing to give E4+E5 Luckily the actual E4 was not subdivided so I could correctly remember the vials with no possible doubt.

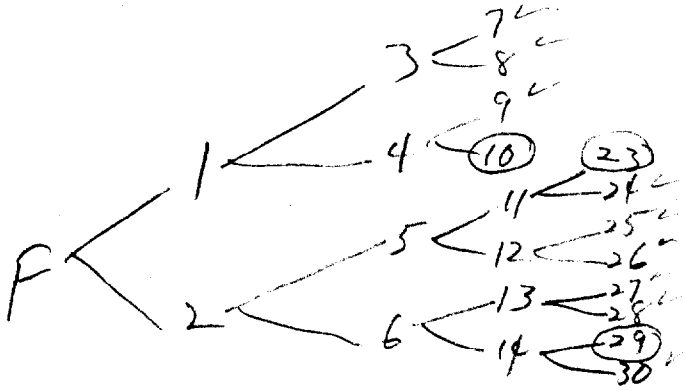
Josh,

I'm still planning another batch for Friday - I don't have too many hopes for the batch either.

The cultures tend to form filaments + then split off cells on the ends. I'm trying to keep records of all filamentous cells + sometimes rather arbitrarily number progeny of filamentous cells indicating how they were numbered.

This is one of the hardest cultures to work yet - has a somewhat longer log period and fewer cells from young EMS calluses actually grow.

MM


F
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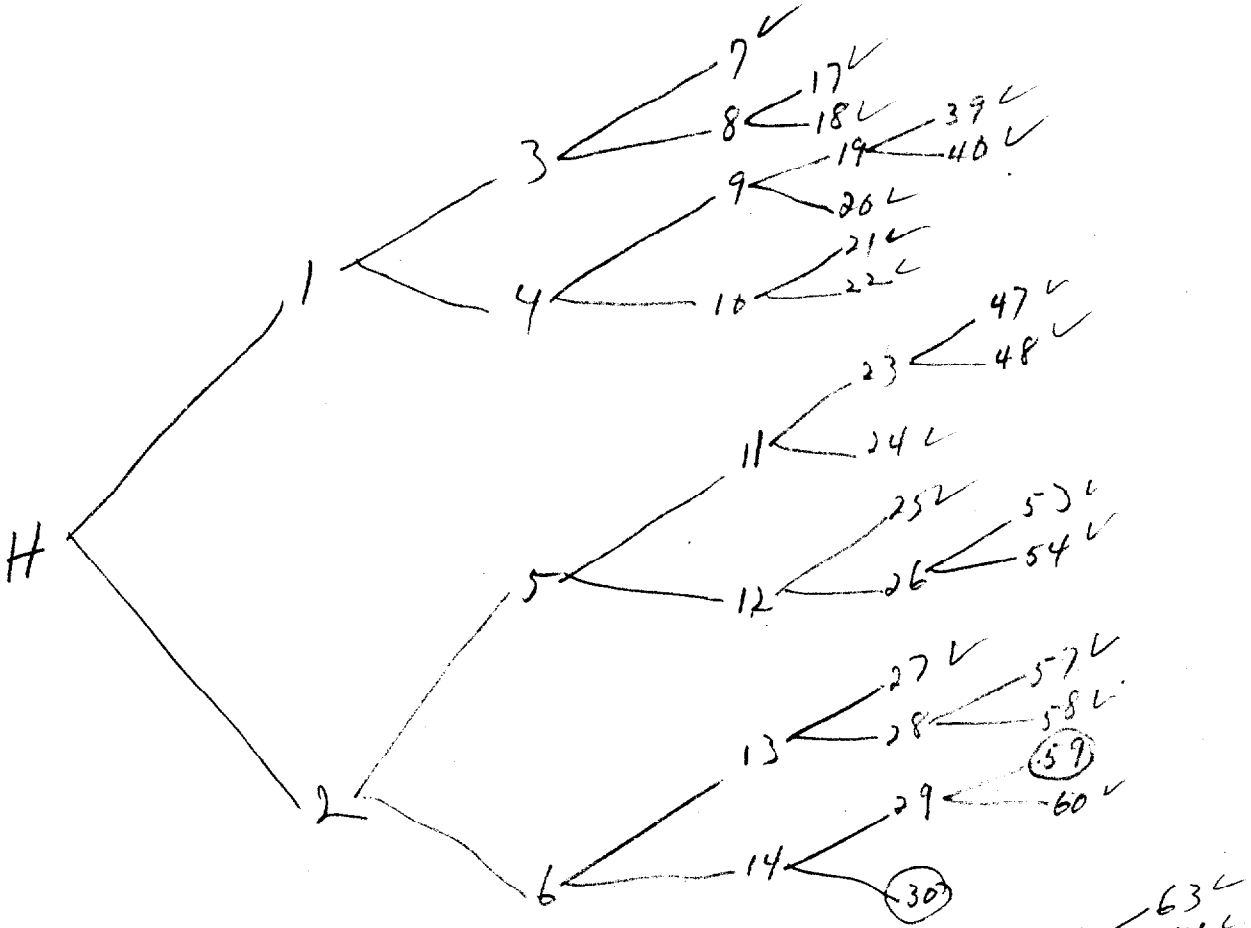
H
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J
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Segregations from H-226

2/18/50

	Lac	Mal	Xyl	Mtl
1		+	+	+
2		+	+	+
3		+	+	+
4		+	+	+
5		+	+	+
6		+	+	+
7		+	+	+
8		−	−	−
9		+	+	+
10		6 +	+ +	+ −
11		+	+	+
12		+	+	+
13		+	+	+
14		+	+	+
15		+	+	+
16		+	+	+
17		+	+	+
18		+	+	+
19		+	+	+
20		−	−	−
21		+	+	+
22		+	+	+
23		+	+	+
24		+	+	+
25		−	−	−
26		+	+	+
2	27	− +	−	+ + ?
2a	28	+ +	+	+ +
3	29	− +	+	+ +
4	30	+ +	−	+ + ?
31		+	+	+
32		+	+	+
33		+	+	+
34		+	+	+
35	− +	+	+	+
36		+	+	+
37		+	+	+
38		+	+	+
39		+	+	+
40		+	+	+
41		+	+	+
42	− +	+	+	+
43		+	+	+
44		+	+	+
45	− +	+	+	+
46		+	+	+
47		+	+	+
48		+	+	+
49		+	+	+
50	− +	+	+	+

	Lac	Mal	Xyl	Mtl
	51	+	+	+
		+	+	+
		+	+	+
5		− +	−	+ + ✓
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
	60	+	+	+
	61 − +	+	+	+
		+	+	+
6		− +	−	0 +
	− +	+	+	+
		+	+	+
		+	+	+
		+	+	+
	− +	+	+	+
		+	+	+
		+	+	+
	71	+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
	81	+	+	+
7	− +	−	−	+ + ✓
		+	+	+
8		−	+	+
		+	0	+ +
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
	91	+	+	+
	− +	+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
	100	+	+	+

(over)

Single colonies of H226 on EMB Lac. 4/pl.
Single-colony chosen from each, and tested on
the sugars indicated. Results indicate strongly
the preponderance of Val+ segregants (Lac_4 - ??)

Formaldehyde – prolonged exposure.

H_2O_2

2/19/50.

A Formaldehyde 8.01 → 8.37 as 690 G. Use suspension of H226 2/19. hold! Sterile!

B H_2O_2 Expose a 10^{-2} dilution of H226 to decimal stage conc. of H_2O_2 beginning with 30% 1:10. 10ml H_2O + .5ml D(-) + .1ml susp. After 15 m. take .1ml samples and spread on EMB lac.

C) Ac_2O H226 1ml + 1ml $CaCO_3$ 10% + 0.1ml 10% Ac_2O/ETOH.

At 10^{-4}, 198 lac – 0 lac v or +! $2 \times 10^6 = 10^{-3}$ survival.

10^{-3} → >1000 lac – 10^{-2} 7 colonies only!

D) assay

10^{-7}: lac v 146, lac – 12 / 156

		% H_2O_2	
	1	3	Sterile
	2	0.3	Sterile
B →	3	0.03	Sterile near center; crowded at periphery. (lac – around center.)
	4	0.003	Crowded!
	5	0.0003	Crowded!

Higher than .03% bacteriostatic on plate. [Should use catalase.]

A) P2 | 3 same plate

		lac v	lac –
6		3	51
A22		27	53
5		13	—
A22		>>13	

Survival = $\frac{5.4 \times 10^7}{1.6 \times 10^9} = 3 \times 10^{-2}$

~~Probably 6% diploid.~~

Suggestion! Do prolonged doses of chemicals differ from UV in permitting a much lower proportion of diploid survivors, conforming to nuclear elimination theory of killing.

Segregants of H226

Febr. 20, 1950.

B. 1:30 Incor. H226 1:10 in Penassay; aerate to 7:10 (6 hours). Plate out on EMB Mal. → But mostly still Lac v! (ca 80%).

A. Streak out single Lac± colonies of H226 (690C) on EMB Mal. Pick single Mal+, Mal− colonies to nmb and spot on EMB Lac, Mal, Xyl, MH.

2/21 (A) 57 Mal− : all Lac− MH− Xyl−

62 Mal+ : 52 Lac+(v), MH+ Xyl+ ... 52: Lac+ MH+ Xyl+

3 Lac− MH− Xyl−

5 Lac− MH− Xyl+

All above tested: V_1^R. [Do H226 V_1^R/V_1^S?]

But segregants are preponderantly parental combinations.

[Tests of phage resistance; mutations are ruled].

(B) Mal− picked (by M.O.) to Mal, Lac, MH, Xyl EMB:

	Lac	Mal	Xyl	MH	L	M	X	MH	L	M	X	MH	L	M	X	MH
1	−		−	−	11 ±	−	+	±	21	−	±	±	±	31	+ ✓	−	+	+
2	−	−	−	−	12 ✓	−	+	+	− ✓	−	−		− ✓	−	+	+
3	±	±	−+	−	13 ±	±	±	±	±	−	+	+	±	±	−	±
4	±	±	±	±	14 ±	±	±	±	±	±	±	±	±	±	±	±
5	±	±	±	±	15 ✓	−	−	−	○ −	±	±	±	−✓	−	+	+
6	−	±	±	±	16 ✓	−	−	−	○ ± ✓	−	+	±	−+	±	−	−
7	−	±	±	±	17 ±	±	±	±	− ✓	−	−	−	±	−	±	+
8	−	−	−	−	18 ✓	−	−	−	−+	±	±	±	−✓	−	−	−
9	−	−	−	−	M ✓	−	−	−	−+	±	−+	●	○ −	±	−	−+
10					20 ✓	−	−	−	30 ±✓	−	+	+	40 −✓	−	−	−
					2 ✓	−	−	−	±	±	+	+	−✓	−	+	+
					6 ✓	−	+	+								

Most of these are poor selections. Investigate the Lac−Mal discrepancies!

But probable pure ✓ shows 6 ## L−M−X+MH+ ; #14 L−M−X−MH− This is a high proportion of M−X+ compared to 693A, but may be due to previous prototroph selection.

Investigate ○. Pick from Mal or Lac

Partial segregations

March 4, 1950.

In older cultures of H226, a number of "partial segregants" have been isolated. See also 687: 9 Mal$\underline{v}$ Lac− were picked up following irradiation. Later, the same was found in ~~2/22~~ control cultures.

693 (2/22) 4 Lac$\underline{v}$ Mal− and 1 possible Lac−Mal$\underline{v}$ isolated from EMB.

3/1

c. See 700. Plating of H226 after growth on EMB Mal.
10 Mal$\underline{v}$ (?) picked. Streak on EMB Mal, Lac.
all were Lac$\underline{v}$ Mal$\underline{v}$.

D. See 700 A1. On EMS Lac, Mal. ≪1% −. Repick and purify on same medium for subsequent testing.
Mal− : 8 ; 3 Lac$\underline{v}$. #2, 3, 8. = ~~700 11, 12, 13~~ 699: 17, 18, 19
Lac− : 14 2 Mal− ; 12 Mal+ No Mal$\underline{v}$.

Segregation of H226
Partial segregation?

2/22/50.

See 693 (B).

1-4 from Lac (693B: 11, 23, 26, 37); 5-9 from Mal (693B: 6, 7, 21, 25, 35).
Streak out on EMB Mal, Lac; EMS Mal, Lac.

	Lac EMB	Mal EMB	EMS Lac	EMS Mal
1	Lac v; Lac −	Pure Mal −	+	−
2	Lac v; Lac −	Pure Mal −	+	−
3	Lac v; Lac −	Pure Mal −	+	−
4	Lac v; Lac −	Pure Mal −	+	−
5	Lac −	Mal +; Mal − v?	n.g.	n.g.
6	Lac −; Lac + (v?)	"	+	+
7	Lac −	"	n.g.	n.g.
8	Lac −	Mal + − v?	n.g.	n.g.
9	Lac −	Mal − v.?	+	n.g.

These isolations leave no doubt as to the occurrence of Lac v; Mal − types. How do they arise? They would represent a persistence of the 2 stage retention noticed by Zelle. A Mal v Lac − has also been picked up.

H226 partial segregation.

6936

2/24

Grow H226 1:100 to saturation in Penassay (aerated)

2/27 Plate at 10^{-5} on EMS, EMB Mal and Lac.

EMS: ca 100 prototrophs ++, +, and −

EMB: Turbid!

2/28 Plate at 10^{-7}(?) on EMB.

Lac EMB: ca 10% deploid; remainder all Lac −

Mal EMB: mostly Mal+; a few Mal−, Mal ⩒.

A. Pick − from Mal EMS. Brush on Mal EMS, streak on Lac, Mal EMB

B. − Lac " Mal EMS " " "

C. Pick Lac ⩒ from Lac EMB. Streak on Mal EMB; save suspensions.

D Pick Mal ⩒ from Mal EMB. ~~Streak~~ Brush on Lac EMB. ≡ only 2 scorable at this time! both Lac ⩒.

Sept 68

~~2~~ + 3/1

A. 11 "Mal-" from EMS.

	Lac	Mal
1	V	−+
2	+−	−+
3	V	−+
4	V	−+
5	V	−+
6	−	−+
7	V	V−
8	V	−+
9	V	−+
10	V	−+
11	V	−+

The frequent occurrence of (Mal− Lac V) among Mal− prototrophs is indubitable. The residue of Mal+ papillae is not explained. In same case a single colony probably contains Mal− and Mal V, Lac V.

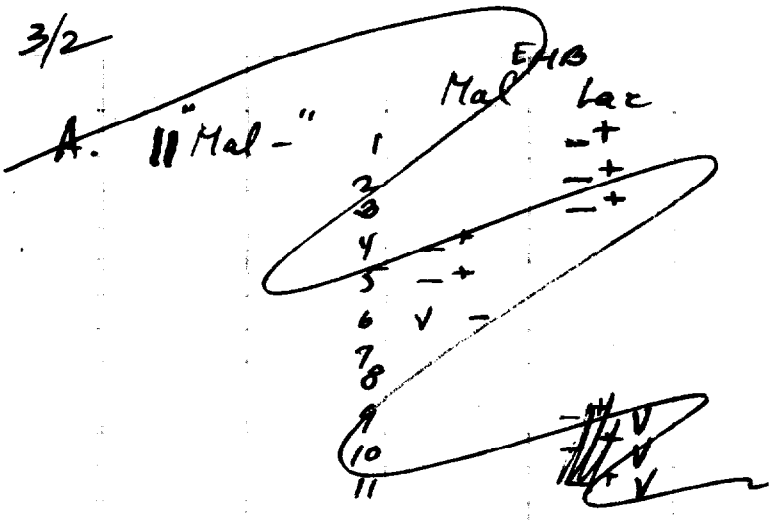
B. 38 "Lac−" from EMS

1	−	+
2	−+	+
3	−	+
4	−+	+
5	−+	+
6	−	+
7	−+	V
8	−	+
9	−+	+
10	−	+
11	−	+
12	−+	+
13	−	+
14	−	+
15	−+	+
16	−	+
17	−+	+
18	−	+
19	−+	+
20	−+	+
21	−+	+
22	−	+
23	−	+
24	−+	+
25	−+	+
26	−	+
27	−	+
28	−	+
29	−	+
30	−	+
31	−	+
32	−	+
33	−	+
34	−+	+

A single essentially Lac− Mal V culture has been recovered here. Apparently, most prototrophic segregants are Lac− Mal+.

	L	17
35	−+	+
36	−	+
37	−	+
38	−	+

3/2



C. Lac v to Mal.

1	V	11	V	21	V	31	V	41	V
2	V	12	V	22	V		V		V
3	V	13	V	23	V		V		V
4	V	14	V	24	V		V		V
5	V	15	V	25	V		V		V
6	V	16	V	26	V		V		V
7	V	17	V	27	V		+		V
8	V	18	V	28	V		+-		V
9	V	19	V	29	V		+-V		V
10	V	20	V	30	V	40	V	50	V

Not well streaked out. The expt. should be repeated.

∴ No Lac V Mal- found in this sample.

Photorecovery of uv effects

2/20/50.

See 690 for set-up. Irradiate 2/19 H226 10^{-5} 5 seconds.

A) No uv

B) uv 5sec.

C) uv 5sec + light 110 mins. (temperature not well controlled)

C: no survivors.

Count A + B at 36 h.

	Lac v	Lac -	
(A)	63	16	!
	91	30	
	74	18	
	228	64	
(B)	65	31	
	79	37	
	30	14	
	174	82	

Increased prop of ⊙ types.

Relatively little effect at 5 secs. (use 10 for following expts.)

2/22/50:

	Lac v	Lac -	
A:	114	9	
	134	2	
	124	6	
	372	17	/ 389
B	124	6	
	74	37	
1+	61	57	
	44	33	
	62	41	
	241	168	
	60	42	/ 102

C.

Lac v	Lac -	
69	10	
74	16	
91	25	
234	51	
78	17	/ 95

Tests for balanced lethals

2/21/50 ff.

Picks lac+ colonies from a variety of treatments to examine for balanced lethals. Streak out on EMB lac, and examine Lewis + for + lability. If any are more or less unstable, restreak. Emphasize isolate lac+ (so this frequency is exaggerated)

690D+ : was lac+.

690D: 28 colonies. 10 Rechecks

E. 17 cols. 10 Rechecks. 1 v- = 695-3

F. 12 cols. 4 (1a,b) Rechecks. 1 v- = 695-2

G. 4 cols. 0 checks.

692A 12 cols. 1 pure lac++ transferred to check. as 695-92A1. others lac+.

689B

Repick from first streaking if any colony not obviously sectoring is seen.

Retests for possible partial segregants

March 6-7, 1950.

			Lac	Mal	Mtl	Xyl
A.	1	= A 169		V	+ −	V
	2	A 189		++	+ −	V
=2/21/50	3	C 30		V	+ −	V
	4	E 116		V	O	∫ − , T
Series.	5	E 121		++ −	V	V
	6	H 17				
	7	A 15		++	V	V
2/24	8	B 21		V	V	++ −
	9	B 36 ✓		++	++	++
	10	C 25		V	V	V
B	11	D 12		V	V	V
	12	D 13 ✓		+(−)	++	++ , 1−
	13	H 22 ✓		++	++	++
	14	I 26		+ −	+ −	− +
	15	J 16		+ −	+ −	+ −
	16	K 16 ✓		++	++	++
	17	K 22		++ −	V	++ −
	18	L 13		++ −	V	++ −
	19	F 10		++	++	++
	20	F 12		++	++ −	++
	21	F 15		++	++	++
	22	F 16		++	++	++
	23	F 19		++ −	++ −	++ −
	24	F 24		++	++ −	++
	25	F 27		++ −	V	++ −
	26	F 28		++	V	++ −
	27	F 29		++ −	++	++ −
	28	F 30		++	++	++
	29	F 42		+ −	+ −	++ −
	30	F 47		+ −	+ −	++ −
	31	F 48		++	++	++

F series (19-31) is peculiar in Lac V Mal+ (very few − segregants). Reisolate and compare reisolated diploid with H226.

Of the others: 2/24: B36, D13, H22 and K16 warrant detailed attention. In 2/21, A189 and E121 should be reisolated.

EMB Mal, Lac.

EMB Lac

Zelle 2/18.

Most of these pedigrees are uninteresting.
However, keep B28, 55, 56; Series D; E 7, 8, 10, 20;
~~[redacted]~~ Series G;

Also keep 680: A15; A33
D: 15–22; 53; 27; 29
E 215–222; 108; 49; 52; 75, 76
G: entire pedigree

sel-
Lac - Mal -

Zelle - single cell pedigrees

Zelle 2/18

		Lac	Mal	
	7	+	+	Spur? Ausreißer: −−, V ++, V, −
	8	+	+	−− V ++, −
	10	+	+	
	12	+	+	
	13	+	+	
	14	+	+	
	19	+	+	
	20	+	+	
	21	+	+	
	24	+	+	
F	15	−	+	
	16	−	+	
	17	−	+	
	18	−	+	
	19	−	+	
	20	−	+	
	21	−	+	
	22	−	+	
	23	−	+	
	26	−	+	
	27	−	+	
	25	−	+	
	28	−	+	
	29	−	+	
G	3	−	+	
	11	+	±	
	15	+	±	
	17	+	±	
H	5			
	6			
	66			
	68			
	67			
	62			
	69			
	71			
	72			
C		all Lac −	all Mal −	

Zelle 2/18

		Lac	Mal	
A	3	+	±	all het
	4	+	±	
	11	+	±	
	12	+	±	
	13	+	±	
	14	+	±	
B	8	+	±	all het
	9	+	±	
	25	+	±	
	26	+	±	
	29	+	±	
	30	+	±	
	47	+	±	
	48	+	±	
	49	+	±	
	52	+	±	
	53	+	±	
	54	+	±	
	55	+	±	
	56	+	−, +	Probably Lac c/c
C	18	−	−	mut
	19	−	−	
	21	−	−	
	22	−	−	
	23	−	−	
	24	−	−	
	27	−	−	
	28	−	−	
	31	−	−	
	32	−	−	
	33	−	−	
	34	−	−	
	35	−	−	
	36	−	−	
	37	−	−	
	38	−	−	
	39	−	−	
	40	−	−	
	41	−	−	
	42	−	−	
	43	−	−	
	44	−	−	
	45	−	−	
	46	−	−	
	109	−	−	
	110	−	−	
D	6	+	+, − ? (pseudo)	Both are −, v; and Mal +, −.
	7	+	+	
	8	+	±	
	11	+	±	
	19	+	±	
	20	+	±	
	21	+	±	
	22	+	±	

3/1 3.2/24

		Lac	Mal	Xyl	Mtl
I	17	+	±	+	±
	18	+	±	+	±
	19	+	±	+	±
—	20	+	±	±	±
	21	+	±	±	±
	22	+	±	±	±
	24	+	±	±	±
	25	+	±	±	±
	26	+	±	±	±
	27	+	±	±	±
	28	+	±	±	±
	31	+	±	±	±
	32	+	±	±	±
	33	+	±	±	±
—	34	+	±	±	±
	47	+	±	±	±
	48	+	±	±	±
	59	+	±	±	±
	60	+	±	+	±
	61	+	±	±	±
	62	+	±	±	±
J	14	+	±	±	±
	15	+	±	±	+
—	16	+	±	±	+
	17	+	±	±	±
	18	+	±	±	±
	19	+	±	±	±
	20	+	±	±	±
	21	+	±	± ?	±
	22	+	±	±	±
	23	+	±	±	±
	24	+	±	±	±
	25	+	±	±	±
	26	+	±	±	±
	27	+	±	±	±
	28	+	±	±	±
K	5	−+? Rev	± Rev	−+	±
	15	+	±	−+	±
	16	+	+	+	+
	18	−	−	−	−
	19	+	±	±	±
	20	+	±	±	±
	21	+	±	±	±
	22	+	±	±	±
—	29	+±+	±+	−+	−+
	55	+	±	±	±
	56	+	±	±	±
	57	+	±	±	±
	58	+	±	±	±

L	Lac	Mal	Xyl	Mtl
2	±	±	±	±
13	+	+	±	±
14	−	−	−	−
23	+	±	±	±
24	+	±	±	±
25	+	±	±	±

Restreak.

Mal A15, [illegible] H22, I26, K16, K22, L13

Xyl A9, A15, F, B21, B36, K22, H22, D12, 13, K16,

Mtl F H22

Restreak small sugars:

A15, B36, B21, C25, D12, D13, F −,

H22, I26, J16, K16, K22, L13

K5 K18 L13, 14.

Lacv Malv

[SIB IS LETHAL.]

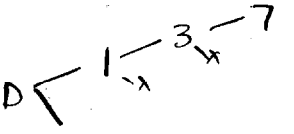
Lacv Malv

32/24

H226

		Lac	Mal	Xyl	MHl
C	61	+	+ ±	±	±
	62	+	+ ±	±	±
D	7	+	+ −+	−+	±
*	12	+	+ ±	+	±
	13	+	+ ±	+	++
	23	+	+ ±	+	±
	29	+	+ ±	+	+
	30	—	+ +	+	+
F	10	+	++ ++	+	+
	12	+	++ 0	±	+
	15	+	++ +	+	+
	16	+	++ +	+	+
	19	+	++ ±	+	±?
	24	+	++ +	+	±?
	27	+	++ 0	+	+
	28	+	++ +	+	+
	29	+	++ 0	+	+
	30	+	++ +	+	+
	42	+	++ +	+	+
	47	+	++ +	+	+
	48	+	++ +	+	+
G	10	+	0	±	±
	13	+	±	+	±
	15	+	±	+	±
	16	—	—	—	0
	17	+	0	+	±
	19	+	0	+	±
	20	+	±	±	±
	24	+	±	±	±
	25	+	±	±	±
	26	+	±	±	±
	29	+	±	±	±
	30	+	0	±	±
	38	+	±	±	±
	47	+	±	±	±
	48	+	±	±	±
	76	+	±	±	±
H	8	+	±	±	±
	16	+	±	±	±
	20	+	±	±	±
	21	+	±	±	±
	22	+	+	+	+
	23	+	±	±	±
	31	+	±	±	0
	32	+	±	±	±
	39	+	±	±	0
	40	+	±	±	±
	50	+	±	±	±

Double



D29, 30, 73.

No Segregants but save F10.

G15, 16

3/1 J. 2/24 Pedigree H226 Zelle

		Lac	Mal		Xyl	MM
A	9	+	±	±	+	±
	13	+	±	0	+	±
	15	+	++	+	+	±
	16	+	++	±	+	±
	18	+	++	±	+	±
	21 ~~23~~	+	±	±	±	±
	23 ~~24~~	+	±	±	±	±
	24 ~~42~~	+	±	±	±	±
	26	+	±	±	±	±
	29	±	±	±	±	±
	30	+	±	±	±	±
	35	+	±	±	±	±
	36	+	±	±	±	±
	46	+	±	±	±	±
	51	+	±	±	±	±
	52	+	±	±	±	±
B	15	+	±	±	±	±
	16	+	±	±	±	±
	18	+	±	±	±	±
	19	+	±	±	—	±
	20	+	±	±	±	±
	21	+	±	±	+	±
	22	+	±	±	±	±
	28	—	—	—	—	—
	29	+	±	0	— +?	±+
	30	—	—	—	—	—
	35	+	±	±	±	±
	36	+	+	±	+	+
	47	+	±	±	±	±
	48	+	±	±	±	±
	49	+	±	±	±	±
	60	+	±	±	±	±
C	15	+	+	±	±	±
	17	+	+	±	±	±
	18	+	+	±	±	±
	19	+	+	±	±	±
	20	+	+	±	±	±
	22	+	+	0	±	±
	23	+	+	±	±	±
	24	±	±	±	±	±
*	25	+	+	±	+	±
	26	+	+	±	±	±
	27	+	↓	±	±	±
	28	+	+	±	±	±
	33	+	+	0	±	±
	34	+	+	±	±	±
	59	+	+	±	±	±
	60	+	+	±	±	±

Relationships uncertain.

Detailed tests on single cell segregants

March 7, 1950

696 A.

A189 } Streaker
E121 } EMB Mal, Lac. Both are mostly segregated Lac−, Mal+ but show some Lac ✓ and Mal ✓ colonies.

696 B.

	Lac EMB	Mal EMB	Revelates (Lac+ [EMS])
B36	mostly −	++	✓
D13	mostly −	++	✓
H22	"	++	✓
K16	"	++	+ ✓ −

	Recover from EMS Lac or EMB Lac		Mal
Fsmic	"	"	
10	− +	++ −	++ ✓ −
12	− +	++ −	+ ✓ −
15	− +	+ + −	✓ ✓
16	− +	++ −	✓
19	− +	− +	✓
24	− +	++ −	✓
27	− +	++ −	+ ✓ −
28	− +	++ −	✓
29	− +	++ −	✓
30	− +	++ −	✓
42	− +	++ −	+ ✓ −
47	−, +	++ −	✓
48	−, +	++ −	+ ✓ −

Restreak Lac+ from EMB Lac where available.

Fsmic may have a higher proportion of Mal+, but this is doubtful.

Keep F10

Feb. 24, 1950.

M226 (2/24) brought to Chicago (Rest Radiobiology and Bio physics).
15 ml in small crystallizing dish, shaken gently, exposed to unfiltered X-radiation. 2 ml samples removed at intervals of 1 min., 10 min., 20 m., 30 m and 40 min. 1 minute = 942 r [(81; 76)]
Plate at various dilutions on EMB Lac. Plates carried from Site B to Site A in cold weather: temperature shock should be considered.
~~Other~~ Residue of aliquots stored in refrigerator in screw vials.

X-ray effects

2/27-28/50.

Material of 4 of C. X-ray expt. mint No. ___. 40 minute (ca 40,000 r) H2x

2/27. Plate 10^{-5} and 10^{-4} on EMS. 10^{-5} on EMB.

EMB Lac

Lac v	Lac -
0	13
8	14 -
1	8
1	12
10	47

Mal:

+	-	v
2	5	5
4	0	5
1	4	4
1	4	1
6	5	1
14	18	16

EMS Lac Mold!

2/28/50.

10^{-4} (4 x 40 - 3)

Lac

v (+)	-
13	115
17	126
30	241

Mal 10^{-5}

+	-	v
67	42	55
5	11	7
5	1	3
3	1	5
2	4	4
3	6	4

Mal is difficult to count, but separation of high Mal+/- colonies is clear.

A) Picks clear Mal+ colonies and streaks on Lac, Mal EMB; ~~Lac~~ Mal EMS

B) Possible Lac+ were picked in Chicago for streaking out in Madison. By error, different doses were not separated. 7: 10,000 r. 9: 20,000 34: 30,000 4: 40,000 r

A:

	Lac	Mal
1	—	+-
2	+-	v
3	—	+-
4	—	+-
5	—	v?
6	—	+-
7	—	+-
8	—	+-
17	—	+-
18	—	+—

	Lac	Mal
9	—	+—
10	—	+-
11	—	v?
12	—	+-
13	—	+-
14	—	+-
15	+-	+v-
16	+-	+v-
19	—	+—
20	—	+-

B: 6 cultures were scored as stable Lac+ after two restreakings. They do not appear to be typical Lac+ Lac+ haploids. Store on slants.

3/3/50

C. Rels 40 Lac - (no apparent +) colonies from plating of EMBLac 40 min (≐ 40,000 r) X-ray H726. Streak on EMB Mal, and brush on EMS Mal.

	EMB, Mal						
1	+	11	+	21	+	31	−
2	−	12	+		−		−
3	+	13	+		−		−
4	−	14	+ −		+		−
5	+	15	+ −		−		−
6	+	16	−		+		+ −
7	+	17	−		+		−
8	+	18	−		+ −		−
9	+	19	−		+		−
10	−	20	+	30	+	40	+

Mal
Mal
Mal
Mal
Mal
Mal
Mal
Mal
Mal
Mal

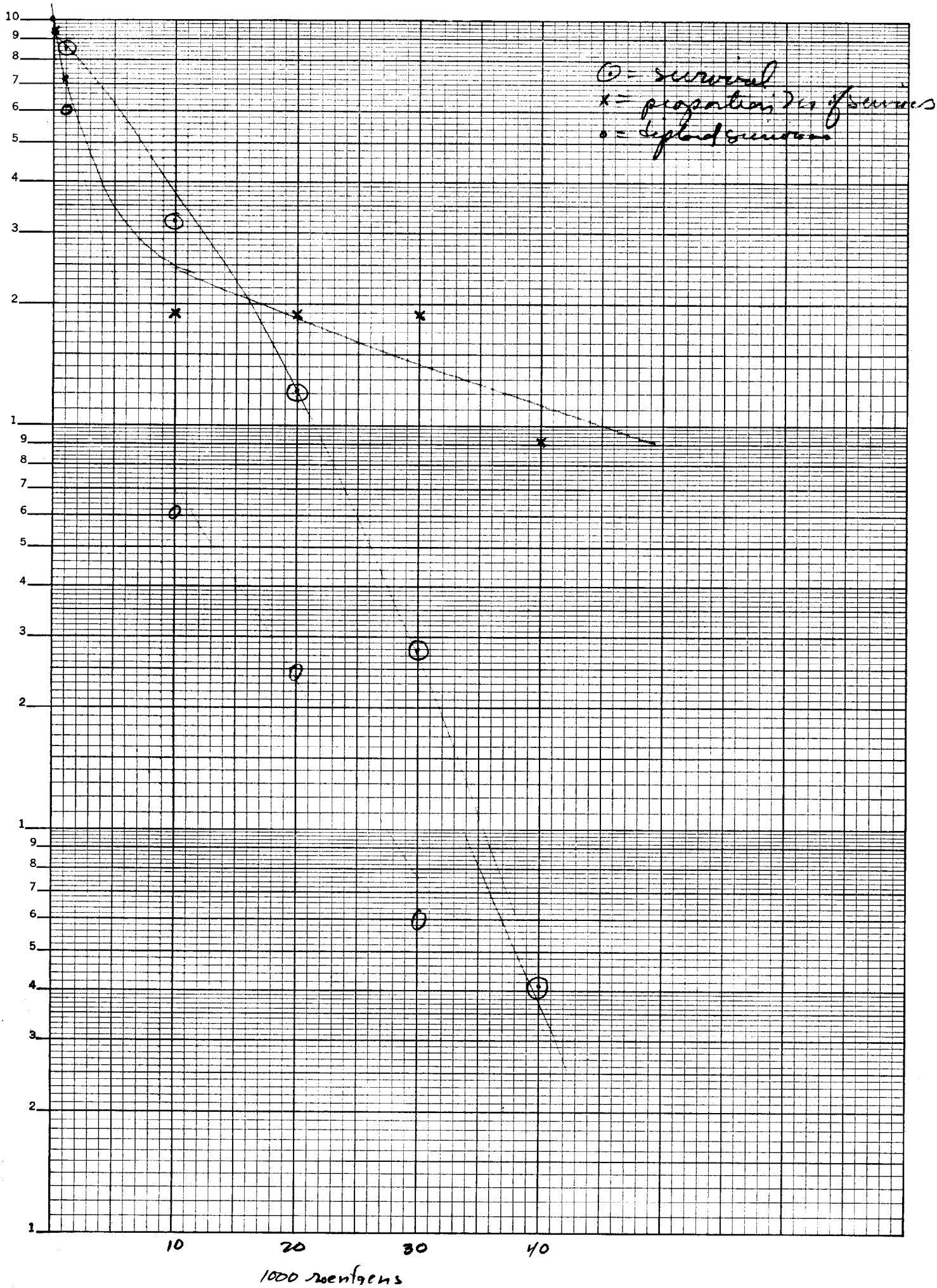
TC.

ca 10% of Lac - are Mal? +/-

A+

	Lac v	Lac −				% v
$0-10^{-7}$	108	7				94
	111	8				
	119	4				
	107	7				
	445	26	471	118	118	94
1 min X-Ray ≐ 1000 r. 10^{-7}	74	29				
	68	29				
	80	23				
	74	30				
	296	111	407	102	102	72
10 min. 2×10^{-7}	17	67				
	15	59				
	17	71				
	11	57				
	60	254	314	39	39	19
20 min 10^{-6}	19	112				
	30	102				
	27	121				
	34	140				
	110	475	585	~~85~~		↑
			146	1,46	14.6	
30 min 1×10^{-5}	75	610	685	.86		19
					8.6	19
40 min. 10^{-5}	9	73				
	1	31				
	3	37				
	3	36				
	18	177	195	.49	4.9	9.3

		Sensorial % v
$10^{-7} \times \frac{471}{4}$	1.2×10^{9}	1.0
$10^{-7} \times \frac{407}{4}$	1.02×10^{9}	.85
$\cancel{4} \times 10^{-7} \times \frac{314}{4 \times 2}$	3.9×10^{8}	.32
$10^{-6} \cdot \frac{585}{4}$	1.46×10^{8}	.121
$10^{5} \cdot \frac{685}{2}$	3.4×10^{7}	.028
$10^{5} \cdot \frac{195}{4}$	4.9×10^{6}	.0041



March 4, 1950.

Many(?) from 693a 2/22/50. 1-4 contains lac⁻ Mal⁻. Pick single lac⁺ from EMS lac and streak out on Mal; put on plants. = ~~699: 1-4~~

6, ■ may also be lac⁻ Mal^v. ~~699.5~~

3/1 693 B. Streak on EMS Mal. ~~699-6.~~

693 A. 1, 3, 4, 5, 7, 8, 9, 10, 11. EMS lac ~~699: 7-15~~

H227 (See 668J) Lac^v Mal⁻. ~~699.16~~

Repurify all cultures on EMS lac or Mal.

699:	From	EMS Mal	Purported: Mal	Lac	Xyl	Mtl	Mal Lac
1	# 693a 6	all+	v v	— v			
2	693B	+, −	v +	— —			
3	H227	—	—	v v✓			
4	693A 1	—, +	—	v v✓			
5	3	—	—	v —✓			
6	4	—	—	v —			
7	5	—	—	v v✓			
8	7	—	—	v v✓			
9	8	—	—	v v✓			
10	9	—	—	v v✓			
11	10	—	— —	v ✓✓			
12	11	—	— —	v ✓✓			
13	693a 1	—	— —	v ✓✓			
14	2	—	— —	v ✓✓			
15	3	—	— —	v ✓✓			
16	4	—	— —	v ✓✓			

P6: Pick 1 colony from EMS and streak out to establish purified stocks for reversion tests, etc. Results in pencil

#1 and 2 Require further purifications for Mal^v lac⁻ type

#5 and 6 " " " " Mal⁻ lac^v.

Abundance of lac⁻ Mal^v is seen.

Partial segregants.

March 10, 1950.

#1 Pick several (EMS) Mal+ colonies to EMB Mal. of 12, #2, 7, 8, 12.
Restreak on EMB Mal as potential Mal^{v} Lac^{-}: Confirmed.

#2 Only Mal+ and Mal- found on restreaking.

#~~5~~ 6 Lac+ (v?) Mal- ~~=6~~. N.G.

#~~6~~ 5 =4 Lac^{v} Mal- OK Lac^{v} Mal-

Retest these cultures:

		Lac	Mal	Mtl	Xyl	Mal+ reversers:
~~1~~	1	—	v	v	v	Type 1
~~3~~	3	v	—	v	v	2
~~4~~	4	v	—	v	v	
~~5~~	5	v	—	v	v	
~~7~~	7	v	—	v	v	
~~8~~	8	v	—	v	v	
~~9~~	9	v	—	—	—	3
10	10	v	—	v	v	
	11	v	—	v	v	1. Mal v Lac v! - and? v tor all clones!
	12	v	—	v	—	4
	13	v	—	v	v	
	14	v	—	v	v	1. Mal+ Lac-
	15	v	—	v	v	
	16	v	—	v	v	
	17	v	—	v	v	
	18	v	—	—	—	
	19	v	—	v	v	
=712A1	20	—	v	v	v	1
A2	21	—	v	v	v	1

P15 699-11 Mal+ Carry as 699-11R1. Lac^{v} Mal^{v}

P17 11b. Lac- Mal+. Segregant reversers.

3a Lac^{v} Mal^{v+} Relatively stable; few sectors! 699-3R1

4a Lac^{v} Mal^{v} Not unusually stable 699-4R1

Balanced lethals

700
C

March 4, 1950.

a) Inoculate 698-0 (control) and 698-40 (X-ray 40,000r) 1:1000 in Penassay and aerate: effort to induce segregation.

b. 698B1-7 are 7 stable lac+ from X-rayed H226.
695-92A is a single pure lac+ from UV H226.

Test balanced lethals. 700=[1-7]; 695: 1-3. Streak on EMB lac, Mal, Xyl, Mtl. Streak EMS lac.

	Lac	Mal	Xyl	Mtl	
1	+V	—	+, [redacted]	[redacted] +,±	} disqualified as stable diploid!
2	+V	—	+, —	++	
3	+	+	+	++	Only #3 is uniform +; others show various changes.
4	+	—	++ sheen	++	
5	+	—	++ sh	+	
6	+	—	++ sh	+	
7	+	+	—	+	
695 "8	+	—	—	—	All grow very poorly on EMS lac.
695 9	+	—	+	+	
695 10	++ sheen	—	—	—	

[redacted]

See 693d.

#1 has sane colonies clearly almost pure lac+, others lac v! Restreak lac+: These give predominantly apparently pure + colonies. A very rare colony may appear -. Pick a type + and use as 700-2.

P7: Plate .1 ml grown culture of 700-3 with T6; also K12
Check nutrition of 700-3.: 3/8 – Meth + [redacted] (~~not prototrophic~~)

700-3 is probably not diploid: a) ~~is~~ gives pure Mal+ non-crossing
b) V_6^R mutants are pure lac+

Search for balanced lethals

700a
A-B

March 5, 1950.

A B.
Inoculate H226 and H226 - X Ray 40,000 into ~~X~~ Permassay and etc.

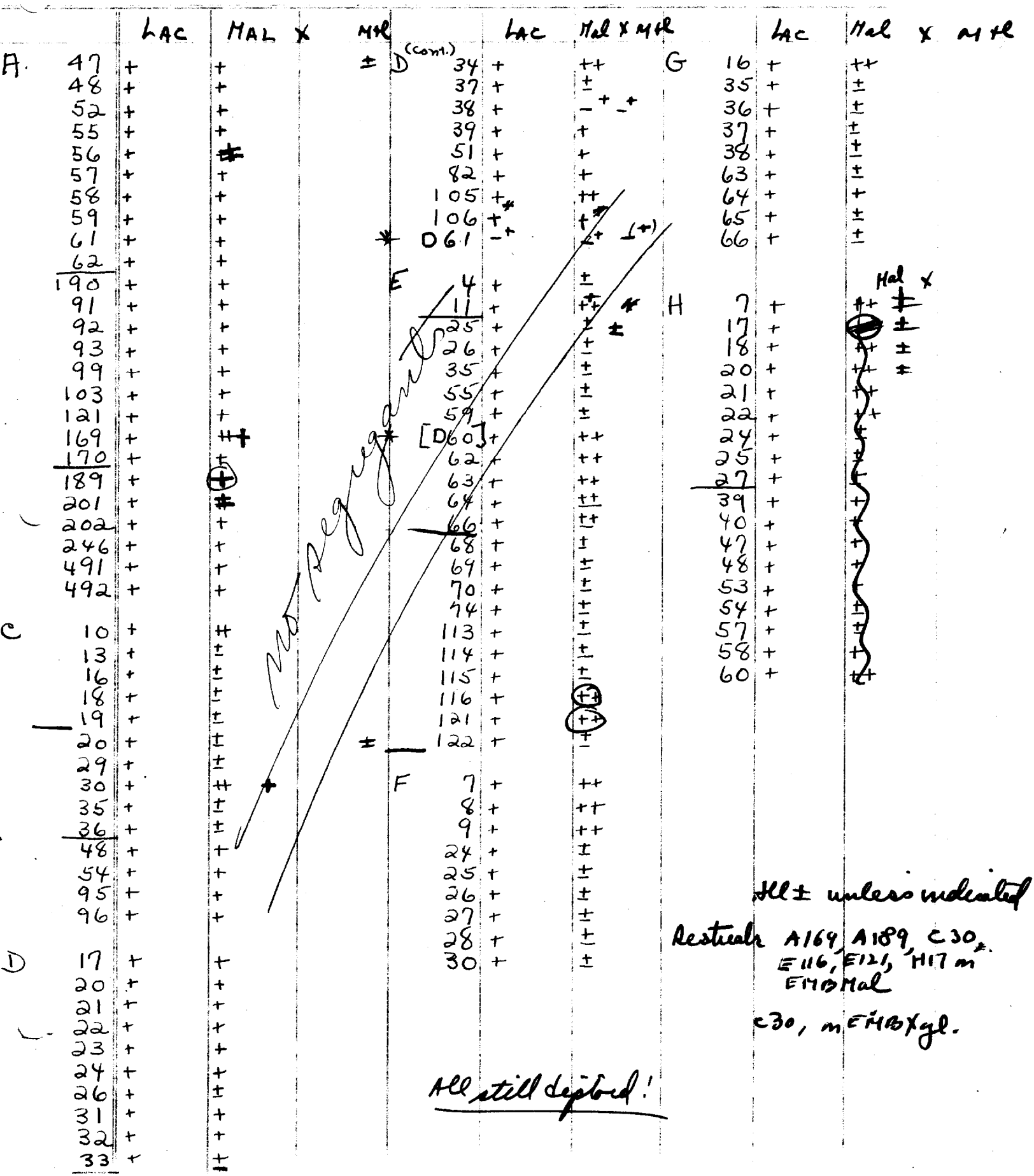
P5: Plate 1st culture. 3P5 Inoculate ca 1:1000 into fresh Permassay.
10P5 Reinoculate (3d culture). also B2.

B1 and B2 on EMB lac < ½% Lac+. Pick few + from B1 and B2
All appear to be Lac+ = 700B: 1-6
A1 Ca. 10%+. Pick 40 + and streak out.

Zelle single cell isolates

~~696~~
702

2/24 - done by Zelle 2/21/50.



All ± unless indicated

Restreak A169, A189, C30, E116, E121, H17 m EMB Mal

C30, m EMB Xgl.

All still depbod!

Chemicals on H226
$\phi_2 I^+ Cl^-$

March 6, 1950.

A. Diphenyl iodonium chloride 2.7 mg/ml in D(o) 10 ml + 1 ml H226 at 37 C. 10 minutes + plate out. dilutions as from treatment tube.

B ~~Acetic~~ allylcide per 692 C. dil. from treatment tube.

C Control. 10^{-7}

D. Pt. $\phi_2 ICl$ 28 mg in 10 ml D(o). Hold 1 ml H226. Incubate 30 mins at 37°. Plate out.

Results.

A: Plates were ~~stained~~ discarded before counted, but killing was << 50%. Some staphidiuging effect?

C: ?? Ca 90% diploid 10^9. [Discarded].

See 704

B: slow growth: hold.

48 hours: Dilution 2. 10 lac− 0 lac v

Dilution 1 82 lac− 1? lac+ Streak out: lac v

Hold plates further:

at 72 hours, no further + appeared; no residual + in centers.

Note: Very low proportion of "residual" diploids with high doses of Ac_2O.

March 8, 1950.

Use once washed H226 (3/5/50) in D(0) in this series. 37°

✓ A. $\phi_2 I^+ Cl^-$ 25 mg / 10 ml D(0) + 1 ml H226. 60 min.

– B. $IAcNH_2$ (Doherty – brown sol'n : must contain free I_2) 50 mg in 1 ml + 10 ml D(0) + 1 ml H226. 25 minutes.

✓ C. Benzoyl Chloride : 2% alcoholic. Add .1 ml to 10 ml D(0); 1 ml $CaCO_3$, + 1 ml H226 10 MINS.

✓ D. Dimethyl sulfate 10% alcoholic Add .1 ml to 10 ml D(0) + 1 ml H226 + 1 ml $CaCO_3$ 10% 15 min.

✓ E. Phenyl isocyanate : 2% alcoholic. Add .1 ml to 10 ml D(0) + 1 ml H226. 10 MINS. 30 to 40

✓ F. Ethyl carbamate (urethane). 2.5 ml 20% solution + 1 ml H226 + 6.5 ml D(0) 10 to 15 (ca 2 hours, 5%).

Results :

A 5 ca 200 ca 85% Lac v. Killing too meagre to be decisive.

B Steals at every dilution. Repeat at lower concentrations! ✓

C 6 ca 100 mostly 2n – Killing too meagre

D 1 ca 100 all lac – Later some delayed Lac v.

E 6 ca 100 mostly 2n – Killing too meagre.

F 2 ca 100 90% Lac v.

Use larger intervals with Benzoyl Chloride ; Phenyl Isocyanate

Chemicals on H226 705

A. H226 3/5 washed. 1 ml in D(o) 7 ml + Propylene Oxide 2 ml 10% STERILE
37° 3 45 to .8 30 !

B. do. 50 mg Ninhydrin in 4.5 ml D(o) .5 ml H226 37°. 50 mins.

C. $IAcNH_2$ 1% 1:10 } D(o) + 1 ml H226 37°. 10 mins. Excessive boiling
D. " 0.1:10 } No boiling.

A. Use shorter treatment; more diluted Pr < O

B. Not haptoglobin 82: ca. 100; 2+.

C. 2; ~~4~~ 5 lacv, 1 lac− ; C1: several hundred; several hundred lacv centri inhibited; periphery lac −.

D. 6 ca 100 lacv.

Chemicals on H226

706

March 10, 1950.

A. Benzoyl Chloride } 70% 1:20 to 400 P/M
B. Phenyl cosiganate } see ~~B05~~ C + E. Steile 48 hours. 410 P/M
.2 ml 2% solutions + 1 ml H226 3/9/50 washed + 10 ml D(0)
+ 1 ml 10% $CaCO_3$ for ϕcocc.

4 hours

C. K_3FeCN_6 M/100 1 ml + 10 ml D(0) + 1 ml H226 10 m.
D Quinine Sulfate 0.1% 1 ml + " " . 5 5
E Iodine M/20 1 ml + 10 ml... 10 m.
F " 0.1 ml 10 m.
G H_2O_2 final concentration 0.03% 10 m.
H H_2O_2 added to Permanganate to 3%. Incubate 5 40 – 8 30.
Add .1/10 to D(0) and add 1 ml H226 10 m. = 6.
I CH_3COCl 1/10 in EtOH. .01/10 ml D(0) + 1 ml H226 H226.

A. A4 Ca 150 ~~cells~~ 90%+ diploid REPEAT!
B Steile

C6 : ca 100 diploid 90%. No killing!
D 6 ca 100 diploid ca 90% Inadequate killing
E 1-6 Steile
F Steile
G 5: 30 Lac – 17 Lac v.
H 6: 6 Lac – 11 Lac v H5: 65 Lac – 36 Lac v. haploidized? ✓
I 6 ca 100 diploid 90%.

(over)

Native peroxide and peroxide-treated broth appear to be equally active as ~~same~~ haploidizing agents.

700·3 x Y10

"Diploid ???"

707

March 10, 1950.

700·3 x Y10 on EMSLac.

Ca 600 prototrophs : all hac+

Chemicals on H226

March 13, 1950.

C. H226 3/9 ~~3/9~~ 1 ml + D(0) 10 ml + $CaCO_3$ 10% 1 ml + CH_3COCl 0.1 ml
Immediate hydrolysis obvious from CO_2 evolution. Keep cold
Compare with tube i same addition, but let stand 4 hours. before
adding bacteria. N.K.

D → N.K.

E Benzoyl Chloride + ~~[illegible]~~ $CaCO_3$. + 1 ml H226 Shake at Room
temperature. ~~[illegible]~~. N.K.
0.1 ml

F. Propylene oxide to 1% 1 ml AD(0) ~~[illegible]~~ 1 ml H226 ~~[illegible]~~

G. Caffeine citrate to 0.3% N.K.

H. $K_2S_2O_8$ to M/200 N.K.

I K_3FeCN_6 to M/100 N.K.

J. I_2 to M/20,000.

		Dil.	Lac v	Lac −	
CH_3COCl	C	6	25	27	Effective (?) CH_3COCl
Hydrol.	D	6	101	14	$CH_3COOH + HCl$
$\phi COCl$	E	6	49	9	Inconclusive N.K.
I_2 >0	F	6	98	17	Inconclusive N.K.
Caffeine	G	6	99	14	Inconclusive N.K.
Persulfate	H	6	117	7	" "
Ferricyanide	I		149	10	" "
Iodine	J	3	41	7	Does not haploidize: 10^{-3} survivors!
		4	8	4	

March 14, 1950

St (K) Benzoyl Chloride (+ DO) $CaCO_3$ Shake at RT 11⁰⁰ AM to 1PM
H226 3/9 STERILE

L. CH_3CHO to 0.5% H226 3/14 unwr. 20 min. 37 <K

St (M.) ϕCHO .1ml /10 " " 4 hours — Shake at RT.
STERILE

St (N.) HNO_2. Add to 10ml M/10 KNO_2 (= .06 ml) 1mM AcOH. Make up to M/50 in pH 4 Citric – KP buffer. Add /ml H226 w. 3/14 in water for effect of HNO_2. cf. O. STERILE } 30 minutes.

O. No KNO_2 – AcOH; buffer only. } OK.

P. H_2O_2 0.03% Dark 5ml H226 3/14 wash / 10. 10 mins Sediment and resuspend.

Q " " Light 30 mins.

R " " Dark control (cool in dark while Q is illumin.)

PIS: K, M, N — all sterile.

	Lac+	Lac−	
L6:	81	51	Possible effect.
O5:	9	6	
4:	91	78	Possible effect!
P6 (no correction for extra concentration)	158	81	} Inadequate killing
Q6	146	47	
R6	106	47	

March 15, 1950

S. MeCHO to 1% H226 3/14 w. 30 m. 37

T Propylene oxide to 2% " " 30 m. 37

U. Ethylene oxide to 1% " " 30 m. 37

W pH4 citrate buffer } H226, washed in water.
X Control, water. } 8$\frac{25}{}$– 15 mins.

			Larv	Lac–	
pH4	W	4:	13	27	
		5:	3	4	
		3			too heavy for accurate score, but – clearly >> lac v.
	X	6	75	8	smeared but countable
(control)		6	76	7	
		6	41	3	The increase in proportion of haploids is beyond question.
$CH_2{<}O{>}CH_2$	U	6	38	44	Definite haploidizations. Many ☿ are ⊙ types.
$CH_3{-}CH{<}O{>}CH_2$	T	6	68	35	" " " "
Acetaldehyde	S	6	25	9	Uncertain?
		55			– Some increase in proportion of haploids??

P16. Y H226 (H_2O) in Acetate-Na-buffer, pH4, M/10. 15 mins.
36 hr. rdg.

Larv	Lac–	
4 12	19	} indubitable augmentation of haploids. a few ⊙. More than control?
3 138	83	

(over)

Z. Potassium hydrogen phthalate buffer
pH 4.0 7/20.

15 mins 37°. H226 (H_2O) 1:10

	Lac+	Lac−
Z3:	108	11

Despite 3 decades of killing, no alteration of haploid-diploid ratio is found. The effects of peroxide sections may be ascribed to acetic and citric acids respectively ($pK_{(1)}$ = 4.76; 3.06

But pK (phthalic) is 2.89, 5.41, so at pH 4 there would be as much free phthalic as citric!

Photorecovery of peroxide-treated E. coli

March 13, 1950.

H_2O_2 to .05% in D(0) 6 ml + 4ml H226 3/5.

Incubate 10 mins., sediment 10 mins. Divide washed suspension into equal aliquots, dil. 1:1 PhB, one to be exposed to visible light 30 minutes. Control left at room temperature.
30° - 40°.

A (light) Strick

B (dark) B1 3 hrs - 1 Lrv

Dose too high!

Partial segregation of H168

3/11/50.

Grow H168 from single EMS Lac colony in D(Lac). Inoc 1:1000 in Y2 and grow, aer., to saturation. Plate out on EMS Lac, Xyl, Mtl. 10^{-6}: ca 500/plate. Pick – colonies and repurify.

EMB: Lac ~~Mtl~~ Mtl Xyl

A. Lac

B. Xyl

C. Mtl

A. 9 – – –

B. 24 23 Lac+ Mtl- Xyl-

1 Lac- " "

C. 16: all Xyl- Mtl- 3 Lac- 13 Lac+.

No lac v ∴ No partial segregation.

No partial segregants formed in H-168

Partial segregation : 17226 712

March 15, 1950.

Grow from 1:1000 in Y2. Plate out on EMS 10^{-5}.

A. Lac. Considerable lac− (ca. 30% of count of 3-500/plate).
Pick probable pure lac− and smear on Luthy EMB Mal, Xyl, Mtl, Lac for discernment of Lac− ... v.
All are, indeed, pure lac−. Scoring of v as against + is possibly uncertain.

1 prob. Lac− Mtl v
1 prob Lac− Mal v
} noted. Recover and retest. Lac− Xyl v Mal v Mtl v
Hold for appearance later. − v v Mtl v.

~~All but 2 of~~ others, 96 Lac− Mal+ Mtl+ Xyl+. 2 ----
of an additional 100, no lac− Mtl, Mal, or Xyl v noted, subject to deficiencies of the brushing technique.

B) Mtl. <1% of prototrophs possible Mtl−. of 20 picked, 6 are Mtl−

c) Mal 4 Mal−

D) Xyl 10 X−

B: 6 tests, 5 are Lac− Mal+ Xyl+ 1 is Xyl slow = #3 (destruct from + or −)

C:		Mal	Xyl	Mtl	Lac
	1	−	v	v	v
	2	−	+	+	−
	3	−	−	+	−
	4	−	−	−	−

D: 10: all Lac− 5 Mtl+ 5 Mtl− 9 Mal+ 1 Mal− all Xyl−

No Partial segregants except C1
Do not bump.

H168 - Mal-revertants.
for hemizygosity test

March 15 ff 1950.

Recsil H168; grow in O(Lac); culture ca 85% Lac v.

A. Streak on EMS Mal to select revertants.
Papilla picked en masse 48-72 hours. Streak on EMS Mal to purify. N21 Pick "single" + colonies and retest

B. As above

C. Broth mainly 3 tubes of O(Mal) and aerate. Growth after 48 hours. Streak out to purify.

D. Single Mal- colonies from EMS Mal as same for revertants.

A: 3 Mal+ prototrophs. 2 are Mal++ Lac v
1 is Mal++ Lac +.
∴ 2 additional tests for hemizygosity of Mal in H168.

C: 3 Mal+ prototrophs all Mal++ Lac v. " "

B 8 Mal+ " " " " "

Acid effects on diploid coli

March 21, 1950.

? See 716

H/26 3/4 15 minutes.

A. Acetate pH 4 M/10
B " pH 4 M/100
C. Phthalate pH 4. M/20
D " " M/100
E Control

This culture apparently contains some lac+. Streak out as 714-A

		Lac v	Lac -	Lac +	
A.	5	620	34	20	?? very little killing !!
	6	2	5	2	
B	6	115	3	6	
C	3	93	3	7	
D	6	29	5	4	
E	7	121	2	8	
	"	133	5	7	

Utilization of neolactose

March 22, 1950

A	58-161	Mal
B	"	Lac
C	W1301	Mal
D	"	Lac

1 cc cells from 4/10 conc. O(O) medium. aerated overnight; twice washed Bacto buffer 1/20. .2 ml 2% substrate

	Flesh	Culture	Substr.
1	10B in Mal.	B	—
2	5B	B	Lac
3	3A	B	Neolac
4	6A	D	—
5	2B	D	Lac
6	4B	D.	Neolac

7/22/50

	ThB	1	2	3	4	5	6
230	82+	05+	42	51	29	32+	2+
235	85	05	40	49	28	32	1
240	90·83	8+	42+	51+	29	32+	1+
245	86	−1	32	40	18	21	−6
255	85	+1	35+	42	21	23	−5 6
305	81	−4	36	42	19	22+	−5
410	81	−4	52+	47	16	22	−10

No fermentation at all!!

H226 segregants for outcrossing

March 22, 1950.

Pick single lac⁻ colonies from 714E7, streak on EMB Mal.

All were pure ~~lac~~ Mal− ! Very likely suspension of H168 was used in this experiment and in 714. This would account for low tailing as H168 is resuspended in buffer.

Appearance of lac⁻ is not unlike H168!

Repeat from H226.

10 Mal+ and 10 Mal− (conjugate) isolated and nutritional tested

	+		−		Lac	Mal	Xyl	Mtl
W1305 *	1	MTL	11	TLB, W130Y	−	− +	− +	− + −
	2	TLB,	12	TLB,	−	− +	− +	− + −
	3	TLB,	13	TL	−	− +	− +	− + −
	4	TLB,	14	TLB,	−	− +	− −	− − −
	5	TLB,	15	TLB,	−	− +	− +	− + −
	6	TLB,	16	TLB,	−	− +	− +	− + −
	7	TB,	17	TB,	−	− +	− +	− + −
	8	TLB,	18	TLB,	−	− +	− +	− + −
W1303 *	9	M	19	TLB,	− (Blue early)	− +	− +	− + −
	10	TLB,	20	TLB,	−	− +	− +	− + −

Check fermentative reactions.

∴ #9 can be presumed to be the recovered B-M− parental W67 type.

Cross with #11, and with W1177 lac+.

Hold W130Y for lac+ reversion for crossing.

Alan Pardee
Roy Y.

H206 single-cell segregants

5-224 = segregant

5-223 = 2n : unisolate

5-86 Lac+Mal+

5-85 2n

20:

Partial segregates:

8 Lac- and 8 Mal- prototrophs from H206 plated each was Lac- Mal-

Studies on single cell segregants
(1226)

Recover from vials sent by MR Zelle and stored in refrigerator.

Series 2/5

A15 * inviable A33 also inviable!

D— some inviable on EMB agar. D15 and D20 are lac-Mal-Xyl-Mtl- others inviable!

E 215 ✓
216 ✓
221 × ?
222 ✓

G 103 ✓
104 × ✓
105 ✓
106 ✓
107 ✓
108 × ✓
109 × ✓
110 × ✓

* Recover from EMB blue agar!

Sib tetrads.

G3
G13 both viable

G9 O/K
G24 O/K

E 219 viable OK
220 n.v.
52 n.v
108 n.v.

[HO] radicals in H226
"Fenton's" Reagent.

March 28, 1950

A H_2O_2 .01%
B " + Fe^{++} (NH_4SO_4) M/1000 } + 1ml/10 H/226, H_2O.
C Fe^{++} M/1000

	Lacv	Lac-
A6	37	22
B6	23	27
C6	112	10

Ferrous by itself has no effect. No very marked potentiation of H_2O_2 observed. Should be repeated under slightly more drastic conditions.

Chemicals in H226

March 31, 1950.

A. Benzoic anhydride 0.4% (2/100 of 2% alc.)[sat'd. I.
in D(0) H226 3/24/.50

B Benzoyl peroxide 0.4% "

C. Acetate buffer M/10 pH 4

D. " " M/50 pH 4

E Phthalate " M/20 pH 4.

F Control 10^{-7} :

		lac v	lac -
F	10^{-7}	9	5
		40	4
		16	5
		65	14
C 3:		8	101 ←
D 5:		5	12
D "4"		1	18
D 3	10^{2+}	171	548
E 2		5	10
E 1		131	70

} any effect?

New ~~heterozygous~~ diploids

April 1, 1950.

A W67 x W945 1 lac+/ 12 plates, ca 100 each. (#18)
B W67 x W950 8 " / 14 plates " " "

For recovery of presumptive diploids, ~~Recisolate~~ (A - single col.) and (B - whole streak) from EMSlac and streak on EMSlac EMB Lac, Gal, Mal.

		Slac	Blac	BMel	BGal	
A18. (from EMB)	A		-, v	+, -?	++	Recisolate from Slac.
	B		v, -	+	+, -, v?	

		Lac	Xyl	Mal	Mel	Sal	Gal	Sal
B 1	A	Lac-						
2	B	N-gr. on EMSlac						
3	A		v	v	v	-	v	Gal-!
	B		v	v	v	-	v	
4	A		=	+ -	++	-	+	++
	B		=	+	++	-	=	++
5	A		+	-	-	=	=	=
	B		+	- +	- →	=	=	=
6	A		v	-	+	=	-	++ =
	B		v	-	+	=	-	++ =
7	A		v	-+	-+	-	-	- -
	B		*			-	-	-
8	A		v	-	-	v	v?	= =
	B		v	-	-	v	+	= =
A 18			v	-	+	+	v	

Keep A18, B3, ~~B5~~, B6, B7, B8 when recisolated!

H		Lac	Mal	Xyl	Mel	Sal	Gal	
233	A18	v	-	v+?	v-		+	
234	B3	v	v	v	v		-	grows poorly
235	B6	v	-	-	-		+	
236	B7	v	-	-	-		-	
237	B8	v	-	v	v?		-	Use for reversion studies

Effect of pH on segregation of H226

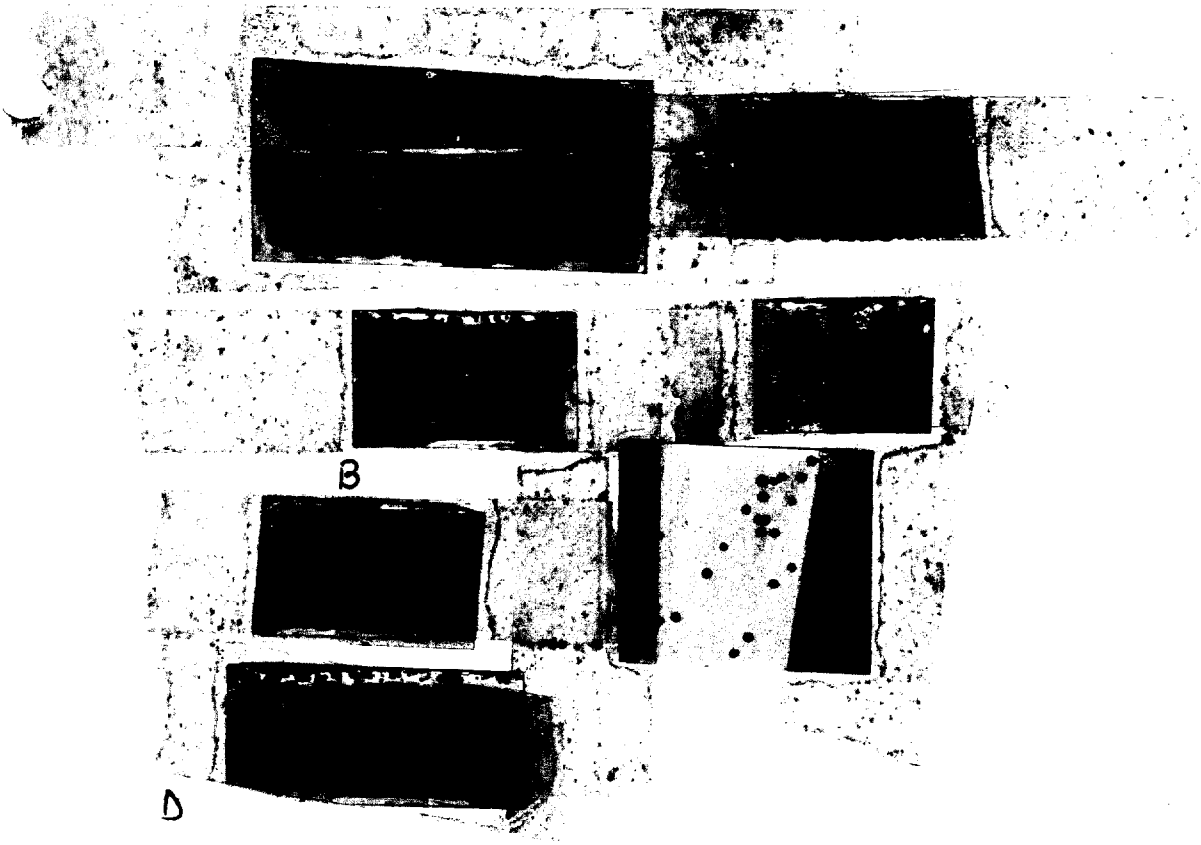
April 2, 1950.

Prepare D(0) + 1% N2Case. Measurement of pH. adjust to various lower pH's.

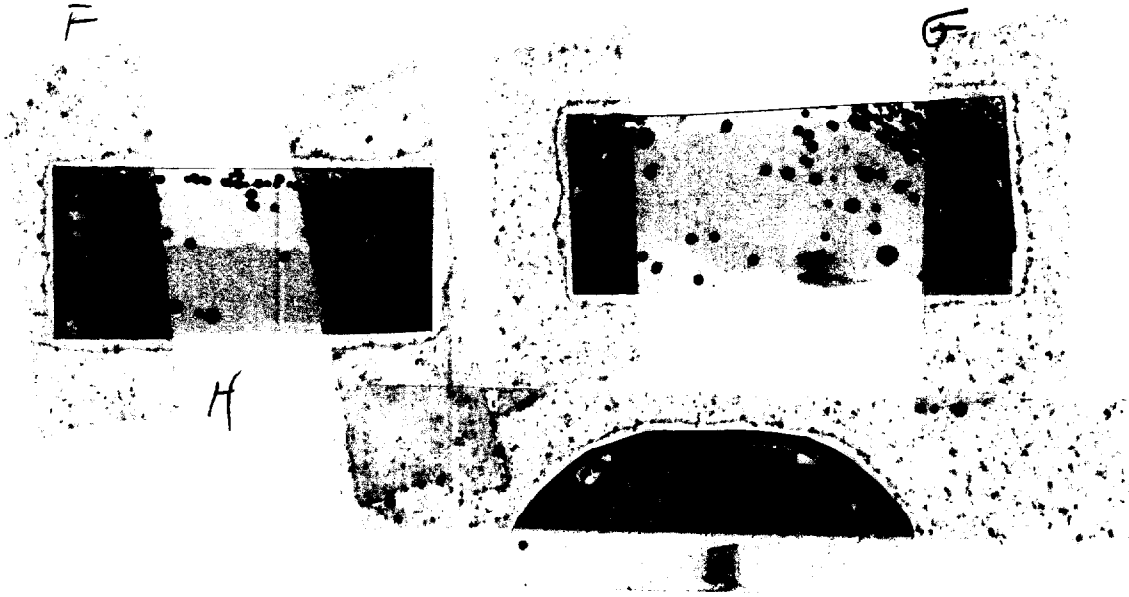
	Medium:	24h.	48hr. gr.	% V.C.
A	D(0) + 1% N2Case	+	+++	70
B	" + .1% glucose	++	++++	50-60
C	+ 1% glucose	+++	++++	<50
D	D(0) +1% N2Case to pH 5.9 with AcOH		+	20
E	" " " 5.0		−	~~tooth~~ Stale
F	D(0) + .1% glucose		++	>80
G	D(0) + .1% lactose		++	85
H	D(0) + 1% glucose		+++	60
I	D(0) pH 6 0.1% glucose		+	65
J.	D(0) pH 4.9 0.1% glucose.		±	all −

Streak out on EMB 24hr at 48h.

Results indicative but should use smaller inoculum.


B
D


F
G


H

April 2, 1950.

"F" W1304 x ~~58-161~~ / EMS Lac.

72 lac+ picked and streaked on EMB lac. None were lac v.

~~Hfrsale - not. lac+ , possibly~~ + > -.

Test on EMS Mal for Mal segregation:

	Mal+	Mal-
lac+	11	62
lac-	10	58

A W1303 x W1304 13 plates EMS lac ca 200/plate = 7600

13 ~~W1303 x W1304~~ 5 + colonies at 80 hours. Hold plates for delayed +.

Pick these and streak on EMS Lac EMB Lac EMB Mal.

6 plates EMS Mal - dilute suspensions: yield fewer
9 Mal - 2 Mal + Streaks + as 6, 7.
Both pure Mal+ lac -.

	Lac	Mal	√Resist.	Lac	Mal	Xyl	Mtl	H
1	v	v		v	v	v	v	240a
2	v	v		v	v	v	v	240b
3	-, v	-, +	v?	-v	-	v	v	241
4	v	v		v	v	v	v	240c
5	v	v		v	v	v	v	240d

Note the prevalence of Mal v here

H241 should be tested for hemi/homo - zygosity of Mal.

44 colonies picked from A as checker after 3 days. Restreak to look for faded lac v. None were after 4 days incubation.

Outcross H226 segregants

April 10 ff. 1950.

E. W1303 × W945. (? Does Gal become heterozygous?)

P16: ~~100~~ 10 plates EMS Lac. ca 200 scoreable prototrophs per plate.

8 possible lac+ or lac v. Pick and streak out on Lac EMS, Lac EMB; Mal; Gal EMB.

#6 = Lac v. #2 = Lac + others Lac −. Remarks as 722-E1 Remarks "−" on EMB.

Mal − Gal −.

of 10 addnl plates (ca 1500...), 4 "+" picked for testing. 2−, 1+ (1 v. E2)
E2 is lac v Mal v check Gal?

G. W1304 × W478. ca 1/3 Lac +. Pick 90 lac+ and streak on EMB Lac.

5 possible lac v. Repick to EMB Lac; Mal EMS Lac.

	Lac	Mal
1	v	−
2	v	−
3	++ −?	−
4	v	+
5	++ −? v?	−

Xyl − Mtl −
E1 Lac v (weak) Gal − Mal −
E2 Lac v Gal v Mal v Xyl v Mtl v

Note: ~~no~~ Mal v.

After additional 24 hours, a number of "above" lac + picked from EMS.

Of 21 isolations, 16 are apparent lac v. Repick these as above.

1	+	−	
2	+	−	
3	+	−	
4	v	−	
5	v	−	
6	v	+, − v?	Mal −
7	v +		
8	+, − v?	+	
9	v	−	
10	v	−	
11	v	−	
12	v	−	
13	+ − v?	v?	Mal v?
14	v	−	
15	+	+	

No definite Mal v but Recheck.

April 20, 1950.

H. W1311 × W67 ("A")
I W1311 × W1304 ("B") } on EMS Lac. → no yield Note 1/5/51 both are TUB.

H 100 Lac+ picked and streaked on EMB Lac
Hold possible ++ in refrigerator.

Pick purified lac+'s to DN2 Glu for spot test of constitutive lactase.

28 Lac+ 26 mpg+ 2 possible mpg− . (11, 18)
Replate these on DN2 Glu for recheck.

Recheck −1 for ~~at~~ prototrophic ~~constitutive~~ lac+.

12 Lac slow 1 possible

Hemizygosity tests: lac - partial segs.

April 3, 1950.

Entrained from 699 - .

Type 1 (Lac - Mal V) : on EMS lac, may eventually assume + appearance.
Select "papillae" from D(o) agar - lactose.

699-1 No papillae found. Colonies remain very small.

A 699-2#. 2 papillae: #1 gave - and Lac V colonies on EMB lac
#2 gave only lac - ! Recheck.

B 699-20. 8 papillae: #1-7 Lac V. #8 Lac -.
more appeared later!
But all of these cultures are Lac - on EMS lactose !!
See below - eventually gives + centre colonies.

These cultures grew slowly to rather large colonies on D(Lac) & obviously account for the poor selection!

C 4/3: Pick single prototrophs from EMS lac A1.

D " 4 " Lac V colonies from EMB lac A1

E From EMB lac B1-7, 1 each.

But after 3 days, colonies with dark centres appear on EMS lac, probably representing "lac +". This appearance develops very slowly.

723C #1 and #3 are weak Lac V on EMB lac and show comparable appearance on EMB lac. Restreak #1 on EMB lac. Transfer to D(lac) slant as 723 A1 ✓: ✓ mostly weak Lac V.

723D More or less typical lac + on EMS; Lac V on EMB. Streak out to compare with 723 A1. A1 and D1 both quite weak lac + on EMB

F 4 new colonies from 699-20 on D(Lac), to EMS lac
after 72 hours, Lac + centres.

Lac heterozygosity tests

April 7, 1950.

723: A + C compound:

Both are Lac- after 24 hours; but give Lac V mosaics apparent in 48 hours on EMB Lac. On EMS Lac, colonies taken at 48-72 hours. This holds for all of this series! How many Lac V may be missed? Or, are these not true reversions?

See 722: no comparable Lac V failed isolated from ~~[illegible]~~ W1303 x W1304

Heterozygosity tests
Mal- partial segs.

724

4/2/50 ff.

From 699:

A. 699-11:

2 papillae: Lac v Mal v Save single Mal EMS+ colony from each as 724A1, A2 OK ✓ Cf. 699-11R1.

an additional 2 papillae to EMS Mal. Reisolate from second. Check(1) as A3. Mal v Lac v ✓

1 gave Mal+; the other only papillae (2d) - Restreak on EMS Mal v/c check as A4: 15 M− X− ∴ ++/−− i.e. "E15" 13 M+ X+

D Grow 699-11 in D(Lac) aer., overnight. Spread on several EMS Mal and D(Mal) plates to obtain additional Mal+ revertants. N10 About 50 cols on 7 plates. 30 picked and streaked out on EMS Mal. Repick single colonies and streak on EMB Mal, D Lac ~~EMS Mal~~; EMB Lac −

#20 = Mal+ Lac− ~~Lac+~~

#25/#26 = Mal+ Lac? each was Lac −

Others are all Lac v Mal v.

Held on D Lac plate

B 699-9. 3 papillae from EMS Mal to EMS Mal for purification. Reisolate pure +, check, and to slants as B1-3 4/6

#2 Lac v Mal v Keep as #1, 3 Lac − Mal+ .724B1 Reject others

C 699-12. 2 Mal+, both give Mal v. 724C1, 2 purified and rechecked. To slants 4/6 Lac v Mal v ✓

Linkage phases of 699-11 M+ recombs. 724a

4/8/50

Streak out A0 – A4 on EMB Mal.

P7. Restreak Mal V. Pick isolated Mal+, – on the same plates and brush on EMB MH:

	MH+	Mal+ MH–	MH+	Mal– MH–	
A0	0	6	17	2	+–/–+
A1	1	5	5	1 (v?)	+–/–+
A2	1	2	5	1 (v?)	+–/–+ ??
A3	0	0	0	1	++/–– ??

P8 8 Mal V from each of above streaked out on EMB Mal to obtain distinct segregants. Pick app. pure+ and – from each quadrant to ~~A0~~. Xgl EMB. one + one –

A0.	Mal+	Mal–	A1	M+	M–	A2.	M+	M–
1	8X–	15X+		X–	X+		–	+
2	1X–	6X– 1X+		X–	X+		–	+
3	5X–	5X+		X–	X+		–	+
4	2X–	5X+		X–	X+		–	+
5	3X–	6X+		X–	X+		–	+
6	3X–	6X+		X–	X+		–	+
7	8X–	8X+		X–	X+		–	+
8	8X–	6X+		X–	X+		–	+

Each of these is +–/–+

A3.	M+	M–
1	4+	4–
2	3+ 1–	4–
3		4–
4	5+	4–
5	2+ 2–	4–
6	4–	4–
7	3+	4–
8	8–	1–

This is very likely in the ++/–– phase. Are the segregations accurately complimentary ??

Rec 724D: —— into ~~#2~~ Permassay

Plate out on EMB Mal for segregants to test linkage phases

Linkage phases of 699-11 Reversions 724b

April 12 ff. 1950.

A 0 trans
A 1 trans
A 2 trans
A 3 cis
A 4 cis
D cis

} See 724a.

	Mal +		Mal −	
	X+	X−	X+	X−
A 4	10	0	0	1
D 1	10	0	0	0
D 2	10	0	1	3
D 3	10	0	0	0
D 4	10	0	0	4
D 5	10	0	2	4
D 6	10	0	0	4
D 7	10	0	0	1
D 8	0	1	10	0
D 9	9	1	0	2
D 10	10	0	0	3
D 11	10	0	0	2
D 12	10	0	0	2

Contras ++/−−

Recheck.

all but D8 had a preponderance of + segregants

D1-12 appear all to be in the cis phase ++/−−. However, since they were recovered from a single plating they might represent recurrences of the same mutation and should be counted as but a single reversion, viz., D.

Reinitiate the experiment by starting cultures from separate single colonies.

E ~~15~~ 22 ~~were~~, independent reversions on Mal, from EMS Mal from single lac+ (EMS) colonies. ~~12~~ 15 were lac−, Mal+ pure (segregated!)
5 were lac∪ Mal∪ (#10, 13, 15)(21, 22) (Recheck 22: maybe lac∪ Mal+)

(See over)

F

[yk rads on:]

		Mal +	Mal −	type	
E	10	10 − 0+	10+ 0 −	trans	
	13	12+ 0 −	10 − 0+	cis	
	15	10+ 0 −	10 − 0+	cis.	
	21	13+ 7−	18 − 0+	cis	
	22	20+ 2 −	18 − 0+	cis	
F	1	10 − 0+	10 + 0 −	trans (Note: Lac −) F1+ trans	see 72Yc (~~Do not cumulate~~)
	2	10 − 0+	5+ 3 −	trans	
	3	6 − 0+	2+ 1−	trans	6,7 o;
	4	11+ 0 −	11 − 0+	cis	
	5	11− 0+	7+ 2 −	trans	

G. From dilute plating of H238 (D Lac) on EMS 14 al.

1-4 o.k.

Cumulative score:

TRANS 𝍸 𝍸 |

CIS 𝍸 𝍸 ||

724 protocols

F8 724 F8 Malv Lacv

~~49" Mal+ Lac− segregated.~~

Definitive

~~G5 Mal+ Lac−~~

~~6 Mal+ Lac−~~

~~7 Mal+ Lac−~~

~~8 Mal+ Lac−~~

G5 9 Malv Lacv

~~10 Mal+ Lac−~~

	Mal+	Mal−	
F6	8+ 2−	8−	cis
F7	9+ 0−	10−	cis
F8	10−	10+	trans
G1	10+0−	7−	} 5 cis!
G2	10+0−	9−	
G3	10+0−	3−	
G4	9+1−	6−	
G5	10+	8−	

recombination score:
9T 1/C

G6	G a	Lacv Malv+	10M− X+	4M+ X−	3M+ X+	TRANS
G7	b	Lacv Malv+	8M−X+	2M−X−	10M+X−	TRANS
G8	c	Lacv Malv+	10M+ X+	9M−? X+		??
	d	Lac− Mal+				

G8 was almost completely M+. Repeat. 11T 1C

5/23 G8 is pure Xyl+ Lacv Malv (partial segregant?)

Associated Mal− should be saved to determine whether there is any correlation of the mutation with partial segregation.

H like G. "1-11" purified. 1,2,4 were lacv Malv; others were lac− Mal+.

	M+	M−	
1	7X+ 3−	10−	#3 had lacv mixed segregant finally separated from lac+'s. Eliminated designations 5-11.
2	9X+ 1−	10−	
4	9+ 1−	8−	Not lacv; Lac− Mal+

Rest of H is all lac−

724 F1 (Lac - Mal v) 724c

May 10, 1950.

Apparently, a partial segregation occurred after mutation from ~~Xyl~~ Mal = to Mal ±, resulting in a Lac - strain. The Lac + and - components present in the finally isolated Mal + prototroph (a direct from EMS Mal) were separated. Each was Mal v. The Lac + was Lac v. The Lac + component must be ancestral; scored for Mal-Xyl linkage phase with remainder of series. Rep Lac - as a partial segregant. F1 + : 7 Mal + : Xyl + 8 Mal + : Xyl - also trans

F1 - ~~gave~~ apparently gives somewhat mosaic colonies on EMB Lac after 48 hours, resembling ~~the "Lac +" colonies of~~ H239. (Lac + trans 699-20 Lac -)

Repick single colonies from EMS Lac (4 colonies)

24h. Each is Lac - Mal v.

60-72 hours. On Lac, definite mosaics ~~colonies~~ with dark centers shown by most colonies. On EMS Lac, some colonies are much darker than others. ~ these.

The "dark" type gives colonies on EMB Lac mosaic at 24-30 hours

The "light" requires 48-72 hours for Lac v.

5/22/50 Streaks of "light" on EMS Lac acquire "dark" papillae: restreak these:

Seaver

Merizygosity tests

Segregates H229.

4/4/50.

1 Mal+ obtained from D(Mal)

A. Streak on EMS Mal (purify); EMB Mal and Lac — mostly Mal+ Lac v.

Verify from single EMS Mal+ colonies. ✓ verified Mal+ Lac v from 4 EMS cols.

B. Streak out on EMB Xyl Test pure Xyl− for Lac+.

31 tests. 2 indicated Lac+. Restreak on Lac, Xyl.: Both Lac v Xyl v

---

A. 1 additional Mal+. Purify on EMS Mal. check purified colony: again: Lac v Mal+ mottled on Mal EMB, but no Mal− colonies or sectors.

726

Zelle 4/4/50

H226 Single cell isolations

Z. 3/31/50

		Lac	Mal	EMS Lac
A	8	+	+	+
	10	+	+	+
	19	+	+	+
	23	—	—	o
	24	—	—	o
	25	+	+	+
	26	+	+	+
	27	+	++	+
	28	+	+	+
—	31	+	+	+
	32	+	+	+
	33	+	+	+
	34	+	++	+
	41	+	++	+
	42	+	+	+
	59	+	+	+
	60	+	+	+
	61	+	+	+
	62	+	+	+
	10	+	+	+
	17	+	+	+
	37	+	+	+
B	20	+	+	+
	25	+	+	+
	27	+	++	+
	28	+	+	+
	29	+	+	+
	37	+	+	+
—	38	+	+	+
	39	+	+	+
	40	+	+	+
	53	+	++	+
	54	+	+	+
!	61	—	+	—
	62	+	++	+

A23-24 A25-26

B61 B62 B29

F21 are segregants. F22

Three sibs are indicated —.

Transfer sibs to D(Lac); also streak out on several sugars.

! Check for partial segregation !

o = n.g.
— = lac⁻ g⁻

	Lac	Mal	EMS Lac
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28	all×	all×	all×
31			
32			
33			
34			
59			
60			
61			
62			
12			
17			
18			
19			
20			
21			
22			
23			
24			
28			
30	all×	all×	all×
31			
32			
33			
34			
56			
111			
112			
119			
120			
121			
122			
2	+	+	
4	+	+	
16	+	+	
17	+	+	

Vac Ural Stac

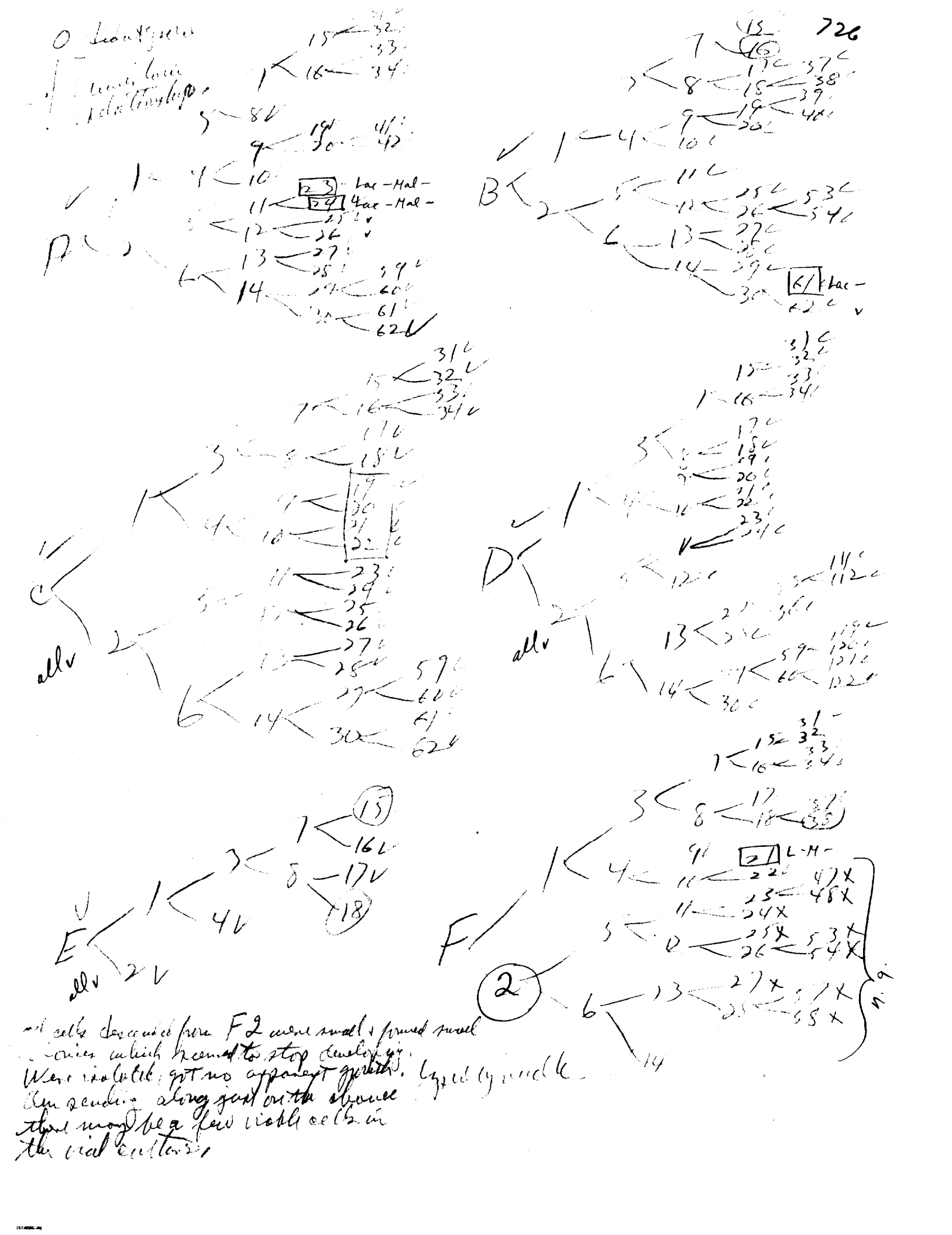
I

10
16
17
18
23
24
25
26
27
29
31
32 all x
57
58 all x
61
62 all x
83
84
85
86
19

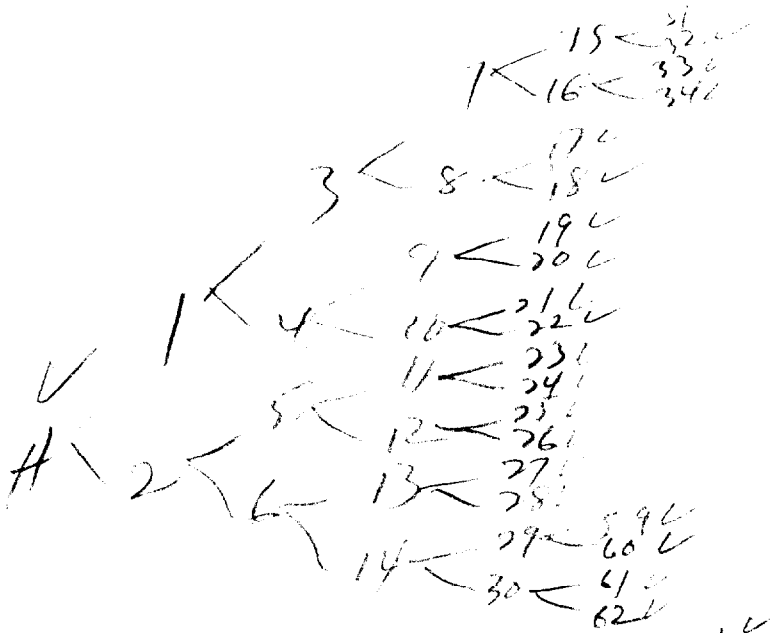
K

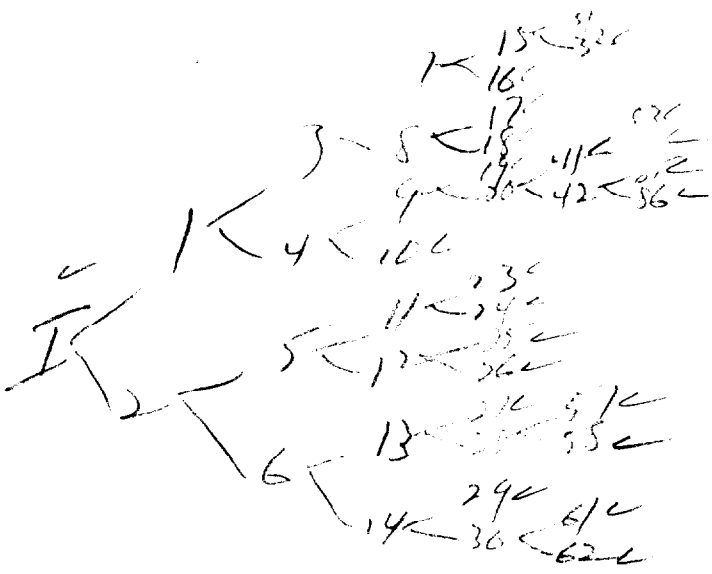
12
16
17
18
19
20
21
22
23 all x
24
27 all x
28
29
31
32
61
62 all x

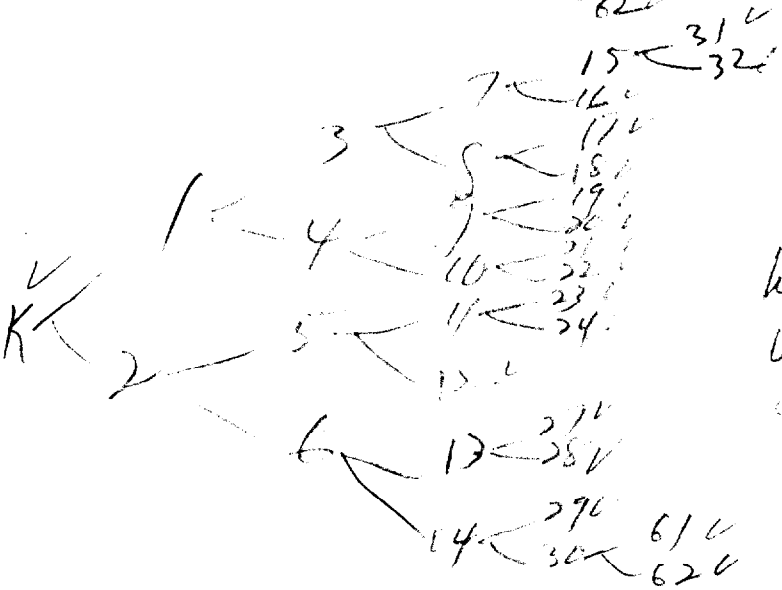
288 ~~screw~~ screw cap vials returned to Zelle.



All cells descended from F2 were small + pinned small colonies which seemed to stop developing. Were viable, got no apparent growth. Can see dividing along just on the chance that may be a few viable cells in the mid section.



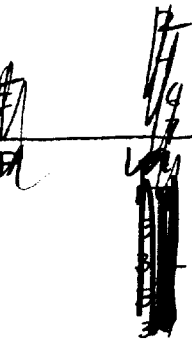




Individuals most of these are hospital rats, they grow too well. Initial inoculum was p. cen 18, from Dairy minimal culture. (Inoculated with an EMS tree colony)

I can put the cloth in the incubator OK. Have two outside waiting for bugs to grow. Will have another clone till next week — transferring again.

Lac Mal EMS Lac



9	+	+	+
17	+	+	+
21	—	+	0
22	+	+	+ Check!
24x	0	0	0
25x	0	0	0
27x	0	0	0
31	+++	++	++
32	+++	++	++
33	+	++	++
34	++	++	++
37	++	++	++
47x	0	0	0
48x	0	0	0
53x	0	0	0
54x	0	0	0
57x	0	0	0
58x	0	0	0

17
18
19
20
21
22
23
24
25
26
27
28
31
32
33
34
59
60
61
62

all x all x all x

Study of Zelle single cell isolations 726b.

A. segregants:		Lac	Mal	Xyl	MTL	Nutr.	
1	A23	−	−	−	−	TLB,	
2	A24	−	−	−	−	TLB,	
3	B61	−	+	+	+	+ MTLB,	Repeat: ✓ no partial segregants.
4	F21	−	+	+	+		

B sibs

1	A25	✓	✓	✓	✓	+	no deviations
2	A26	✓	✓	✓	✓	+	
3	B24	✓	✓	✓	✓	+	
4	B62	✓	✓	✓	✓	+	
5	F22	✓	✓	✓	✓	+	

C doubtful ✓.

F 31	✓	✓+	no deviations.
32	✓	✓+	
33	✓	✓+	
34	o	o	
37	✓	✓+	
B 27	✓	✓+	
34	✓	✓+	
41	✓	✓+	

April 7, 1950.

Take up cultures described on p. 700.

A. P7 Streaked out 698B1, B2 700-2. P8: all are Thal-.

1 698 B1 #2 is Lac+, with sheen for most part.
2 B2
3 700-2 = 698B2! #1, 3 are apparently mixtures of Lac+, Lac-.

Replica apparent pure +.

① 6 colonies streaked out. Each throws off ca 1% Lac-! No Lac v.

② 4 colonies " ". Mostly +, some v.?, a few % -.

③ " " " . 1% -; mostly type +.

Test ~~Xyl2~~, Lac-'s on Xyl, Mtl; Nutrition of parent.

Check nutrition of single + colonies from ①, ③
Also inoculate in O(N2Ease) lac for irradiation.

1. 8 Lac- : Mtl+ Xyl+
2. 6 Lac- : all Mtl+; 5 Xyl-; 1 Xyl+.
3. 6 Lac- : Mtl+ Xyl+.

See 731

April 9, 1950.

Grow 727-1 and 727-3 in D-Y2-plus. aer. est. 10^{10} yield.
Dilute each 10^{-4} for irradiation. 20 secs 30cm.
Dilute to 10^{-6}. controls .05
UV .1 of this dilution/plate.

	"+"	"v"	s	−
1. Control.	117	13	1	31
	107	10	0	25
1 UV=x	227	40		46

Negligible killing. Repeat expt. with higher doses and better populations

---

Mal+ segregants of 727: 1, 3

~~Sel~~ Pick papillae from EMB Mal and purify. No growth Mal v.
Pick to Lac EMB; streak out on EMB Mal.

MA = 7271
MB = 727.3

A: 10 picked and purified 4 Mal+ from EMB Mal brushed on EMB lac
3 and 6 were lac− # 10 a, b lac− c, d Lac+.

B. All 7(4) lac+. Streaks out on Mal EMB for hemi zygosity tests.

A: all Lac ++,− Mal pure++. ∴ 727-1 and 727-3

B: all Lac++− Mal pure++. are pure Mal−

Hemizygosity tests: [redacted] H237 728

4/9/50.

see 720. From W67 x W950. Lac v Xyl v Mal− Gal− MA? St?
check from slant. Incr. D(Lac) 10 ml for heavy suspensions.

H237 may not be heterozygous diploid: it does not give typical Lac v.

One Mal+ 3 Gal+ each pure +.
but lac appearance peculiar. Recheck H237: appears to be Lac +.

Irradiated 17226 on EMS Lac

April 9, 1950.

20 secs. 50 cm Irradiate 17226 r/3 at 10^{-4} in H_2O.
Dilute ~~to~~ to ~~10^{-5}~~ 10^{-6}; plate .1 ml

A Control

B UV.

EMB Lac

	V	—
A	32	15
	35	22
	28	15
B	2	12
	6	7
	6	16
	2	15

Not a highly pure suspension.

EMS Lac ——— A - too many lac -

G. J.

Fermentation of neolactose

April 8, 1950

A 58-161 /100ml Y2 1%lac
B " 3x25ml " agar
C W1301 /100ml "
D " " agar ".

	Flask	Cells	substrate
1	2A	B	—
2	9B	B	glucose 10mg.
3	3A	B	lactose "
4	4B	B	neolac "
5	6A	D	—
6	10A	D	glucose 10mg
7	11A	D	lactose "
8	14A	D	neolac "
T	1		

This experiment was designed to determine whether lactose-adapted cells could utilize neolactose.

Since 58-161, fully lactose-adapted, ferments neolactose ¼ as rapidly as it does lactose, the non-fermentability may be due to a block of adaptation comparable to lac: Bugal on lac. —: However, the falling off of the fermentation may speak for inhibition by allolactose, or a relatively high K_s for neolactose.

	T	1	2	3	4	5	6	7	8
12[20]	137+39	22	17+	−2	25+	50	30	20	7
12[25]	~~89~~	23	17+	−1	25	50	26-28 (−30')	21	8
12[30]	140	27	95	34	30+	58+	−85 85 cl	54	11
12[35]	140	26	103	90	34	55	145	106	16
12[40]	141	27	269 (+211) 295 to 84	186 217 to 69 148	51	58	244 +130 283 to 68	203 242 to 75 [167]	41
12[45]	143	30	155	124 − 114	77	60	136	129	77
12[50]	145	33	251 +488 273-96	213 239-48 329	105	62	230 +338 261-53	214 245-64	117
12[55]	142	33	165	98	131	63	110	106	154
1[28]	143	34	249 +645 252-99	172 200-150	159	64	210 251-79	208	204
1[0]	144	34	181	214 379	208	66	+510 127	304? 306-71	261 265-112 153

	T	1	2	3	4	5	6	7	8
1 15	148	35	250	274	229	64	258	147	141
			272-110 +883	282-88 888	247-122 (125)		269-82		
1 25	150	40	154	170	154	66	164	228	181
		+931	232-114	219-126 881				263-118	
1 35	149	40	150	162	198	70	249	172	234
							269-73		247-104 296
1 45	150	43	205	219	230	70	118	231	140
1 55	154	46	246	262	261	75	161	275	180
			+ 254-110	267-119 829	273-116 282			285-113	
2 05	154	43	129	131	134	71	199	136	215
2 15	152	45	147	151	163	74	204	163	241
2 25	150	47	159	161	187	76	217	168	270
									278-115
2 35	150	43	162	163	204	77	230	165	130
2 45	154	48	172	166	221	77	235	165	148

4/14/50. Cells had been kept in refrigerator

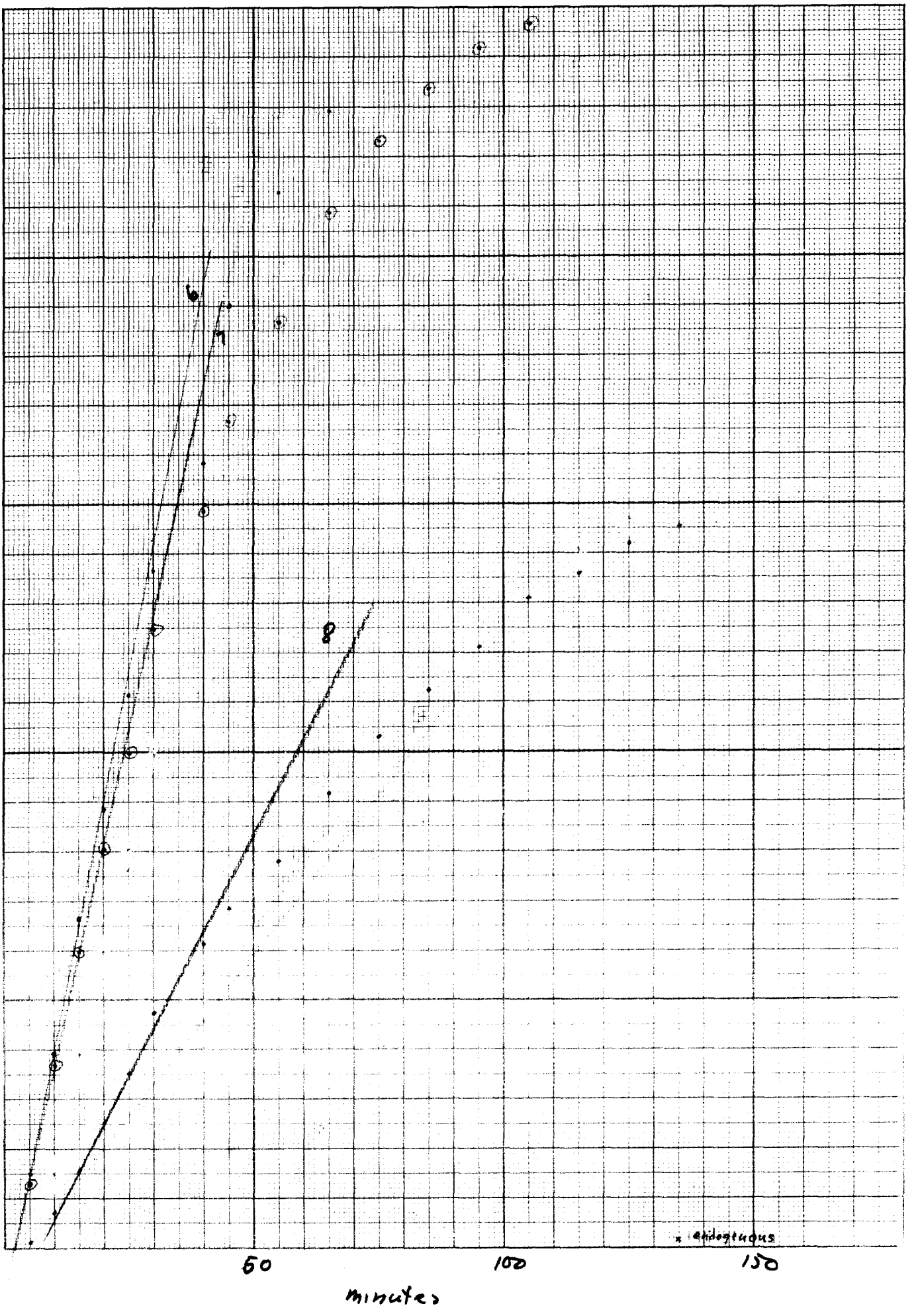
Estimate optical density at 4200 Å. dilute in distilled water

A	10^{-2}	287
B	10^{-2}	281

min.	T	Th	1	2	3	4	5	6	7	8
0	1230	0	0							
5	1235	0	-1	68	56	4	-1	60	52	5
10	40	-1	-1	173	151	20	-1	158	148	29
15	45	-3	0	268	235	44	1	263	239	63
20	50	-5	1	362	322	70	1	355	~~325~~ 322	101
25	55	-2	4	456	401	99	5	446	~~398~~	141
30	1300	-3	4	539	474	126	5	545	499	190
40	10	-4	3	623	565	174	6	633	594	246
45	15	-8	0	688	621	191	0	760	668	275
55	25	-10	3	782	724	239	0	851	747	313
65	35	-9	4	867	810	284	6	937	837	367
75	45	-12	4	919	864	313	2	999	893	413
85	55	-14	5	958	905	342	1	1040	935	451
95	2 05	-14	2	985	922	372	1	1078	968	486
105	15	-12	6	1005	944	~~364~~ 343	6	1085	997	514
115	25	-12	8	1117	954	427	8	1098		543
125	35	-10	6	1022	958	446	11	1113		568
135	2 45	-14	7	1028	957	459	7	1114		582
Flask Const.			1.84	1.64	1.69	1.81	1.58	1.60	1.70	1.58
Initial linear rates:		mm	~~1224~~	1224	1080	262		1230	1080	
per hour.								~~1964~~	~~1870~~	390
		μl/h.		2007	1825	474		1968	1836	616
		N/L	=	.24						.335

W1301/Lac

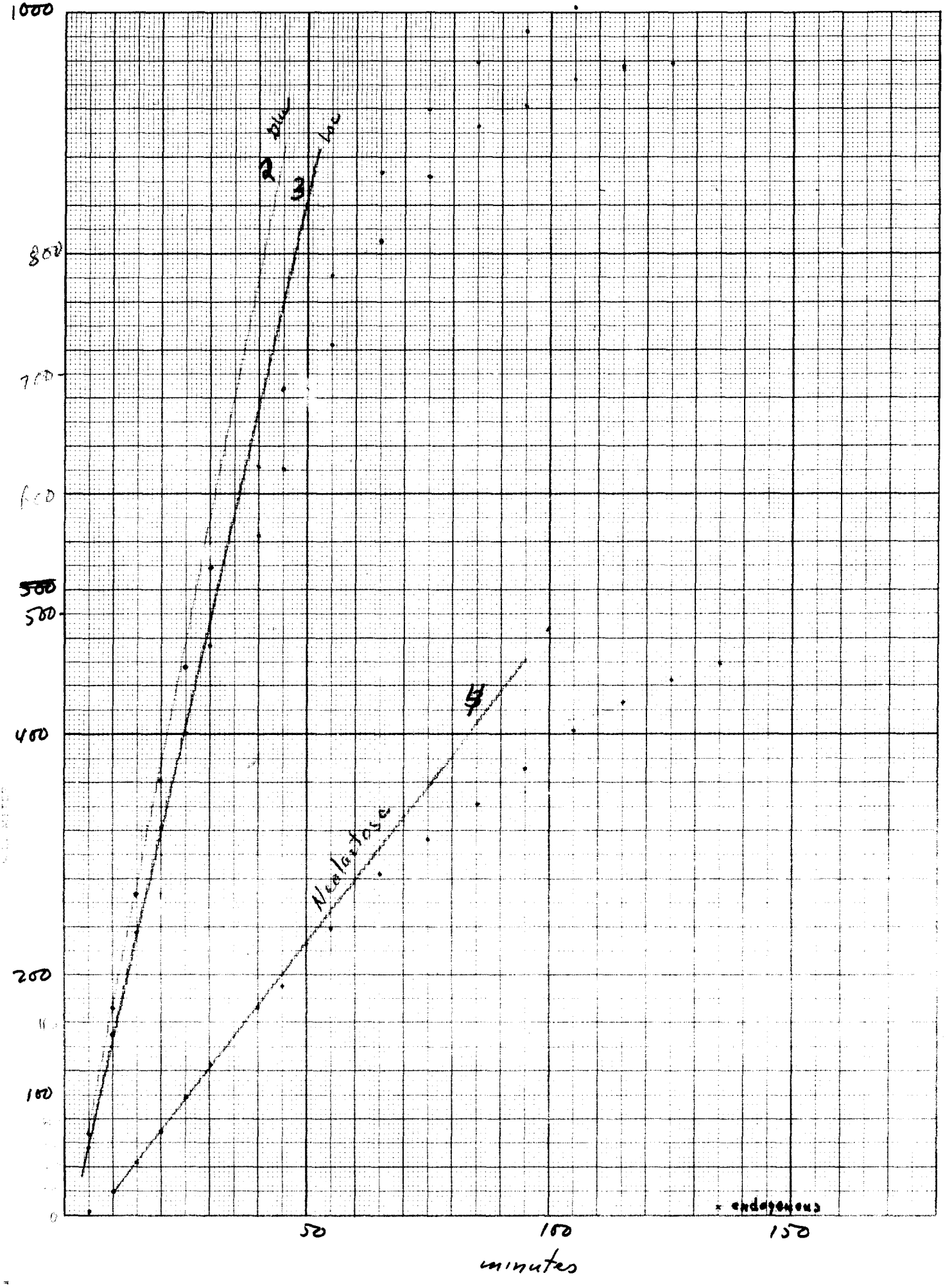
730



mm.

58-161/Lac

730



Nicotine response

730A

April 10 (9?) 1950.

	Cells	Substrate	Flask
1	3	—	12A
2	3	Glu	13A
3	3	Lac	5B
4	3	Nicot	2B
5	6	—	10B
6	6	Glu	6B
7	6	Lac	7B
8	6	Nicot	8B

3 = 58-161 / Nicotine N2 agar

6 W1301 " " "

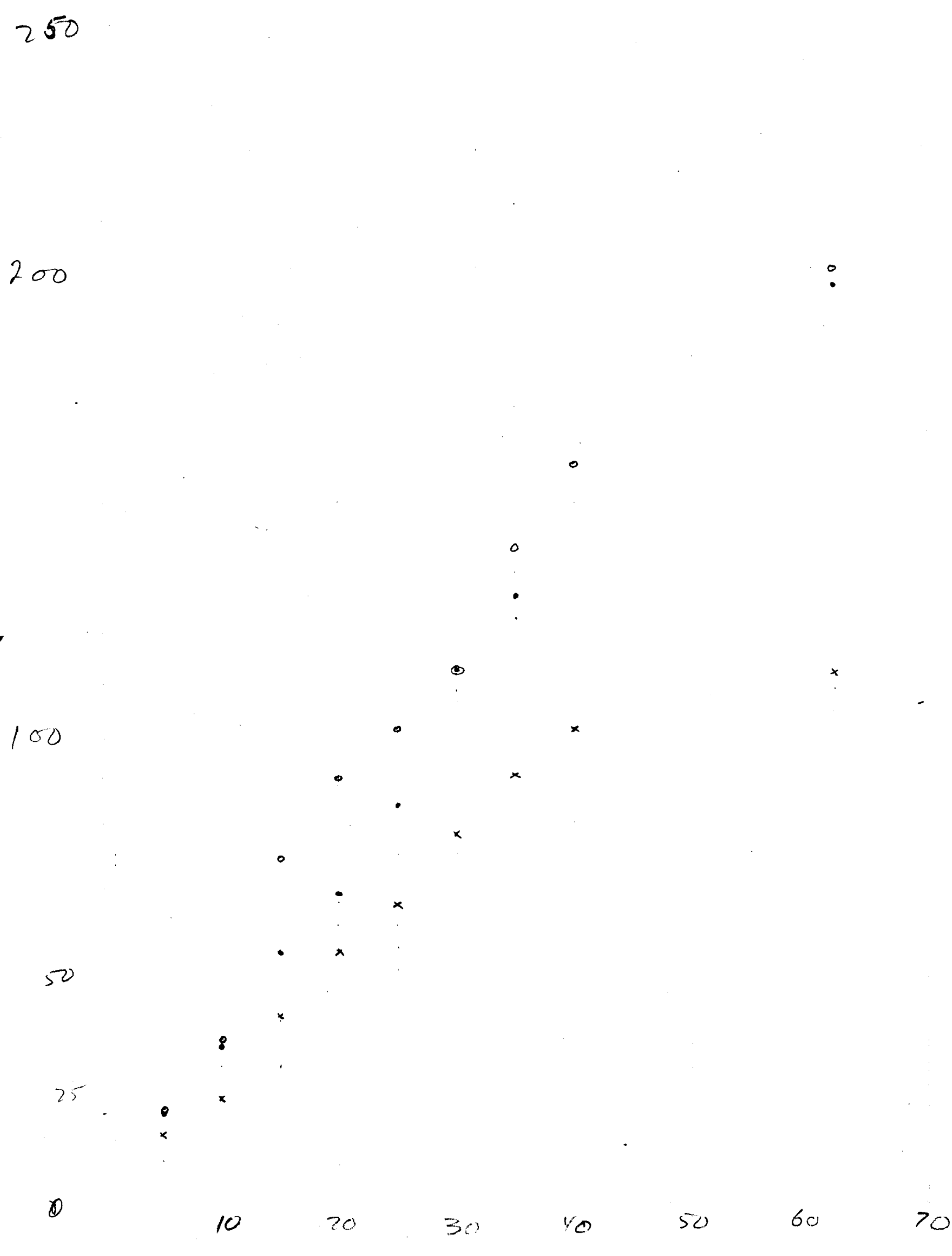
barometer very low!

	T	1	2	3	4	5	6	7	8
310	149	68	45	61	75	9	39	60	68
320	147-50	64	45	61	75	9	39	62	72

TIP

320	149	64	47-	58	73	4	34	57	62
335	150	68	68	65	79	12	58	74	70
340	151	68	83	65	78	11	72	82	70
345	156	74	108	71	83	17	95	105	76
350	156	73	121	70	83	16	115	118	81
355	159	75	143	74	86	17	136	131	81
~400?	159	75	170	76	90	22	157	145	86
~405	164	81	192	80	91	22	189	164	94
~410	165	82	(14) 191-112	81	92	24	218	175	97
" 415 (4322)	156	72	181 (15) 182-131	73	81	14	250 256-67	187 193-95	88
" 445"	158	77	182	79	87	17	119	119	99

98
57
41


250
200
100
50
25
0
10
20
30
40
50
60
70

Calculations

	T	Th	—	Gluc 1	Lac 2	Molac 3	—	Gluc 6	Lac 7	Molac 8
330	0	0	64	47	38	73	4	34	57	62
335	5	1	+3	20	6	5	7	21	16	7
340	10	2	+2	34	5	3	5	36	23	6
345	15	7	-1	54	6	3	6	54	41	7
350	20	7	-2	67	5	3	5	74	54	12
355	25	10	-3	86	6	3	3	92	64	9
400	30	9	-2	114	9	6	9	114	79	7
405	35	15	-2	130	7	3	3	140	92	17
410	40	16	-2	+32	7	3	4	158	102	19
432	62	17	-13	196	-2	-3	7	199	113	9
445	75	7	+6	265 (83)	14	7	6	257	153	30
				•				o	x	

data in the curve

See Protocol for repetition 730B

Outcross "stable diploid" 731
see 727

April 10, 1950.

Cross 727-1 and -3 as follows., on EMS lac

A	1-	x	Y10	Streaks of 727-1 and -3 streaked out. 99%+.
B			W677	
C	3		Y10	
D			W677.	

A and C gave good yields, almost all lac+. B, D gave ~~poor~~ ~~yields~~ moderate yields, lac-:lac+ ca 3:1

Pick + and streak out on EMB lac.

A: 100+. No distinctive lac v but some colonies have lighter, possibly mottled ~~ed~~ centers. Mark these for further purification. Repick all to EMB Mal, MH, Xyl.

B

C. 7~~8~~+ As A.

D 2 tested: Hold in cold

A. Spot-tests of purified lac+ [1-6 excl. 3. all Xyl+ Mtl+
20, 21, 22, 40, 53, 54, 55, 56, 93 Mal –
others Mal+.

2. All Mtl ++. All Xyl+ #~~8~~5, 87 Mal – 87 Mal-+?
others Mal+
+Hold Mal plates in refrigerator.

Treg 727-3 x W588

A. 6 restreaked on lac EMB as possible from lac V.

Each of these threw off carriedable lac - and has appearance suggesting a rather stable lac V.

C. Ditto. C3 destinctly variegated. } Each is pure Mal +.

B. 12 EMS lac + picked and streaked on EMB Lac Colonies resemble those of A + D. #1, 2 are like C3

D. like B.

Kept on EMS Lac

Pick single "4" colonies and restreak on EMB....

B:	lac	Mal	Xyl	Mtl
1	++ -	—	+	+
2	++ -	—	+	+
3	++ -	—	+	+
4	++ -	—	—	—
5	V	—	—	—
6	++ —	—	+	+
7	++ V	—	—	—
8	++ —	—	+	+
9	++	—	+	+
10	++	—	+	+
11	++ -	—	+	+
12				

D:

	Lac	Mal	Xyl	Mtl
1	++ −	−	++	++
2	++ −	−	−	−
3	++	−	++	o
4	++ −	−	++	++
5	+ −	−	−	−
6	++	−	+	+
7	++	−	+	+
8	++	−	−	−
9	+ −	−	+	−
10	++	−	++	+
11	++	−	++	+
12	+ −	−	−	−
13	+ −	−	−	−
14	++ −	−	++	++
15	++	−	++	++
16	++ −	−	++	++
17	+ −	++ −	++	++
18	++	++ −	++	++
19	✓	− −	−	−
20	++ −	++ −	++	++
21	++ −	++ −	[illegible] −	−
22	+ −	−	++	−
23	++	−	++	++
24	++	−	++	++
25	++ −	−	[illegible] −	−
26	++ −	−	++	+
27	++ −	−	++	+
28	++ −	−	++	+
29	++ −	−	++ ++	+
30	+ −	−	−	−
31	+ −	−	++	+
32	++	−	++	+
33	+ −	−	−	−
34	++ −	−	++	++
35	+ −	−	++ ←	[illegible] −

Constitutive galactosidase of W1301

April 18 ca., 1950.

A 58-161 Glu — 2 plates each D(x2) + glucose 0.1%
B " —
C W1301 Glu — growth in 10 ml H_2O. .1 ml in 10 ml
D " — Residue in spotplate for dying.

A	383	403 (20m)	5.8	Add benzene to each tube and refrigerate
B	209	236 "	3.1	
C	388	++++	180	
D	262	++++	144	
—	001	014		

C and D are too active to assay at this dilution. Use .01 ml/10

1u = Δ=100 in 20m.

				Units/ml
C'	048	150 (10m)		180
D'	027	113 "		144

E, F are suspensions from 4/15. Dilute benzene treated suspensions 1:100, use .2/10 E = 58-161 F = 1301 /no sugar

	Di	Donpg (10m.)	
E	003	017	?
F	004	222	>2000

ca 150 before activation. See Manometric Protocols 4/12.

4/15 See manometric protocols 4/12 called.

∴ The "constitutive" lactase differences persist through "activation".

4/20. Qualitative test (spotplate) for galactosidase in W1301 grown in D (BMTLB,) — various sugars.

Glu	+++
Lac	+++
Mal	+++++
Gal	+++
Ara	— ?
Mtl	++++
Stl	++++
K. Dna.	+ -?

April 18, 1950.

W1301

A. DN2 — 6 plates Use ca 1/5 in 20 ml

B. DN2/Glu 7 plates. Use ca 2/5 in 20 ml

Hold 24 hours. Run manometry 4/19. See Protocols.

Bottle's experiment, the cells (B) were tested on a variety of substrates glucose, galactose, maltose, lactose, (but not arabinose.) were rapidly utilized by B. Later that PM, with same suspensions, xylose, sorbitol, mannitol and gluconate were tested, and were not utilized:

+ A	— B
glucose	arabinose
galactose	xylose
lactose	mannitol
maltose	sorbitol
	gluconate

This was taken to mean that W1301 is preadapted to sugars A, because previous work had indicated that K-12 was not preadapted under these conditions. But see 4/20.

Cells (A) also utilized lactose and maltose, but rather more slowly in relation to glucose.

Cell density of suspensions used. Dilute: .1/10 ml

	Di	D_{onpg}^{10}	Δ	Δ/Di
A	128	370	236	1.84
B	160	560	397	2.48
Δ_{onpg}		019		

W1301 : Synthetic medium.

April 18, 1950.

~~Synthetic~~ Grow 20h. aer. 37°. D (Lac or Glu) + BM 1/10

R.A.

			Di	Dorpg	$\lambda^{corr.}$	Di 1:10	Δ/Di
1	58-161	Glu	016	033	1	208	—
2	"	Lac	034	190	142	321	~~4.3~~ 4.43
3	W1301	Glu	020	620	585	228	25.6
4	"	Lac	036	434	385	332	11.6
	—		~~4~~	017			
5	58-161	Glu	208	200 (20m.)	—		—

Thus W1301 produces a "constitutive" lactase on very simple medium.

Selection against W1301 is anticipated in non-lactose medium. Transfer successively in D(BMTiB.) glucose, lactose, and maltose. Streak out on EMBLac. 1st transfer from W1301/Glu above. 0th: pure lac+

0th: colonies from EMBLac picked to DN2 Glu. Spot test for constitutive lactase:
12 colonies: all onpg+

1: 12 from Mal to DN2 Glu } all onpg+ 58-161 4 colonies
4 Lac
4 Glu
onpg−.

2: EMBLac − all lac+.

3: " " . Test from DN2 Glu to onpg. 4 each: onpg ++

Galactosidase in W1301: Synthetic medium with various carbon sources.

~~[redacted]~~ 734a.

April 20, 1950.

W1301 grown on ~~[redacted]~~ D BATTLE, ... overnight ĉ aeration

	Sugar	.1/10 Di	Donpg	1/10 D	Δ	Δ/Di 10 R.A.	Galactosidase spot test
1	Glu		269	157	240	15.3	+++
2	Mal		930	146	900	62	+++++
3	Lac		372	149	340	23	+++
4	Arab		119	156	90	5.7	±
5	Mtl	10	452	146	420	29	++++
6	Stl		875	153	840	55	++++
7	Gna	5	128	160	100	7.9	+
8	Salac		320	168	290	17	+++
0	—		021				

fermentable ĉ Incl Na2CO3 at 10 m.

Repeat expt. with fresh cells. 0.1/10

		Di	10 Donpg	1/10 Di	Δ cor	Δ/Di 10
1	Glu	0.8	202	114	197	17
2	Mal		538	223	533	24
3	Lac		147	180	142	7.9
4	Arg		109	208	104	5.0
5	Xyl		179	182	174	9.6
6	Succ. 2 ml		373	120	376	15

234 -2

as above.

Verification: Preadaptation of W1301 to maltose, galactose

735

April 20, 1950.

Flask	T	Cells	Sidearm (10 mg.)	uncorr. Δ 40 m.
9B	1	A	—	1
2B	2	A	Glu	300
3B	3	A	Gal	167
5A	4	A	Mal	202
10A	5	B	—	1
6B	6	B	Glu	107
4A	~~8~~	B	Gal	94
8A	8	B	Mal	80

A = 58-161 2 plates DN2 Glu } 10 ml.
B = 1301 1 plate " . }

2 ml per flask. M/20 $NaHCO_3$ CO_2.

Time → / Flask ↓

	200 ±	210	225	240	317	uncorr. Δ 40M.
T	155	152	155	152	154	-3
1	13	11	15	13		1
2	18	72	203	>310		
3	43	91	186	217		
4	47	85	162	246		
5	52	52	52?	50	54	
6	54	76	115	158	265	
7	61	78	111	152	244	
8	59	77	104	136	205	

Galactozidase: Use .1 ml / 10 minutes

	Dil	10 mg. Dialyzed
A	128	870
B	160	560

	Δ 40 mg	019.	Δ	R.A.
A	319	329	023	~~.072~~ .072
B	132	273	137	~~1.04~~ 1.04

Preadaptive Galactosidase tests on F.2.L. "suppressor" cultures

April 19, 1950.

Grow from EMB colony to Permassay 10ml. Wash once:

See EZL code

	Di	Dongg	
A	010	−	
B	002	−	
C	009	−	
D	007	++	W716 B
E	2DIE 0/9	±	W716 C
G	017	++++	Read diffuse ?? between Lac and Mal
L	019	++++	
M	016	++++	

see 734/-1

Quantitative readings interrupted by vacation.

Repeat "O" from DN2/Glucose = W716B. in ca 10ml

		Di	Dongg 20
4/20/	D	083	1070
	—	0	019

W1301 x W945

April 20, 1950.

"C" W1301 x W945 on EMB Lac

+, slow, – colonies seen.

Purify.

28 Lac+ — 2 possible npg – — no! Both are npg+
Replate on DN2 glu

12 Lac slow — 1 possible npg – — But very slow
Replate and streak out EMB Lac

Store purified cultures on NSA slants

Restreak purified Lac – prototrophs and hold on EMB Lac.

5/6. Pick separated – and Lac+ revertants (corresponding) to T(0), for test for constitutive lactase

"W1312" is not a prototroph! Repeat cross! (W1301 x W1177
W1301 x W1178)

---

ca 50 Lac – allowed to revert and Lac+^{R} tested from D(0) glu for constitutive lactase. None were Const+.

Conclusion: Const locus is closely linked to Lac, or Const+ is epistatic to Lac–.

TRY: Lac,– on EMB Neolactose

Preadaptation of 58-161; W1301

737

A- 58161
B= 1301 April 21, 1952.

	Cells		Flask.	0	↓ 340	350	400	410	435 +
					Time →				
1	A	—	B	26	25	23+	28	27	25+
2	A	Glu	A	75+	75+	101	156+	168	257
3	A	Gal	"	70	70	93	127	157	212
4	A	Mal	"	47	47+	64	88	106+	163
5	B	—	"	31	25+	26	29+	27	28
6	B	Glu	"	20	20	58	99	136	242
7	B	Gal	"	23	19	49	84	114+	201
8	B	Mal	"	56	54+	77	101	122	187
Thermobarometer.				136+	136	140	140+	139+	140
				0	↓				
9	A	Lac	9B	34	TIP 35	40	38	41	40
10	B	Lac	11A	61	67	97	118	150	179
Thbm					140	142	137	141	139
					440 ↑	452	500 (-1)	510	520

∴ 58-161 is preadapted to galactose, maltose, glucose but not to lactose. W1301 is preadapted to lactose.

K12?
Y10?

A, B gram on DN2 Agar

Cf. K-12, 58-161, 1301 for preadaptation

April 22, 1950.

Flask	Cells	Subst	12 50	100	↓ 105	110	120	130
10 A	K-12	Glu	08	09	29	83	174+	297
2B		Gal	50	51+	68	113	179+	202
3B		Mal	31	29	34	50	88+	147
4B		Lac	58	59	60+	60	63	67
5B	161	Glu	41+	42	58	114	209	>300
6B		Mal	54	53+	61+	82	140	224
13A		Lac	51+	52	58+	60	64+	64+
8B	1301	Glu	67	68	76	128+	216	310
9A		Lac	28	29	30+	83	166+	246+
	Thermobar		134	135	136	138	143	137+

2 plates DN2 Glu/10 ml 2 ml each.

∴ K-12 grown on D(N2), Glucose is preadapted to galactose and maltose, but not to lactose. This speaks for a medium influence previous work with cells gave no such preadaptation. Compare the media used!!

Bacterial densities: .1/~~ml~~ 9 4200Å.

		D.	Galactosidase spot
	K-12	329	±
A)	58-161	460	−
B)	W1301	400	+++

Assay A and B for galactosidase. Then add benzene and store overnight.

Separate aliquots. To A; B' add ~~toluene~~. Octyl alc.

Preadaptation of K-12.

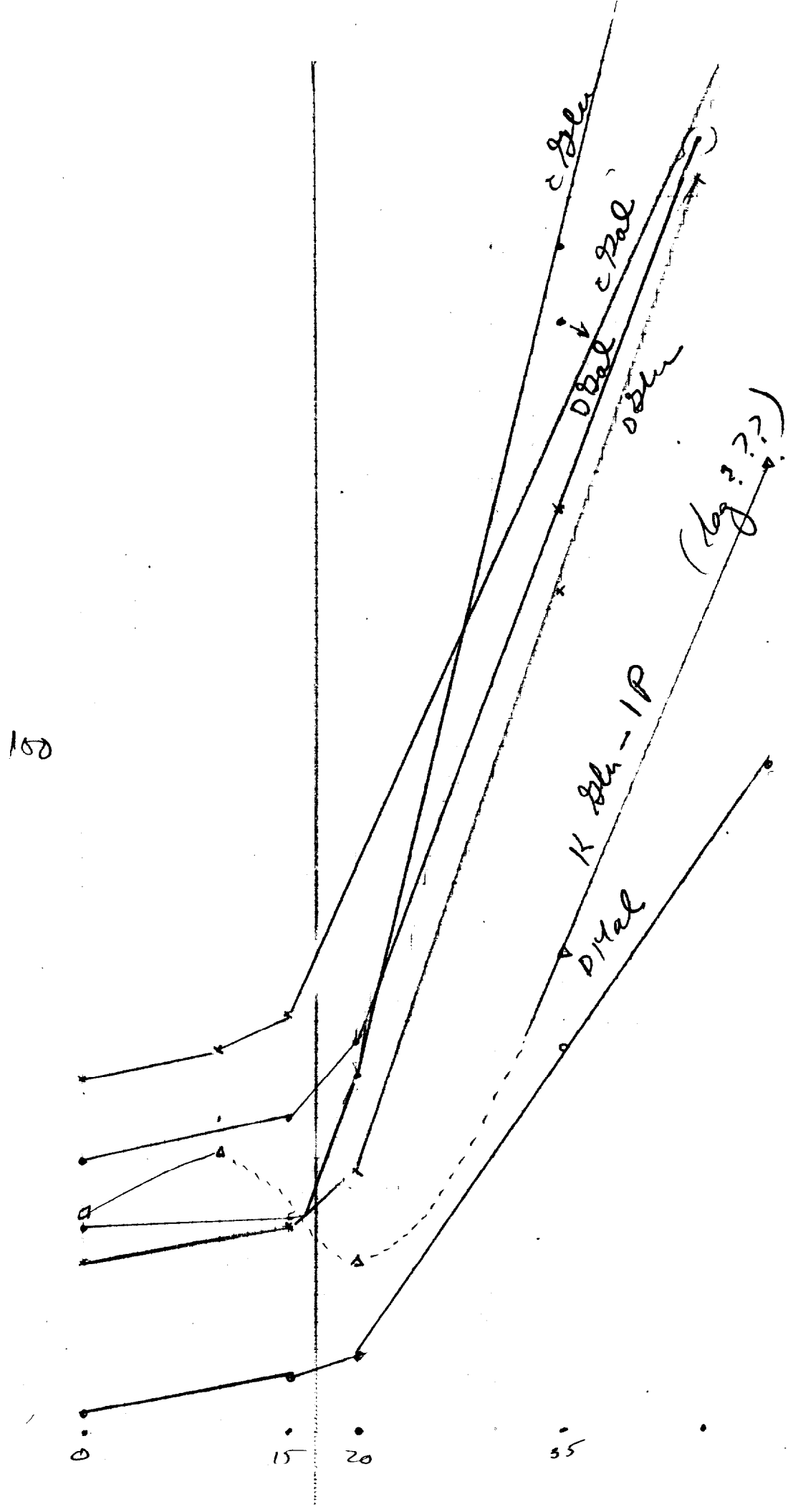
April 25, 1950.

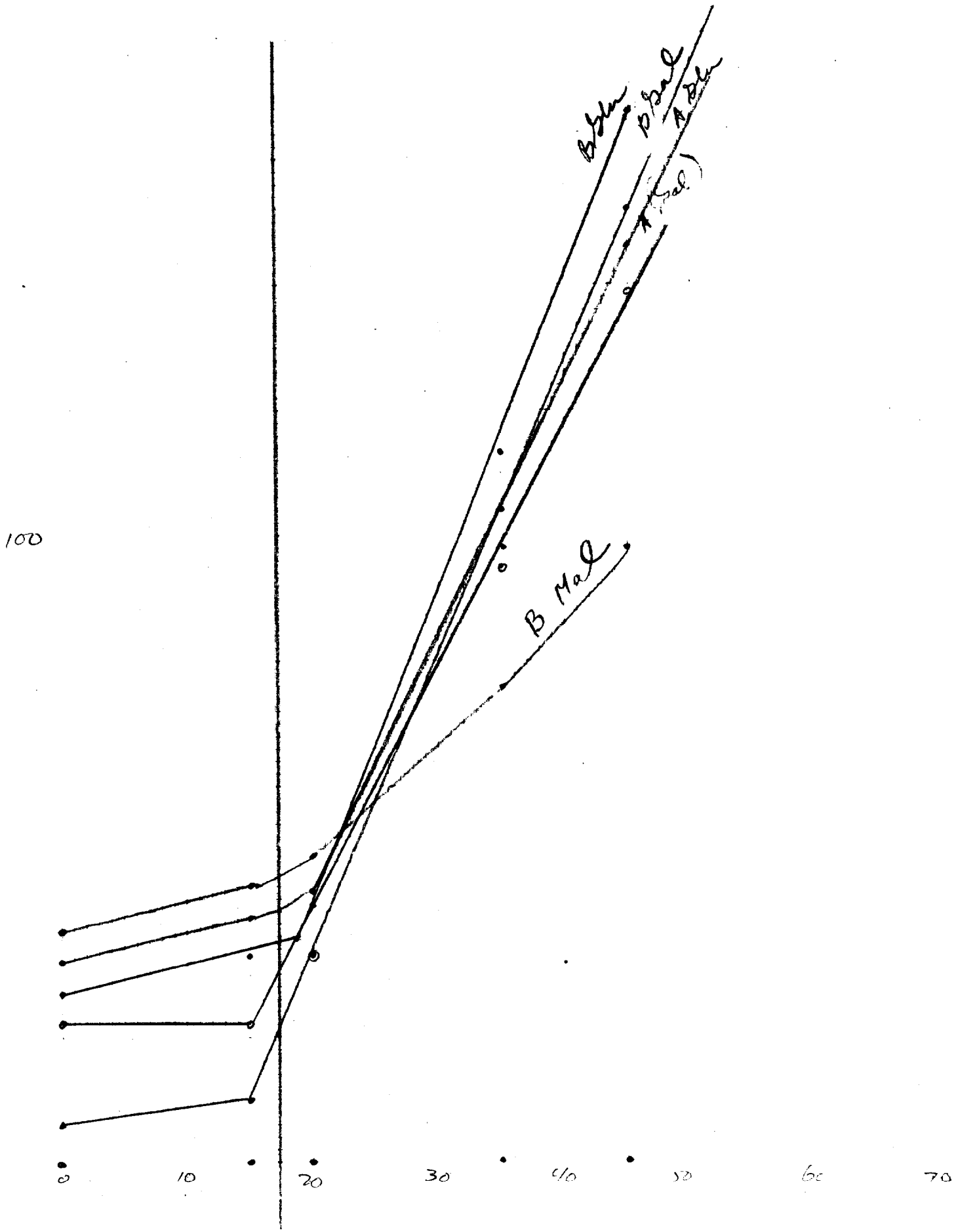
A	Perm.		100 ml
B	"	Agar	100 ml (3 plates)
C	D N2	Glu .1%	"
D	"	Agar	"

Flask	Cells	Side 1	Side 2	0	15	(1) ↓	20	35	45
				4:30	4:45		4:50	5:05	5:15
B 1	A	Glu		06	10		33'	104	152'
A 2	A	Gal	Mal	22	22		33	95'	139
6A 3	B	Glu		27	33		41	113'	167
7A 4	B	Gal	Mal	32'	39		43	98'	146
A 5	B	Mal		37'	44		49	76'	98
3A 6	C	Glu		30	31		52	173	252
4A 7	C	Gal	Mal	40	46		57'	162	188
A 8	D	Glu		25	30		38'	123	188
B 9	D	Gal		52	56		61	135	183
A 10	D	Mal		03	8		11'	56'	97
A 11	D	Glu-1-P 5 mg (.25 ml)		32	41'		25	70'	141
3B/12	TB			135'	137'		137/ 140	140	low pressure day!

These data show a clearly constitutive glycolysis of maltose and galactose from E. coli K-12 harvested from Permassay! They also show glycolysis of glucose-1-phosphate (having simple phosphatase!)

(See Lebiedeff on metabolism of E. coli)


100
0
15
20
35
c Glu
c Val
D Val
D Glu
K Glu - 1P
(log ? ? ?)
D Met


B blw
B Bal
A Blw
A (Bal.)
B Mal
100
0
10
20
30
40
50
60
70

Irradiation of #226

April 25, 1950.

Dilute #226 stock (from D Lac 4/24) 2×10^{-6}.
Retain aliquot; UV aliquot 20 sec. at 50 cm. Plate .05 ml (−) (uv)
on EMB Lac EMS Lac EMB Mal

A. Unirradiated 10^{-7}
B Irradiated 2×10^{-7}.

A	EMB Lac	V	−	Mal EMB	"+"	Vars	−
	1	45	29		9	17	1
	2	22	18		8	17	3
	3	61	37				
	4	51	32				
	5	16	10				

B	EMB Lac (2x)	V	−			
	1	11	27	3	8	1
	2	6	36	7	3	3
	3	2	12	12	18	4
	4	13	27	2	3	1
	6	40	23	10	3	0

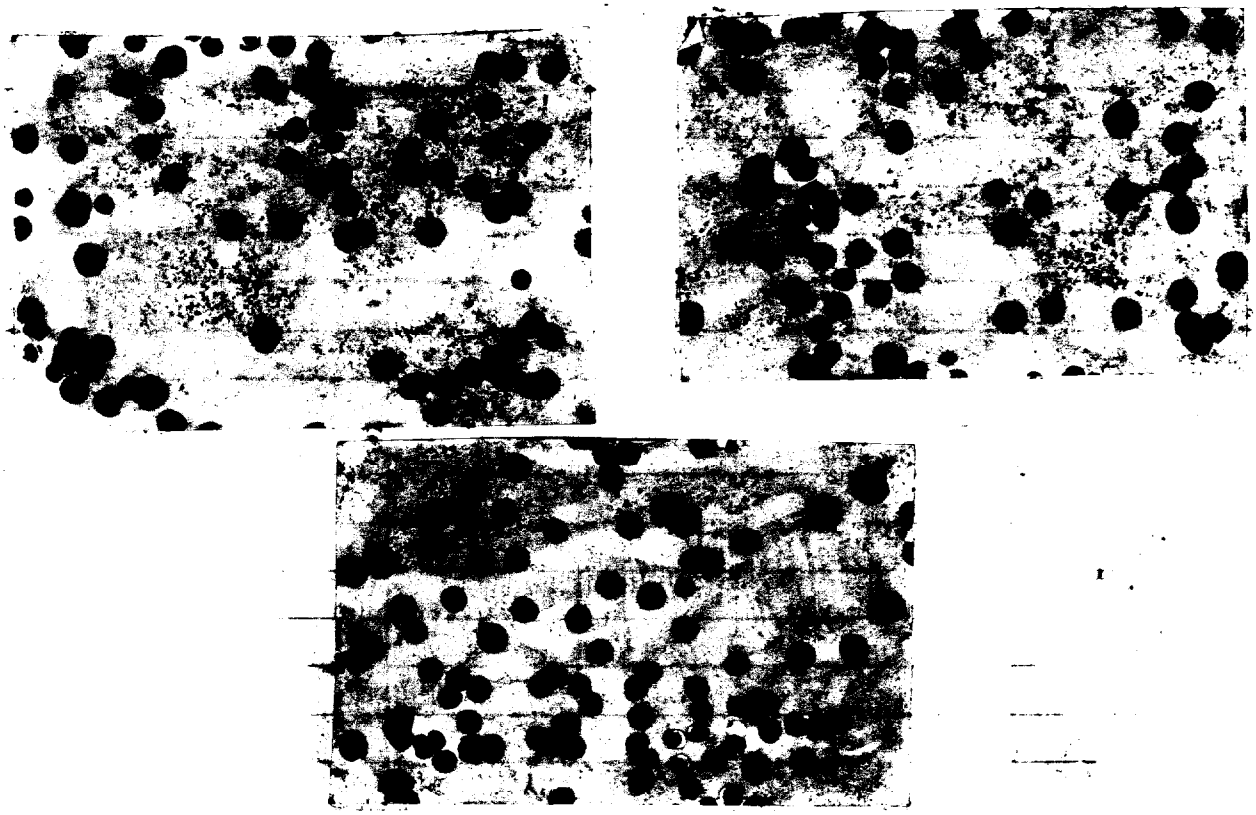
EMS Lac:

A 12+ 0−; 33+ 2− 0 Sectored.

B
0+ 1−
3+ 1−
5+ 3−
2+ 1−
4+ 2−
1+ 0−
1+ 1−
0+ 1− 1 sectored.

16+ 10− 1 sec.

Repeat for larger number.



A

B

Irradiation of H226

April 30, 1950.

Dilute to ~~10^{-4}~~ 10^{-4} for irradiation. A. Control. Plate at 10^{-7}

B. 20 secs UV. Various dilutions.
("7" = 2×10^{-7})

Plate on EMB Lac; Mal EMB Lac; Mal.

A. EMB Lac

v	–
116	8
128	18
115	8
121	19
480	53* / 533

EMB Mal. 2 plates.
+ and v not distinguishable with certainty.
No – seen.

* 4, circled, later scored as Lac v

B EMB Lac

B7 24h.

24	58
47	63
71	121

} all v are delayed.

Mal: See above. All are + or v.

B7 42h.

34	53
~~48~~ 52	~~36~~ 45
86	98

Jerad. H226

May 3, 1950.

A. EMS Lac	+	−
	128	0
	130	0
	94	0
	88	0

Mal	+	o
	129	0
	155	0

B	EMS Lac	+	−	sect.
	"B7"	33	25	10
		32	20	5
		30	18	6
		46	19	10
		141	82	31

Note "induced" lac - "mutation".

B6: too dense to count well. However, on each of two EMS Mal, no - or see were seen! Streak out Lac see on EMS Lac

---

H226 from A; EMS Lac : + were streaked out as EMB Lac, etc. Each of 8 colonies was

Mal++ Xgl+ Mtl+ Lac+ ! This is, then, a partial segregant! Pick to slant as 739-1.

Reisolate type H226 from slants.

Kinetics and stability of cellular lactase

April 25th. 1950

Harvest X-12 from aerated Y2 Lac (1%?) after ca 18 hours. Wash 2x and concentrate from 100 to ca 10 ml. Remove ca 2-3 ml and toluene with benzene at room temp. for autolysis overnight. (B). Assay remainder with orpg M/2000 in Na P buffer M/50 pH 7.5 [Stored overnight in refrigerator]

On basis of preliminary assay, dilute A 2/50 and B 1/500 to give convenient ranges of activity in 20 minutes.

Assay system: 5 ml H_2O 3 ml M/15 Na P buffer 7.5 1 ml cells. 1 ml substrate (or H_2O). Add 1 ml Na_2CO_3 M/1 to stop reaction. Use dum scale of spectrophotometer. Time with stop watch, with 30 sec. interval between additions. Run in 38° "Precision" water bath with motor stirrer. Preliminary turbidity gives variance of tubes. Use volumetric pipettes. Remove from bath to room temperature when Na_2CO_3 is added.

Check, time sequence with A.

(1)

	Time	D:		D_{orpg}	Na_2CO_3 ①	+1 hr	$A^{corr.}$	A^{corr}_{D}
1	5	100		134		133	080	84
2	"	095		220		220	166	161
3	"	098		353		357	299	298
4	"	097		458		462	404	403
5	"	099	(085) (081)	045		038		
6	"	−008	(+004) 000	001		013		

no orpg / no cells

These data are clearly non-linear !! (presumably due to "activation" ~~of~~ during assay !)

Corrections

① = −009 / −045 / −054

② = 021 / 038 / −059

(2) ~~Incubate cells in buffer prior to assay (12[45] to 4[30] PM)~~

April 26, 1950.

A Y2 –
B Y2 0.1% glucose } agar 1 plate each. A, C to 7 ml
C Y2 1% glucose B to 10 ml

Flask	Cell	Sub	5'	10'	15'	TIP ↓ 25'	30'	35'	45'	55'	A200m + Substrate
B	1 A	Gal	51	51	55	64	78	89	117	155	91
A	2 A	Glu	37	34	37	51	68	78	109	150	99
"	4 B	Glu	63	57	61	76	97	114	161	218	132
"	5 B	Gal	52	44	47	57	75	91	134	175	118
"	6 B	Mal	45	33	38	48	54	58	76	101	53
"	7 C	Glu	54	45	48	71	95	115	166	209	138
"	8 C	Gal	39	27	32	53	73	92	136	131	78
"	9 C	Mal	47	36	39	47	46	43	50	39	—
"	10 B	Glu 1mg	7	− 3	− 3	7	30	47	106	143	136
"	11 B	Glu .5mg	23	16	13	20	40	54	114	107	87 ≡
	TB		169	171	171	176	180	180	189	189	13

Use 2 ml. per vessel. + .1 ml 10%

Readings by P. Phandi.

Note: Utilization of maltose by B but not C. Galactose utilized by each of them! (at a good rate). Experiment needs repetition!! Galactose is preadaptive! (purity of galactose ??)

Utilization of galactose; maltose
K-12 from Y2

April 28, 1950.

A. Y2 — 0.1% glucose agar — 2 plates
B Y2 glucose 1% — 2 plates
} to 2 ml per vessel
ml. .1 ml 10% substr.
Both Galactose and Maltose Recrystallized.

Flask		Cells	Substr	Time → 0:00	10	20	↓ 25	30	35	45	100	A_{24}
1A	1	A	—	05	07	07	9	6	8	12'	15	3
2B	2	A	Glu	32'	35	35	65	121	191	310	—	24
3B	3	A	Gal	44	49	47	48	48	48	56	66	8
4B	4	A	Mal	45'	52	50	58	66	81	116'	308	58
5B	5	B	—	37'	46'	42'	44	44	44	50	52	6
6B	6	B	Glu	16	27	22'	55	121	185	296'	—	24
9B	7	B	Gal	46	58	54	53'	57'	55	61	69	8
8B	8	B	Mal	54	65	62	65'	71'	69	73	79	8
		Th Bac.		146'	156	151	151	154	152	155	157	4

Thus K12 from .1% glucose ferments maltose ca 1/5 - 1/4 the rate of glucose; from 1% glucose, at only a negligible rate! Galactose is not used significantly by either, previous discrepancy presumably due to impure galactose.

W1301 - Preadaptation to galactose

May 1, 1950

-W1301 and 58-161, each harvested from 3 plates DN2 Glu. 1 Wash and resuspend in ~~25~~ 30 ml. 1/20 $NaHCO_3$. Flasks 2 ml cells, .1 ml 10% ~~substrate~~ in sidearm.

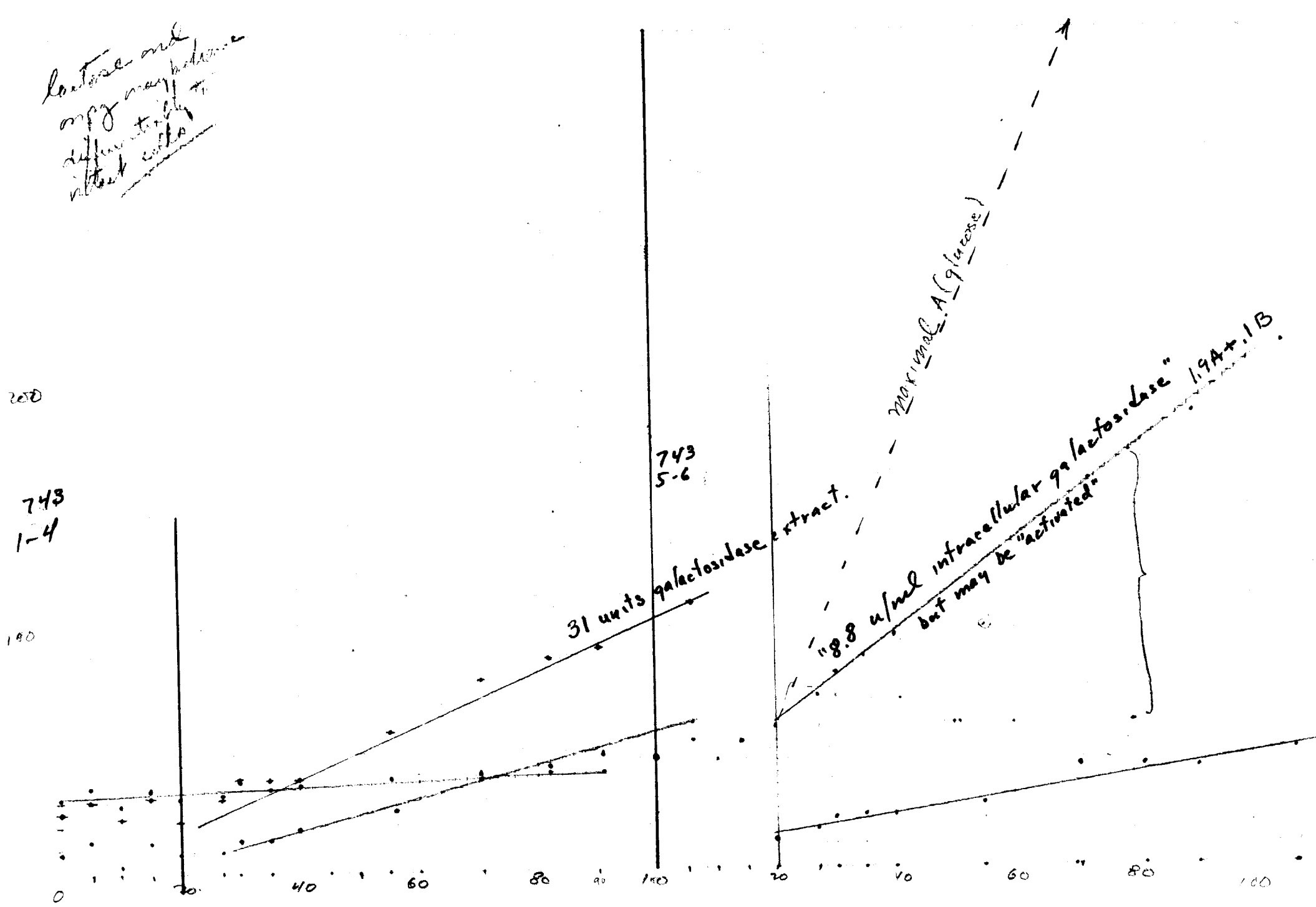
			0	5	10	15	25	30	35	45
1A	—	161	55	57'	59'	57'	61	64	62'	61
2A	Glu .2 ml	161	48'	50	61	93	153	183	209	262
3A	Gal	161	13	10	10	17	20	20	23	21'
4A	Lac	161	51	52'	51'	55'	59	58'	61	60
6A	~~Lac~~	1301	60'	65	63	70	71	71'	72	70
5A	Glu	1301	62	64	77'	119	181'	206	236'	290
7A	Gal	1301	76	74	70'	84	82'	82	84'	85
8A	Lac	1301	24	27	28	69	127'	150	177	224'
	Th Bar		133	135	132	138	139	136	140	139

W1301 is preadapted to lactose but not to galactose (galactose should be accumulating!) Hr uses lactose as fast as glucose.

1/10	Di	Donpg		Δ
A	252	260 (10 min)		—
B	249	473 (6 min)	ca 220	∴ B shows an activity of $\frac{4 \times 2.2}{1} = 88$ u/ml.

(Test by B exposed to conditions of Warburg vessel.)

Judging from efficiency of B in mixture with A, B contains approximately 7x the amount of lactase needed to keep pace with glycolysis, assuming equal potency within and outside the cell.


743
1-4
743
5-6
maximal A (glucose)
31 units galactosidase extract.
"8.8 u/ml intracellular galactosidase"
but may be "activated"
1.9A+.1B
200
100
0
20
40
60
80
100
20
40
60
80
100

M243 segregation pattern

743
b

	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl
(1)	+	−	+	+	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	±	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	+	−	+	+	+	−	+	+	−	−	−	−
	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
	−	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	+	−	−	+	+	+	+	−	−	−	−				
	−	−	−	−	−	−	−	−	+	−	+	+				
(2)	−	−	−	−	−	−	−	−	+	−	+	+	±	−	−	−
	−	−	−	−	−	−	−	−	+	−	+	+				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	+	−	−	−				
	−	−	−	−	−	−	−	−	+	−	+	+				
	−	−	−	−	−	−	−	−	−	−	−	−				
	+	−	+	+	±	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+
(3)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
(4)	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	−	−	−	−	−	−	−	−	+	+	±	+	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−				

Gal − Mal − MH Xyl − −
+ + + +
= predominant

All− 108
All+ 6
Gal− +++ 15
Gal+ − − − 5
Xyl/+++
7:0 2

Segregation of H243

May 1, ... 1950.

Picks 8 colonies of H243 from EMS Lac and inoculate into Penassay. After 48 hours, plate out on various media: Dilution

(1)

7	EMB Lac	142 total.	4 clear Lac⁻, A number (±5) of others are faded lac⁻ reminiscent of Lac⁻ - Lac⁻.
	EMS Lac	3+ 0- (some minute: hold)	
	EMS Gal	7+ 1-	
	EMS Mal	2+	
6	S Lac	12+ well defined. several small - hold.	
	S Gal	11+ "	
	S Mal	18+ 6-	
5	S L	++ numerous poorly defined -	
	S G	ca 20 -	
	S M	30 -	

---

(2).

7	B L	196 total	1 Lac⁻.
	S L	0	
	S M	0	
	S G	2+	? - hold.
6	S L	7+	Many small -?
	S G	12+	" "
	S M	1+	
	B L	3+	Many -.
5			
	S M	181+ 31-	2 sec.
	S G	26-	2 sec?

---

(3)

7	B L	84 tot.	2 Lac⁻.
	S L	2+ very +	
	S M	2+ 1-	
	S G	1+ 1-	
6	B L	13 +	Many small
	S G	25+ 4-	" "
	S M	23+ 1-	
5	S L	Many large+.	
	S M	194+ 75- ✓	
	S G	184+ 62-	

Segregation of H243

May 3, 1950.

(4). 7 BL [redacted] SV (1 faint)
SL 4+
SG
SM

6 SL 11+ many small
SG 18+ 1- many small.
SM 11+ 7-

5 SL [redacted]
SG 12-
SM 147+ 33-

Partial segregants. Pick EMS: Gal- and [redacted] Mal- to EMB; [redacted] Lac for partial segregants.

Repick lac+ and streak out on EMS Lac, EMB Lac, Mal, Gal for verification and resolution

(4) Gal- : 6 tests 2 Lac+
Mal- : 22 tests 2 Lac+

(5) Gal- : 33 tests 9 Lac+
Mal- 37 tests 8 Lac+

Segregants: Pick Lac at random and test on four sugars:
(1) Mal Gal Xyl Mtl

Partial segregants

May 6, 1950.

Restricted possible Gal- or Mal- Lac+/- from EMS Lac+.

(3)

EMS →	Lac	Mal	Gal	EMS Lac
1	+?	+-	V(+,-)	
	V	V	V	
	V	V	V	
	V?	—	—	
5	+(V?)	+-	[illegible]	
6	V	V	V	
7	V	V	V	
8	V	V	V	

	Lac	Mal	Gal	Stac
9	+(?-?)	[illegible]-+	—,+	
10	V	V	++	
11	V	V	V	
12	V	V	V	
13	V	V	V	
14	V	V	+V	
15	V	V	V	
16	V	V	+V	
17	V	V	V	

#4, 5, 9 → 1, 2, 3. should be looked [illegible] again. How can the — appearance be accounted for? Each appears to be Lac+ pure, Gal-, Mal-, B_1- } probably many Lac+ crossovers.

(4)

1	V	V	V
2	V	V	V
3	V	V	V
4	V	V	V

M242 Reversions (Mal+ — purified and selected:

1-8: pure Mal+ app. Lac- (i.e., segregated) both old.

Repeat Gal and Mal- from EMS (#1, 3, 4) (ca 3 each)

	Gal-	Mal-
Gal EMS	all -	7+ 7-
Mal EMS	12- 1+	13- 1(-+)
Lac EMS	all -	

Kinetics of cellular lactase.

May 1, 19[illegible]

6 plates [illegible] 1% to 10 ml. 9 ml. [illegible] P.O.
1 ml = dilute to 10. (1:10) = .6 plate / 10 ml [illegible] 10 units to
2 parts [illegible] incubated under benzene [illegible]
[illegible] cores).

Dry [illegible] 10 mg.
Extract [illegible] 5 [illegible] of suspension of dry cells (744)
Hoo[illegible] 4.5 ml [illegible] mucous [illegible]
Make preliminary assay [illegible] kinetic curve
Extract hexadecyl [illegible] 5 m. x 40

Use 1 and [illegible]

Intact cells. [illegible] Di [illegible] Dilution 15

Dry cell susp. [illegible] = .01 ml

Benzene cell susp. [illegible] 6 m

Note very low [illegible] cells may have de[illegible]

744E Add buffer to [illegible]

Enzyme [illegible]

		Di	Dilution		
1	744 A	.192	500	20m.	ca 10 × 3 = 30 u/ml
2	744 B	.022	358	5m.	ca 50 × 4 × 3 u/ml = 6
3	744 E	.059	1470	5m.	

① mg equivalence of bacterial density unit. Let 1 BDU = quantity of bacteria giving gst. tons of .10 in 10 ml.

Then 276 mg dry cells were obtained from 9 ml of a suspension which had a density of $10 \times \frac{1}{.1} \times 1.39$ per ml $= 9 \times 139$ BDU.

$\therefore$ 1 BDU $= \frac{276 \text{ mg}}{9 \times 139} =$ 217 γ/mg.

Discard this expt.

Manometric efficiency

May 6, 1950.

Cells harvested from D V2 1% Glu (stored 24h.) 200 ml agar.

Suspend in ca 40 ml $NaHCO_3$ M/20.

Dispense 2 ml (D) per flask. + added cells.

Flask	Vessel.	Sidearm (10 mg.)	TIME → 0	10	35	↓ 40	45	50	55
B	1	D —	33	34	39	37	43'	43'	41
B	2	D glucose [17]	−02	−07	[18] 06'	[15] 20	103'	167	219
B	3	D lactose	42'	42	49'	50	52'	51'	48
B	4	D + .1ml A lactose	48'	46'	54'	60	64	66	67
B	5	D + .1ml D lactose	24	23'	30'	34	37'	37'	37
B	6	.1ml A lactose	23	22'	25	24'	25	25	25
8B	7	.1ml D lactose	34	32'	35	38'	38	35	32'
9B	9	Th Bac	44	43	41'	49'	48	45'	43

	65
1	38
2 [29 / 71]	78
3	47
4	74
5	41
6	26
7	32
TB	44

Inadequate lactase!

May 5, 1950.

200 ml DN2 Lac 1% K-12 harvested. Wash; suspend sediment in 10 ml. Dry 9 ml (D) aliquot; Dilute 1 ml to 10 (A). Treat ca 2 ml of A with Benzene (B). 40M —

Preliminary assays (see 744.)

A: 30 u/ml. Dilute 1:15
B: 50 × 4 × 3 units/ml, ca. = 600 u/ml. Dilute 1:200

D: 430 mg dry cells (two stage drying).
∴ Calculate 1 BDU = $\frac{430 \text{ mg}}{9 \times 192}$ = .248 mg (Cf. 744 .217

Assume about .23 mg. eg.

D) Prepare a 2% suspension of dry cells. Remove aliquot for use, and dilute 1:10 (2 mg/ml) (D) (2× in anti.)

X_1 Extract remainder 3 hours and spin sediment. (Re-extract = X_2)
Yellow, viscid, opalescent supernatant

Cells

Kinetics of ~~[redacted]~~ un-treated cells. Dilute ~~[redacted]~~ 1/15

	Final conc		Corr A	Corr B	Σ Corr	Δ = V (392)	1/V	1/S
1	M/500	422	79	042	121	301	33.2	500
2	M/1000	339	79	021	100	239	41.8	1000
3	M/2000	260	79	010	89	171	58.5	2000
4	M/5000	177	79	004	83	94	106	5000
5	M/10,000	140	79	002	81	59	169	10000
6	0	079				↓ (Would be 0 in theory)		
7	M/1000 No cells	021						

Terminate with Na_2CO_3. Read within a few minutes.

~~10 r~~ 20 minutes 37° Cells fresh (a few hours in H_2O)

NaP M/50. 10 ml volume + 1 ml Na_2CO_3.

Volumetric Quant Technique

K_s (cells) = 6.3×10^{-4}

V_{max} ≐ 1/25.5 ≐ 392 (3.92 units / ml suspension.)

The cell suspension ~~extracted~~ was 1/15 × 1/10 × 1/9 × 430 mg/ml $= \frac{430}{1350} = 318$ r/ml

(Calculated density unitage : $\frac{2}{3} \times 192 = 128$
≐ 318 r
in perfect agreement, as demanded!)
Lower o.d. due to alkali).

Therefore V_{max} was 3.92/.318 = 12.3 u/mg.

Kinetics: Benzene treated cells.

May 5, 1[illegible].

B) Dilute ~~1:100~~ 1:200 to place in convenient range for assay.

M/—	Dons	Corr	A	1/A	D'	Corr	A'	1/A'
500	482	049	433	23.1	490	039	451	22.2
1000	460	028	432	23.1	461	022	439	22.8
2000	403	017	386	25.9	400	013	387	25.8
5000	293	011	282	35.5	300	008	292	34.2
10000	200	009	191	52.3	197	006	191	52.3
	007				004			
					002			
					033			

H_2O M/500 no enth

See 744 for correction factors.

Repeat readings using a single tube D'

$$K_S = 1.25 \times 10^{-4}$$

Note difference in V_{max} : Cells are $\frac{200}{15}$ conc

$$V_{max} = \frac{1}{21.0} = \qquad 476$$

$$\text{Absolute activity} = \frac{476}{392} \times \frac{\cancel{200}\ 40}{\cancel{15}\ 3} \times 12.3 \qquad 199$$

744a cells

744a cells

180

150

100

50

0 500 1000 K_s 2000 40 6000 8000 10000

$K_s = 6.3 \times 10^{-4}$

Manometric assay of lactase

May 1, 1950.

Dry cells of K-12 harvested from ca 100 ml Y2 Lac, P_2O_5, room temp. Yield: ca 35 mg. Triturate and shake in 3.5 ml H_2O. Sediment and retain supernatant.

a) Preliminary assay in mmg of extract (rather weak! – no pancreatic salt treatment?) → 77u/mol

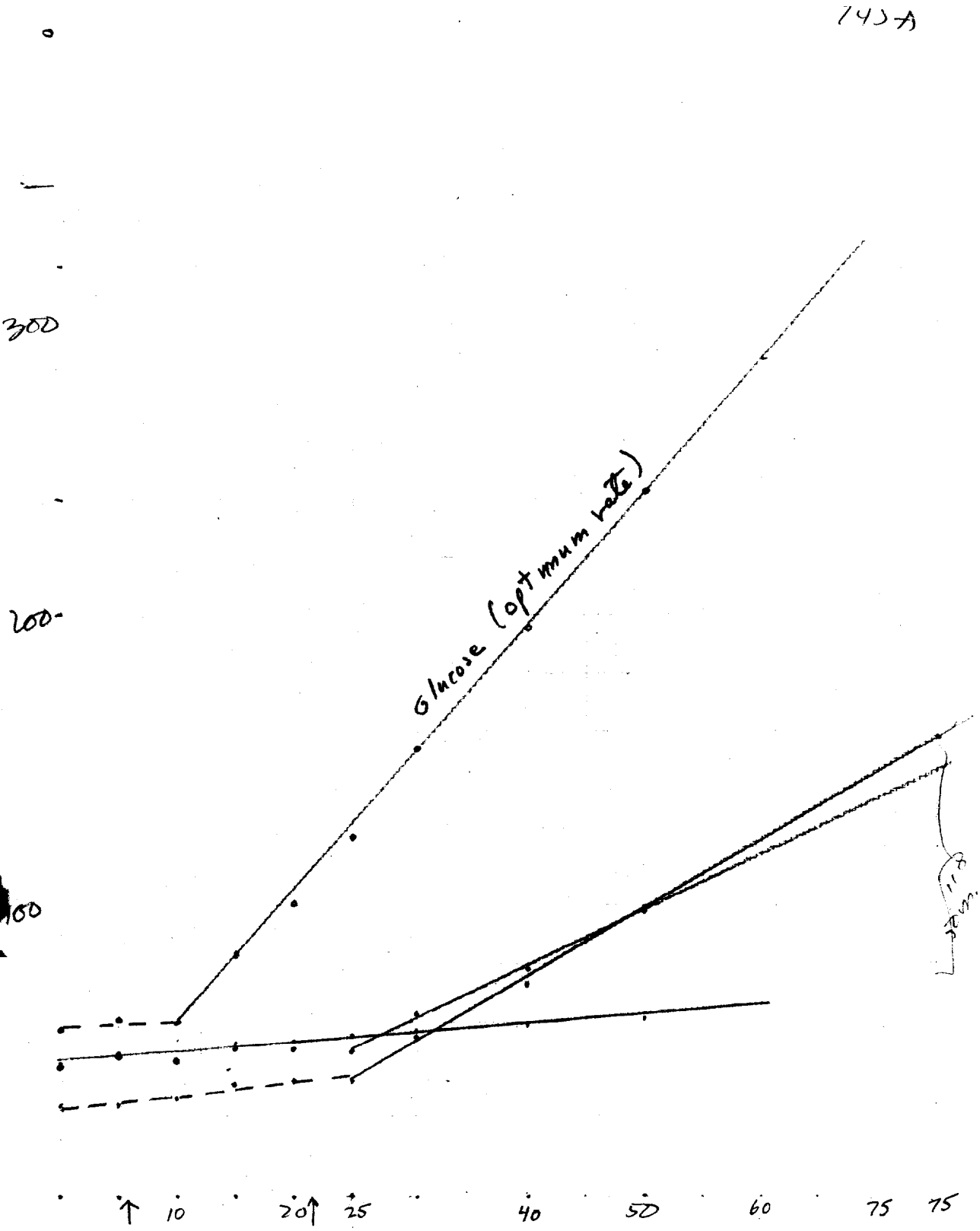
b) optical density of 742-A cells (for assay!)

See 742

Use 2.0 ml 742A cells. Time →

	Side 1 / Side 2	0	5	① 10	15	20	27	30
1	lactose 10mg —	31	36	28	35	32'	33'	38
2	" Extr .2ml	8'	14	3'	13	8'	09'	14
3	" " .4ml	25	30'	23	32	22	31'	39
4	glucose Extr .4ml	28'	35	29'	38	32	34'	40
5	1.9ml A 0.1ml B - lactose	46	54	46'	53'	59'	72	82
6	1.9ml 0.1ml B lactose	8'	18	7	16	12'	16'	21
TB.		157	162	156	162'	[illegible]	160	163'

	35	40	55	71	82	91	106
1	35	37	39	41	41	41	47'
2	14'	18	26	39	43'	48	61
3	39'	39'	58	80	88'	93	112
4	37	37'	36'	42'	42	37'	40
5	88'	97	101'	161	177	189	217
6 growth?	22'	22	26	42	42	41	47
TB	164	162	161	169'	168	163	167


300
200
100
Glucose (optimum rate)
10
20
25
40
50
60
75
75

Manometric assay of lactase

May 4, 1950.

Use same cells (742 A!) for assay of glucose liberated.

	Cells 2 ml	Substrate 2 ml		0	5		10	15	20	25
C 1	A	Lac	—	46	49'		47	53	54	55'
A 2	A	Glu	—	58	61		60	83'	101	124
A 3	A	Lac	744	45	48		45	51	51	50'
A 4	A	Lac	"	31	32		34'	39'	40	40

31	40	50
57'	59	61
154	196	243
63	78'	98
55	73	99

Glucose A = 228 / 50 min

Lact

Lactase A = 118 / 50 min.

(opt.)

Should steady state be reached at a suboptimum level?

Manometric Efficiency

May 8, 1950.

3 plate 2 plate

Harvest K-12 from DNase .1% Glu (G) and Lac (L).

~~Resuspend in 5 ml NaHCO3 buffer.~~

~~↓ cell density computed (from opt. density)~~

Resuspend in 20; 15 ml respectively.

Optical density: (.1/10)

(9 ml)

	O.D.	mg/ml	Dom g
G	360	8.3	
L	291	6.7	

Use 1 BDU ≙ .23 mg as conversion factor

Repeat .03 ml/10

L 091 122 !

very low activity!

Need of $NaHCO_3$?

or use of tolue ?

Effect of Bicarbonate buffer.

Manometric assay.

May 9, 1950.

Cells harvested from 2 plates each D/2 Glu; Lac (.1%) and resuspended in 10 ml H_2O. From "2"; aliquot A diluted 1:1 with H_2O; B with M/10 $NaHCO_3$. Let stand (under CO_2) at room temperature from ca 3PM – 8PM. Assay in M/50 Na P 7.5 ...

Assay (.1/10) 20

		Di	Dou[illegible]
Putrescine and [illegible]	A	207	258
	B	191	271
	—	-009	012

2 ml B (Lactose) cells.

B { B 1 Sidearm Lac
B 2 Glu
B [redacted]

A { B 4 A cells - Glu
A 5 " Lac
B 6 A 2nd B 1nd Lac

A 7 A —

A 8 TB

	T→0	5	10	15	20	30
1	22	22	22	24'	28	39
2	25'	24	30	42'	59'	91
4	45'	47	52'	59'	74	94'
5	25	28	28	25'	30'	30'
6	65	69	68	64	72	71
7	54	59'	59	56'	63	60'
TB	121	127'	126	124	131	127

	40
1	41
2	114'
4	107'
5	25
6	66
7	54
TB	122

No bicarbonate effect, but these cells have very low lactase activity!

Irradiation of H226

May 9, 1950.

A - Control 10^{-7} H226 resolated from single colonies.

B - UV 20 sec. Dilutions indicated. Irradiate the 10^{-4} dilution. ϕ 30 ϕ 14.

"7" = 2×10^{-7} ~~$6 = 10^{-6}$~~ ~~$6 = 10^{-6}$~~

EMB Mal

	+	−	V	
A.	19	1	131	Since "7" may be V.
	10	3	122	
B	64	20	142	Not accurately countable.
	87	28	146	Other plates not counted

EMB Lac

	V	−	
A	188	17	
	212	10	
	400	27	
	~~200~~	14	
B	127	108	Many colonies ⊙
	134	80	
	80	128	
	341	316	
	170	162	

Pick Mal − colonies and test for Lac V. See 747A.

Pick whole Lac − colonies and streak out for Mal+/− content.

~~Ps~~ tests

32 Mal+ Mal − Mal+, −

14	13	5

+	−	+ and −
18	9	5

B2 did not grow on synthetic (EMS)
A and B1-3 appear to be Mal- Lac v.

See 7/7D

H226 : UV.

747A.

a) Picks apparently pure ~~Lac~~ Mal- colonies to EMB Lac, for "partial segregants". (1, 2, 4)

A] 4 Mal "–" : 3 Mal- Lac– #3: Mal-, +?; Lac –+? Restreak (747 A) has –v in both Lac and Mal

B] 38 Mal "–". 13 of these have apparent Mal+ in streak, usually not so clearly evident Lac+. REISOLATE

← 3 possible Mal- Lac v. Restreak: 747AB-[1-3]. May be Mal- Lac+ appearance for some colonies

Remaining 22 are apparently pure Lac- Mal-.

b) SECTORED COLONIES

Pick from Mal sectored colonies (EMB), the – sectors possible, and the + correlate. Streak on EMB Mal; Lac:

Mal "–"	Mal "+"	Lac "–"	Lac "+"
–	+	–	+
– *	+	–	–
–	+	–	+
– *	+	–	–
– *	+	–	–
– *	+	–	–
– *	+	–	–
– *	+	–	–
– *	+	–	–
– *	+	–	–
– o	+	+	–
+	+	–	+
+	+	–	+
– *	+	–	+
– *	+	–	+
+ *	+	–	–
–	+	–	+
+ *	+	–	–
+	+	–	+

Mal "–"	Mal "+"	Lac "–"	Lac "+"
– *	+	–	+
+	+	–	+
+ *	+	–	–
– *	+	–	–
–	+	–	+
+	+	–	+
– *	+	–	–
+ *	+	–	–
– *	+	–	–
– *	+	–	–
+ *	+	–	–
+ *	+	–	–
– *	+	–	–
+ *	+	–	–
+	+	–	+
+	+	–	+
+	+	–	+
+	+	–	+
+	+	+	+
– *	+	–	–
+	+	–	+
+	+	–	+
+	+	–	+
+	+	–	+
+	+	–	+
+	+	–	+
+	+	–	+
+	+	+	+

Mal "–"	Mal "+"	Lac "–"	Lac "+"
–	*	+	–
+	*	+	–
–	*	+	–
–	*	+	–
+		+	+
–	*	+	–
+	*	+	–
–	*	+	–
–	*	+	–
+	*	+	–
+	*	+	–
–	*	+	+
–	o	+	+

– denotes absence of visible + .

These data are of limited value owing to the difficulty of identifying a minute fraction of Lac v in a Lac – streak (picked finally to plate). * types would appear to represent Mal+/Mal– mixed colonies & Lac v.

o might be Mal– Lac v — Restreak as 747c 1-2

#1 is Lac – Mal –, + , prototroph

#2 is Lac v Mal v

Pick Mal- streaks from c) and check on Xyl EMB

40 *
2 o

19 Xyl+ 17 Xyl– ! (High crossover frequency?)

EMS Platings

747B

May 12, 1950

Colonies.

		+	−	sectoral
EMS Lac		74	0	0
		101	0	0
		140	1	0
EMS Mal		49	0	0
		155	0	0
		141	2	0
		134	1	0
B	EMS Lac	104	15	12
		82	9	16
		101	13	9
		98	23	20
		120	15	11
	Mal	102	4	11
		89	6	10
		65	5	14
		65	5	3
		102	4	12

5/22. Pick − and sectoral −, + from Mal EMS to Lac EMB, Lac S, and Mal B.

Lac	Mal	* Lac	Mal
−+	− ++ +	* +	− − +
−	−	* +	+ − +
−* +	−	−	−
−	+ ++ +	−	−
−* +	−	−* +	− − +
−	−	* +	−
− +	−	· −	−
−	−	** +	+ − +
−* +	−	** +	−
* +	+ ++	* +	−
−	−	−	−
		* +	+ − +
		* +	+ + −
		−	+ − +
		−	+ − +

Study * from EMS Lac to EMS Mal. 6 "− " and 7 "+" pairs (sector)

Analysis of these plates was interrupted (by weekend vacation trip and lack of assistance)

Experiment to be repeated on more appropriate scale. Test for:

a) auxotrophic Lac; Mal v

b) Partial segregants in intact and sectored Mal− (EMS) colonies.

c) Carefully examine "induced haploids" for trace residual diploid (Cf. 747A-[B]).

UV-induced partial segregants

747D

May 16, 1950.

1-3 = 747B-B1-3 4 = 747B-A1

a) from EMS (excludes 747D2)

b) from EMB Lac.

		EMB Lac	EMB Mal	EMS Lac	Mal Xyl MH
a.	1	V	—	+	
	2				
	3	V, —	+, —, v?	+	not part. neg.
	4	V	—	+	

				EMS Lac		
b	1	V	—	+		
	2	V, —	V	no growth	(Methionine less not part. neg.)	— V +
	3	V	V	+		
	4	V, —	—	+		— —

5/18 c Repick 3/4 single Lac V colonies from 2a, b. Restreak on EMB Lac; Mal and determine nutrition: Methionine less.

Repick single Lac V colonies to Mal EMB to obtain crossers, in hemizygosity test.

SUMMARY:

#1.	Lac V	Mal- Xyl V MH V	Prototroph
#4	Lac V	Mal- Xyl- MH-	Prototroph (spontaneous)
H244 #2	Lac V	Mal- Xyl V MH +	Methionine less.

PROJECT:

1. Hemizygosity of Mal- in 1, 2, and 4
2. Identification of triploids in outcrosses of #2.

5/24

747E. Autosis ~~#~~ segregants of H244 (Xyl⁻) to Y10.

a

M+ Lac- Mal-Xyl- Mtl+ × Y10

1.

H244 Reversion hemizygosity test

#2. Segregation: Streak out Lac v. on EMB Lac

a) Test 5 Lac - on Xyl EMB and BATLB, Each was M- Xyl+

a' Test 5 Xyl - Lac - Each was M+ =747E1

5/21/50. b) Test addnl. Lac - on Xyl EMB: 34 Xyl+ 15 -.

Check Xyl - on BATLB.

H244M+

c) Brush on Mal EMB. for reversions (hemizygosity tests): ~~33~~ 23 Brushes.

5/21 36 Papillae picked from these and streaked on EMB Mal, Lac

~~#1,4: Brush on EMB Mal for reversions.~~

5/22 Where predominantly + or v on Mal and Lac, repick single ~~Mal+~~ Lac v to Mal ~~Lac~~ for verification as possible Lac v reversions.

5/23 27 groups sampled. Mostly Mal -. 4 groups had ~~Mal+ Lac~~ Mal+ : 1/4[b] ; 1/4[c] ; 3/6[a] ; 1/3[d]

Restreak each of these on Lac, Mal EMB, and restreak single Lac v colonies:

	Lac	Mal	Pick - to EMB Xyl.			M+ may be v
			Mal-	Mal±	Mal+	
1	- v	+ v -	4X-	1X±	9X+	} CIS
2	v	+ v -	5X-	1X+	7X+	
3	v	+ v -	7X-	~~1X+~~	1X+	
4	v	- v	3X-	5X+	2X+	TRANS??

W1301 crosses.

May 8, 1950.

A × 1272
B × 1178 } EMS lac
C × 340

Picks 100+ from B and streak out on EMS Lac for Lac v. Yields very low on A. C considerable.

c) 73 Lac+ picked to EMS Mal. All +.

c) v. good yield. Lac+ and Lac slow (incubator at 36+° - threshold for Lac_3^t).

20+ and 20sl picked to water susp.

and spotted on EMS lac, B Lac, ~~Mal~~ Glu

20 Lac+ : ~~Mal~~ Glu

20 Lac sl : ~~Mal~~ Glu -. Are any of these const Lac+? (at 30°?)

~~Pick to DNZ Glu for further test.~~ Picks from EMB Glu for spot plate tests with onpg.

Glu -:

onpg +	1, 3, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, ~~20~~	70%
onpg -	2, 5, 7, 9 12, ~~19~~	30%

11, 12.

The onpg + glu - cultures must represent the genotype Const + Lac_3^t. Purify to verify temperature behavior. Streak out the two types. Pick from EMS Lac. on EMB Lac.

~~Inoculate NZB with #~~ 11, 12, 1301 and 58-161. ~~Incubate at~~ 30 and 40°.
of 16 Glu + cultures, 10 +, 6 - on onpg.

---

see 749:

Test various suppressor stocks for constitutive lactase: (escape from Glanto)

W252: very strong +++ on onpg.

are lac 3 constitutive?

May 11, 1950.

B 100 lac+ picked and streaked on EMB Lac.
Idiosky; 2 improbable lac v. Picks four colonies and restreaks
88 further lac+ picked and streaked: 1 definite Lac v.
Of previous set, # 1 is Lac v, others probably not. Keep as
748B1 and B2. Brush on DN2 tube for spot test.
onpg spot tests: Both B1 and B2 are constitutive.

C: Segregation of Col among ~~Lac+~~ Lac+ (from EMS spots to DNB onpg spot plate tests

1-12 5+ 7- (5, 6, 9, 10, 12) These data not very informative.

#11 (npg+) is lac+ at 44° Both are Gal —.
#12 is Lac — " "

May 11, 1950.

Test various suppressor strains for constitutive lactase:

a) scrape growth from (old) slants for npg spot plate:

	W	npg	Constitution			
1	251a	±	W108	SLac I	+ Lac_3 −	(ferments Lac + Sal) L+ M+ D −
2	252	++++	W108	SLac	"	L+ M− D −
3	327	−	W108		"	M+ L− D −
4	329	−	W108		"	M+ L+ D −
5	349	±	58-161	LOL +		
6	7160	+	Y70	Lac_1 − Su+		
7	716E	+	"	"		
8	1301	++	58-161	Lac+ Const+		

b) Inoculate into Penassay and incubate 11AM — . Assay. After 36 hours, spot plate test with one drop of growth culture.

251	−
252	+++
328	−
329	±? Repeat:
349	−
396	−
397	−

Lactase economy

May 13, 1950.

Harvest K12 from 200 ml aerated Y2 .1% lac, wash, into 10 ml H$_2$O

Galactosidase assay: .02 ml / 10. Rel. Act. ca 1.3

Original suspension has ca 800 u/ml lactase.

Di	Donpg (20 M.)
132	309

K12 / Glucose from 5/11/50. used for assay

For manometric assay, dilute this suspension 1/1+2, to a final

borate buffer of M/20. Cell densities (.1/10) Dilute to 1/1+1 (clearance

L	180
G	200

L suspension should have ca 250 units of activity / ml. Incubate at 38°C

Flask	Side 1	Side 2	4[25]	4[32]	4[35]	4[40]	4[45]	5[00]
1	G	glucose	39'	40 39	42	48	49'	
2	G	lactose	31	30	32'	33'	33	
3	G	lactose .2 ml L	43	42	43,	47	46	
4	G	lactose .1 ml 7+4 extr.	55	55	58,	58,	58	
5	L	glucose	43	43,	53'	108'	159	
6	L	lactose	46	45'	48	83	117	
A 7	G	—	63	64'.	66',	62'	65	
B 8	L	—	42	44'	45'	40	45	
B 9	—	—	133	135'	133'	129	133	

6 ugm glucose

N.G.

Hemizygosity tests: HZ42

May 14 ff 1952.

HZ42 is a culture giving weak lac+ (v?) colonies which may segregate very infrequently. In previous tests, Gal+ or Mal+ revertants were usually pure lac−.

3 Mal+ } from EMS. Apparently pure +. No sign of
4 Gal+ } segregation on lac. Gal+ are strange lac+. Hold in incubator.
No sign of segregation.

Lactase Efficiency

4370 units split $\frac{17.45 - 5.91}{2.18}$ mg in 20 mins.

1 unit = 6×10^{-5} mg. per minute / ml

~~= .06 r/min. of 45 r/minute each.~~

1 unit = .6 r/minute

Lactase assay on lactose (Barford) 752

May 18, 1950.

K12 harvested from 10 plates (= ca 400 ml) BNZ .3% Lactose, washed and resuspended in 15 ml H_2O. Store equal aliquots in water (A) and in water under benzene (B) (shake 3-4 hours). (overnight in refrigerator. Assay with onpg

		Di	Donpg	Acor	Assay stock susp:	mg/ml
1/10 dilutions from stocks.	.1 ml A	(176)	301	123	123 u/ml	
	.1 ml B.	110	++++		>	28.3
	.01 ml.	011		437	4370 u/ml.	

LACTOSE ASSAY SYSTEM:

1 ml stock suspensions + 2 ml 10% lactose + 1 ml M/5 Na P buffer + 5 ml 10% $CaCO_3$ + 2 ml H_2O. Incubate 20 mins. Boil 2 minutes. Sediment and assay supernatants (1 ml)

	"Cells"		Assay
1.	A	no lactose	0
2.	A		4.84
3.	B	no lactose	0
4.	B		17.45
5	Novello lac		5.91
6		Glucose 1 mg.	2.18

The boiling with $CaCO_3$ appears to cause appreciable lactose hydrolysis, but the increased hydrolysis of benzene-treated cells is quite apparent

←

Lactase activity measured on lactose

Preliminary

Substrate = 2% lactose. All experiments in M/100 NaP buffer. 7.5

a). 1 ml 744 extract (= 880 units/ml) + .6 ml 10% lactose + .15 ml NaP M/5 + 2.55 ml H_2O @ 37° 3 38 – 8 45

---

744 extract 880 u/ml

1 ml extract + .6 ml 10% lac + .2 ml NaP M/5 + 1.2 ml H_2O.

Ca. 5 hours incubation.

Add 4 ml Benford's Cu reagent to 1 and to .1 ml.

	N/100 ml KMnO4
Glucose 1 mg	2.47
lactose 10 mg	<< 1 drop
Assay .1 ml	5.67
Assay 1 ml	36.0

This is the equivalent of complete hydrolysis. Repeat with lower unitage; shorter time.

5/18/50. System as above: .1 ml extract .6 ml 10% lactose .2 ml NaP 2.1 ml H_2O to 3 ml volume.

Sample .1 ml from time to time into 5 ml Cu reagent.

9 25 0

1 15 .5 ml sample 3.65

Glucose 1 mg 2.43 Take 2.4 as standard.

" " (1 ml boost H_2O) 2.13

∴ sample contained $2 \times \frac{3.65}{2.4}$ mg "glucose"/ml = 3.04 mg/ml.

∴ 88 units enzyme · 230 minutes split $3 \times 3.04 \times 10^3 / 360$ μ moles lactose

1 unit = $\frac{9{,}120}{88 \times 230 \times 360}$ μM/min. = 1.25×10^{-9} moles/minute.

= .45 γ/minute

Trial Run.

Calibration of method.

(1)	1 mg glucose	3.35 − .63	2.72
(2)	10 mg glucose	23½ × 20.10	33.3
(3)	10 mg lactose	2.5 − 1.86	1.86
(4)	100 mg lactose	13.5	11.86

later: titrate with Setaphonic C, no phosphoric acid

Constitutive – lac, Recombinations from heterozygotes

May 22, 1950.

748 B1 and B2 – Each, 8 lac v colonies, streaks out on EMB lac.
Note lac+ colonies ± + ± sheen. ~~Brush~~ Brush on DN2 Glu, test on onpg spot plates. npg tests not correlated with sheen, but some are constitutive; others not. (Possibly a negative correlation (sheen + npg –; sheen – npg +).)

		sheen	npg	Nutrition
B1 – 1		+	–	
		+	+	
	①	–	+	M –
		–	+	
2		+	+	
	②	+	+	TLB,
		+	+	
3		+	+	
		+	+	
	③	–	+	++
		–	–	
5		+	–	
		–	+	
6	④	–	+	++
		+	+	
		+	–	
7		+	+	
		+	–	
		+	–	
		+	–	
8		+	–	
		–	+	
		–	+	
	⑤	–	+	M –

		sheen	npg
B2 – 1		+	+
		+	–
		+	–
		–	+
		–	+
		–	+
2	+		–
	–		+
3	+		–
	+		–
	–		+
	–		+
4	–		+
	–		–
5	+		–
	+		–
6	+		–
	+		+
7	+		–
	+		–
	–		+
	–		+
8	+		–
	+		+
	–		+
	–		+

	sheen	npg	
	+	–	
p1 +	7	7	
–	8	1	

B2 –	+	2	12
	–	11	1

Use 1, 2, 5 in crosses. Sec over

753-1 × Y10
753-2 × SD-161
753-3 × Y10
} on EMS Lac

Ca 250 prototrophs from each. All Lac+.
∴ None of these is Lac, − Cst+

Brush Lac − segregants on EMS Lac.
Repick and separate −, + revertants and pick to E DN2 Slu. Lac− components are all npg−.

B1: + comp.

	npg
1	−
2	−
3	+ 1
4	+ 2
5	−
6	+ 3
7	+ 4
8	−
9	−
10	+ 5

1318
1320

B2

	npg
1	±
2	+ 6
3	+ 7
4	+ 8
5	±
6	+ 9
7	−
8	+ 10
9	+ 11
10	−
11	+ 12

Restreak Lac− which give npg+ revertants.

Retest
1 −
2 +
3 +
4 +
5 −
6 +
7 −
8 −
9 −
10 −
11 +
12 −

Classification from brief growth on DN2 probably incorrect. Do retests (accurate DN2)

W1301	++++
W1318	−
1319	−
1320	±

Reduced invertase activity.

Irradiation of H226

754-0

May 22, 1950.
Fresh D(Lac). Dilute 10^{-6}. A: control B: UV 20 secs.
0.1 ml per plate.

P22

A. EMB Lac

V	−
170	18
162	19
332	37

EMB Mal

+	V	−
33	139	7
10	122	4
43	261	11

Many of the Mal + in A may be V

B, the Mal V were typically much
Mal − than in A.

B EMB Mal

+	V	−
40	42	16
24	26	13
11	18	6
40	80	15
26	25	7
38	24	10
43	94	14
222	219	81
32	31	12

EMS ~~Mal~~ Lac

+	−
150	0
150	0

A. EMS Mal

+	−
118	1
39	2

May 23, 1950.

B EMB Lac

36-40 hours.

	V	−
	25	33
	29	52
	27	52
	38	58
	23	72
	35	73
6	187	340
	31	57 /68

EMS Mal

	+	−	S
	28	3	6
	24	20	16
	29	8	6
	26	12	9
	32	18	10
5	139	73	47 /
	28	15	9 / 52

Notice very high proportion of protoplasts in yield! Test by picking at random from EMB Lac.

5/24/50.

a) Pick Mal− from B and ~~streak~~ streak on EMB (EMB Mal) Lac ; Xyl; EMS Lac

b) Pick Lac v from EMB Lac (B) to water; spot on EMS Lac; EMB Mal for partial segregants.

c) Pick apparently pure Lac − from EMB Lac to water. Spot on EMS Lac. After 4 days, none of 40 spots was Lac+. 6 Lac− prototrophs. These colonies may be presumed to be pure

A25. a) ① . Pure Mal−. Spot from water scrap. 35 tests: EMB Mal, Lac; EMS Lac

1 Mal− Lac+ ; remainder are Lac−. Hold EMS plate. Streak out Mal−Lac+ on EMB Lac as 754a1

① Brushes from Mal− and Mal +/− . (From middle, no suspension). [LEFTHAND IS MAL− COMP. I

✓=growth
+=Lac+

Xyl EMB	Lac EMB	Lac EMS
−	−	
−	−	
−	−	
−	−	
−	−	
−	−	
+	−	
−	−	
−	✓	✓
+	+	−
−		−
+		−
+	+	−
+		−
−	+	−
−	+	−
−	+	−
−	✓	✓
+		−
−		−
−	+✓	−
−	+	−
−	−	

X		L	B	L	S	
−	✓	−	✓			+
−	—	−	−			
−	+	−	−	≠		
−	+	−	−+	✓—		✓—
−	−	−	−	✓—		✓—
−	+	−	✓			+−
−	✓	−	−			
−	+	−	+	#		+
+	+	−	−	—		—
−	✓	−	−			
−	+✓	−	+	✓		✓
−		−				✓
−		−				✓
+		−				
+		−				✓
−		−				
−		−				
−		−				
+		−				
−		+				754a2.
+		−				
−		−				
+		−				

Subtotal: 47 tests: 1 Mal− Lac v

Mal− : 35 Xyl− : 12 Xyl+

Total 2/82.

	Lac	Mal	Xyl	EMS Lac	Nutr
a1	v	—	—	ng	M−
a2	v	—	—	ng	M−

May 25, 1950

b) 120 Lac^V picked to Lac EMS; Mal EMB.
All Mal+(or v) except #'s: 8, 10, 17, 23, 59, 66, 67, 73, 82, 84, 88, 117 [12/120]
which are Mal−, predominantly. Save suspensions.
All formed colonies on EMS by 24 hours except:
~~5~~, 6, 11, 14, 20, 21, 22, 24, 30 32, 35, 36, 38, 40 43, 48, 49, 50 54, 55, 58, 60
61, 66, 68, 69, 70, 81, 82, 83, 87, 88 ~~FE~~ 91, 93, 100 106-110, 113, 114, 116, ~~118~~ 120
Hold additional 24 hours.

Restreak Mal− suspensions on EMB Lac; Mal; EMS Lac.

c) EMS Lac: Hold EMB Mal: 29 agg. pure +
6 agg. pure −
6 clearly mixed.

Mark gave EMS root

d) As a) but from A

e) As b) but from A

f) 3 Mal− from A, EMS Mal. to EMB Lac, Mal, S Lac } See 756.

g) Mal− fototrophs from BT. Brush to EMS Lac

c754

May 26, 1950.

b)

	Lac	Mal	EMS	Mutation
~~8~~	✓	—	+	
10	✓	—	+	
17	✓	—	ng	L-
23	✓	—	+	
59	✓	—	ng	TL-
66	✓	—	ng	TLB$_1$-
67	✓	—	+	
73	✓	—	+	
82	✓	—	—	
84	✓	—	—	
88	✓	—	ng+	M-
117	✓	—	—	

Thus about 10% of the surviving Lac✓ colonies are Mal- Lac✓.

From a) one infers that 2/82 = ca 3% of the surviving Mal- colonies are Lac✓.

cf. d, e, f.

see 754b

See 755.

c) Held

d) Mal- from control plates. Also sectored colonies.

7 pure Mal-: 6 Lac- 1 Lac✓ (prototroph.) Restreak as 754d1.
✓Mal- Lac✓ prototroph

14 sectored colonies. Mal- of each is Lac-.

e). Lac✓ from control plates: to EMB Mal, EMS Lac 89 tested.

3 are Mal-, prototroph. Restreak. 1 Mal+ (nv) non-prototroph

754e1-3 1 Mal- Lac✓ 3 Mal✓ Lac✓ | 754e4 Lac-✓Mal+,-,✓
- Mal- Lac✓ ✓prototrophs. non-prototroph.
≡ T- L-

f: Each of 3 is Mal- Lac✓ prototroph. Save 754f1.

g) ~~f~~. Replate H226 ~~on Lac~~ on EMB Mal: 6 plates. Pick pure Mal- to Lac

g) 42 Mal- prototrophs from B. 19 are Lac+.

29 sectored pairs. Mal- component of 11 pairs is Lac+.

To be verified. Pick possible exepts, and their sibs, to water and spot on EMB Mal, ~~EMS~~ EMS Lac.

May 27, 1950.

h) 13 pure Mal− colonies : 2 Lac ± ; 13 Lac− Retest the +.
2 pairs Mal−/+ tested Lac of the recorded Mal−/pure Lac v, but also scored Mal+, and presumably Thal v.

g.

June 1, 1950

25 Lac+ av cultures reisolated as autotrophs. Streak out on various sugars to determine further characteristics

	Lac	Mal	Xyl	Mtl		
1	v	v	—	—		
2	v	v	v	—		
3	v	v	—	v+ ?		
4	v	+ v?	—	—		
5	v	v	v	v	M	call it B12
6	~~—~~ v	+	—	v		
7	~~—~~ v	v	+	v	FL	B13
8	v	—	v	v		
9	v	v	+	v		
10	+?	v	v+	v	M	
11	v ~~+?~~	v	v	—		
12	v	v	v+	v	M	
13	v	v+	+	+		
14	v	—	v	v		
15	+?	v	+	v+		
16	v+? ~~—~~	v	v	v	M	
17	—v	+	+	v		
18	o	o	o ~~—~~	o		
19	-v	+	~~—~~ v	—	L	
20	++ +?	—	+	+		
21	v	+	+	+v	M	B14
22	v	—	v	—		
23	v	v	v	v	M	
24	v	—	—	v		
25	v	+	v+	+		

See 756

Restreak v as possible use for outcrossing. Determine mutations.

June 2, 1950

Separate 7 Mal sec colonies of which Mal- component is lac v.

	EMB Lac	EMB Mal	EMS Mal	EMS Lac	
1 −	v	−		+	
+	v	v	++	+	
2 −	v	−		+	ξ unstable - throws off Mal-lac v prototrophs.
+	v	v	++ −	+	
3 −	v	−		+	
+	v ⊙	++	++	+	
4 −	v v	−	−	+	
+	− −	v	++	−	
5 −	v	−		+	
+	v ⊙	v	− ξ?	+	
6 −	v	−		+	
+	v	v+	+, −	+	
7 −	−v	−		+	
+	−v	v+	++	+	

2, 6 Mal+ (EMS) may be segregating Mal− prototrophs (Lac v?)
Verify from single Mal+ colonies (EMS).

3 may illustrate a segregation from Mal v into Mal+ and Mal− lac v.
Verify from EMS Lac.

4 seems to suggest another type of separation. Lac Mal v ⇄ lac v Mal− / Lac− Mal v

5 may be confused: Mal− presumably was streaked on EMS Mal, for Mal+.
Verify from EMS Lac. ✓

6/3/50 ✓ 3 → Mal+ lac v and Mal− lac v

4 → Mal v Lac− and Mal− lac v

6/4/50 5 → pure Mal+

6 → each of 4 tests pure Mal+ prototrophs

2 → 1-10% Mal− prototrophs in each of 8 tests. Restreak; slant as 754g2; test Mal− on EMB Lac 4/10 were lac v.

A. Lac v ≒ ~~total~~ population ≒ prototrophs.

Auxotrophs = 1%

Mal- lac v = 2%

Mal- prototrophs = 2% (all lac v)

B. Lac v = 35.5% Prototrophs = ca 2/3? p Kill = ca 88/180 = 50%

	/Total	/ lac v	/prototrophs	/original total //	
Auxotroph lac v	7.4	21		3.7	
Mal- lac v	3.5	10		1.8	
Mal-	15.5			8	3.5
lac -	64.5			33	10
Mal- prot. lac v			12.7		

The predominant effect is to make haploids pure for Mal, lac. The residual lac v have a high proportion of perturbations. (should be checked on other characters).

754

B UV. Mal- (EMB) 81/522 = 15.5%

Lac- (EMB) 340/527 = 64.5% Lac v = 35.5%

Mal- (EMS) 73/259 = 28.2%

a) Mal- (EMB): 2/56 = 3.57% Mal- Lac v.

Total fraction = 3.57 x .155 = .55% intact Mal- Lac v.

b Lac v. 25/120 are auxotrophic: $\frac{25}{120} \times 35.5 = 7.4\%$ auxotroph

12/120 are Mal- Lac v = 3.5% Mal- Lac v.

c. Mal- (EMS). 19/42 are Mal- Lac v = $\frac{19}{42} \times 28.2 = 12.7\%$ of prototrophs are Mal- Lac v.

754

A. Control: Mal− = 11/315 = 3.5% EMB Mal

Lac − = 37/369 = 10.0% EMB Lac

EMS: Mal − = 3/157 = 1.9%

d 7 Mal− from EMB Mal : 1 Mal− Lac v.

∴ H226 suspension is ca .5% Mal− Lac v.

e. Lac v from EMB Lac /89 : 2 Mal− Lac v / Mal v Lac v non prototroph

This gives estimate of 2/89 = 2.2% Mal− Lac v.

1/89 = 1% auxotroph Lac v.

f. Mal− prototroph 3/3 is Mal− lac v. This gives estimate of ≅ 1.9% Mal− Lac v (prototroph).

Summary: controls. Take 2% as Mal− Lac v (prototroph)
1% as Lac v (auxotroph)

$$3080\ u/ml = V_{max}\ of\ \frac{1.25 \times 3080}{27} / mg.$$

Lactose activation in Lacto-12

K-12 harvested from 6 x 40 ml DN2 .3% lactose agar. Wash and resuspend in + 10 ml H_2O. (A). 3 ml aliquot of (A) shaken with benzene 2 hours. Stored overnight in refrigerator.

a) onpg assay (before storage). in Na P M/30 7.5

RH/69.6 7.7 u/mg

RA 23.9 1432 u/mg

	ml cells or extract	9 ml Di	20 min. Donpg	Δ cor	Assay
A	.01	129	221	090	A = 90 u./ml
B	.01	076	>910 6 mins.	corrected:	
B	.001	010	332	308	B = 3,080 u/ml.
—		-004	011		

b) Manometric assay. Dilute 1.5 ml (A) with bicarbonate to 10 ml M/20. 2 ml cells; 0.1 ml 10% substrate in sidearms.

Flask	Subst.	3^{20} ↓	3^{25}	3^{32}	3^{40}	3^{50}	4^{00}	4^{15}	4^{27}	4^{37}
2A	Glu	26'	40	62'	91	124	157'	213	258	294'
3B	Lac	34'	41'	60'	87	118	147'	197'	239	270
6B	—	31'	36'	39	37	39	37	40	40	41
7A	ThBar.	- 24	29	29'	29'	30	27	29	32	31

Increments:

	T	TB-	A	B
		0	0	0
3^{32}	7	-	30	16'
3^{40}	15	0'	50'	45
3^{50}	25	2'	81'	74
4^{00}	35	0'	117	105
4^{15}	50	3'	169'	152
4^{27}	62	3'	214'	194
4^{37}	72	4'	250	224

A: 215 mm/hour k = 1.84 (glucose) 37°

B: 190 mm/hour. k = 1.78 (lactose) 37°

∴ A ≐ 17.7 μM CO_2/hr ≐ 1.27 mg glucose/hr.

B ≐ 15.1 μM CO_2/hr. ≐ 1.09 mg glucose/hr.

(Assuming 1 μM glucose ≐ 2.5 μM CO_2 - Stokes - J. Bact. Feb. 1949)

$Q_{Glu}^{CO_2}$ = .128

$Q_{Lac}^{CO_2}$ = .110

original suspension A) 33 ~~x 1 mg~~ 1γ/ml

33 x .3 = 9.9 mg / Warburg flask.

27 mg/ml original suspension

c) Lactose hydrolysis. System: 37° 10 minutes.

2% lactose 2.5 ml
Cells 1.0 ml
NaP 4/5 7.5 .5 ml.

Terminate Reaction by adding .5 ml 5% $ZnSO_4$. Clarify with .5 ml .15N $Ba(OH)_2$. Sediment and decant clear supernatant. Dilute 1:10 in water, and take 1 ml for assay, after Caputto, Leloir and Trucco, Enzymologia, 12:350 (1948). To read optical density, read the reduced Ho reagent at 1:10 dilution at 520 mμ in spectrophotometer. All readings of same single cuvette.

6.6 mg/ml

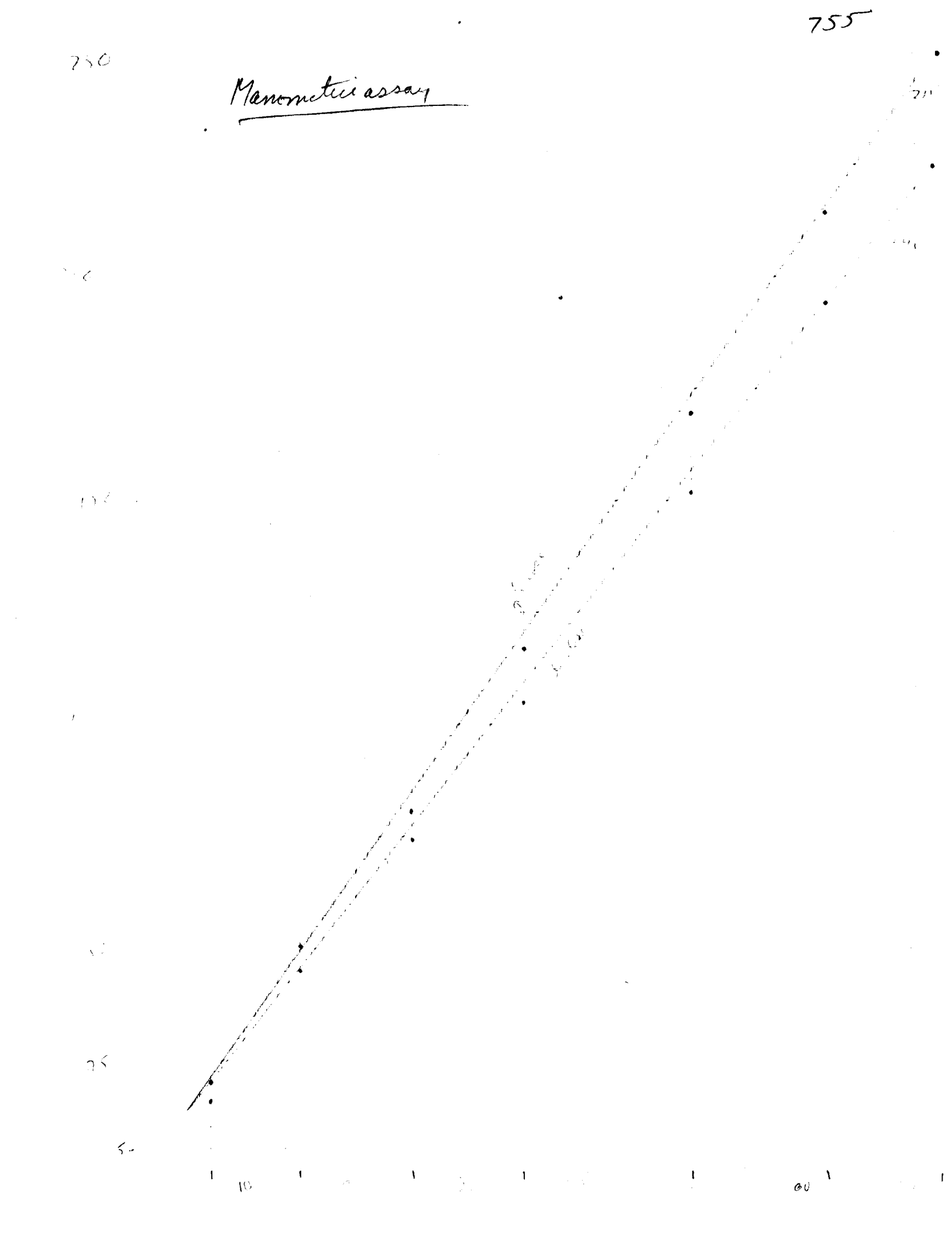
		Density
1.	A. No lactose	004
2.	B. No lactose	000
3.	A. lactose	002
4.	B. lactose	110
5.	— lactose	003 ~~110~~
6.	Glucose (1 ml 2% = 4 mg/ml).	107
7	Blank. + reagents.	003

Thus, 1 ml B in 5 ml liberated 4 mg glucose / ml in 10 minutes.

∴ 1 unit ≐ 20 / 3080 × 10 mg / min.
= .65 γ / min.

No accumulation of monose by A could be detected. It can be assumed to have hydrolyzed ~~at the rate of 1.09/6 × 10/1.5 × 1/2 →~~ 121 γ / ml ($\frac{33\ \gamma}{5\ ml} \times \frac{10}{60} \times 110\ \gamma/\text{mg max}$), ignoring temperature coefficients. Activation of 4/.12 = 33 × re lactose. Activation re enzyme was 3080/90 = 34 ×

Manometric assay



Auxotrophic partial segregants.
FROM H226

May 29, 1950.

	#		Nutr.	Mal	Xyl	M4l	Agent
	H244	M	~~B ~~	–	V	+	uv
T54	A1		M	–	–	–	uv
	A2		M	–	–	+	uv
	B3		L	–	V	V	uv
	B5		TL	–	+	–	uv
	B6		TL	–	V	–	uv
	B11		TLB,	–	–	–	uv
H245	E4		~~M~~ TL	V	V	V	spont
~~H246~~	F1		~~H~~	–	–	–	spont.
	H244M+		M	V	V	+	

Attempt crosses of H245, H246.

~~B11~~					
B12	M	V	V	V	uv
B13	TL	V	+	V	uv
B14	M	+	+	+	uv

Irradiation of H226

4 single colony isolations of H226 (A-D) used to start D(lac) cultures.
A-D are 10^{-7} controls
A_x-D_x are UV 20 secs. at same dilutions. UV and EMS at 40 hrs. Others at 24.

A.

EMB Lac	v	−	EMB Mal	+v	−
	264	17		280	4
	291	24		258	2
	317	15			
	872	56		538	6
		928			

~~EMS Lac~~

EMS Mal

+	−
322	2
185	0!
184	0
691	2

EMB Lac	v	−	Mal	+v	−	EMS Mal	+	−
B.	311	12		215	2		321	0
	334	12		276	4		254	1
					4			
	645	24		491	6		575	1

C.

	217	8		260	5		248	1
ca ⅓ pl.	62	3		228	4		178	1
	202	17		274	2			
	481	28		762	13		426	2

D.

	1	smeared or crowded - uncount.	(.2)	487	4		419	0
		236	23	283	2		131	0
		395	25	260	2			
		635.48						

Essentially homogeneous.

	EMBlac			EMBMal			EMSMal			
A	V	—		~~2)~~	V +	—	sec.	~~+~~	+	—
	88	76			201	29				
	143	97		(omit summarized part.)	114	12	17	135		30
	80	93			260	25	24	161		26
	311	266								
B	1+ 70	70			199	14	8	101		23
	94	98								
	135	87								
	299	255								
Cx Dx	ditto.									

June 2, 1950.

Pick lac⁻ survivors from $A_x - D_x$ to water suspensions. Get 120 on EMS ~~lac~~ Mal, Xyl, Mtl and EMS lac.

Stac	Mal	X	Mtl	Stac	Mal	Xyl	Mtl								
1 +	V	+	V	11 +	+	+	V	21 +	V	+	V	31 +	V	+	V
2 +	(−)	+	V	⊕	+	+	V	(0)	+	+	(−V)	+	V	V	V
3 +	V	V	V	⊕	V	+	V	+	+	+	V	+	V	+	V
4 +	V	V	V	+	+	+	V	+	+	+	V	+	V	+	V
5 +	V	(−)	V	⊕	+	+	V	(0)	+	(−)	V	+	V	+	V
6 +	+	+	V	⊕	V	+	V	+	+	+	(−)	+	V	+	V
7 ⊕	V	V	V	⊕	V	+	V	(0)	V	+	V	+	V	+	V
8 ±	V	+	V	+	V	(−)	V	+	V	+	V	+	V	+	V
9 +	+	+	V	⊕	V	V	V	+	V	+	V	+	V	V	V
10 +	+	+	V	20 ⊕	+	V	V	30 +	V	+	V	40 +	V	V	V
41 +	V	+	V	51 0 +	+	+	V	61 +	V	+	V	71 +	V	+	V
2 0	(−)	+	+	+	V	+	V	+	V	+	V	+	V	+	V
3 +	+	+	V	+	V	+	V	(0)	V	+	+	+	V	+	V
4 +	+	+	+	+	V	+	V	+	V	V	V	−	+	+	V
5 +	+	(−V)	V	(0)	V	+	V	−	+	V	V	−	V	V	V
6 (0)	+	V	V	(0)	V	V	V	+	V	V	V	(0)	+	V	V
7 +	+	V	V	+	V	V	V	+	V	+	V	+	V	V	V
8 +	+	V	V	(0)	V	+	V	+	+	+	V	+	+	+	V
9 +	V	V	V	(0')	+	+	V	+	V	+	V	+	V	V	V
50 +	V	V	V	60 0'	V	+	V	70 +	+	+	V	80 +	V	(−)	V
81 +	+	+	V	91 +	+	V	V	101 +	+	+	V	111 +	+	V	V
2 +	V	+	V	(0)	V	+	V	+	V	+	V	+	V	+	V
3 +	+	+	V	(0)	+	+	V	+	+	+	V	+	+	+	V
4 +	+	+	V	+	V	+	V	+	+	+	V	−	V	+	V
5 +	V	+	V	(0+)	+	+	V	+	+	+	V	+	+	+	V
6 +	V	+	+	(0)	V	(−)	V	+	(−V)	+	V	+	+	+	V
7 (0)	V	+	V	(0)	V	V	V	+	V	+	V	+	V	V	V
8 ±	(−)	+	+	+	+	+	V	+	+	+	V	−	+	V	V
9 +	V	+	V	+	+	+	V	+	+	+	V	+	+		V
90 +	V	+	V	100 +	+	+	V	110 −	V	+	V	120 +	V		V

11 possible fermentative deviations.

19 additional auxotrophs. ~~check on EMS lac −~~.

30

See 759.

Some sour bituothangens are established →

lethal mutations →

Zelle 6/5

M	Blac	Small	Large	Burst
7	—	+	+↓	+↓
8	—	+	+	+
9	—	+	+	+
10	—	+	+	+
23	—	0	+	+
24	—	0	+	+
25	—	0	+	+
26	—	0	+	+
27	—	0	+	+
28	—	0	+	+
29	—	0	+	+
30	—	0	+	+

L				
23	+↓	+↓	+↓	+↓
26				
27				
30				
49				
50				
51				
52				
57				
58				
59				
60				

E				
6	+↓	+↓	+↓	+↓
11				
12				
15				
16				
17				
18				
19				
20				
21				
22				

G				
1	+↓	+↓	+↓	+↓
5				
6				
7				
8				
9				
10				

Note. sterile H_2O added to all empties

788

Zelle 6/5/50

C	Blac	Smal	Bryo	BMH
7	− ?	+	+	+ ↓
9	+	+		
11	+	+		
14	+	+		
21	+	+		
35	+	+		
36	+	+		
37	+	+		
38	−	−	prot −	++ ?
45	+	+	+	
46	+	+		
51	+	+		
52	+	+		
53	+	+		
54	+	+		
56	+	+		
58	+	+		
111	+	+		
112	+	+		+ ↑

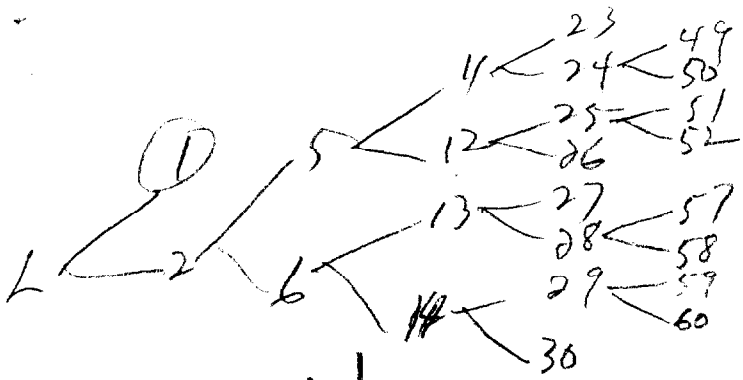
B	+ ↓	+ ↓	+ ↓	+ ↓
7				
17				
18				
19				
21				
22				
23				
24				
25				
26				
59				
60				
61				

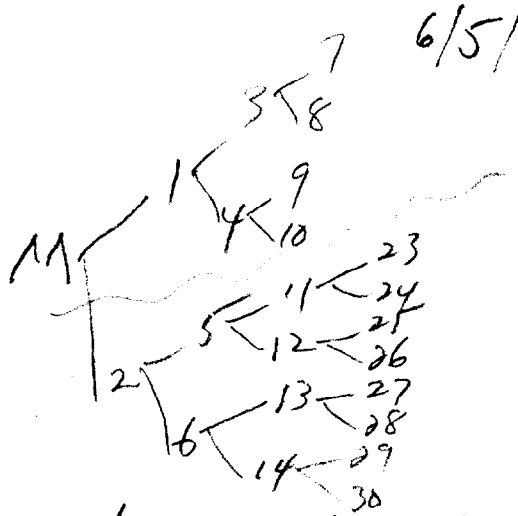
Zelle 615

D	Blau	Mal		
7	++	++	++	++
9				
11				
17				
21				
27				
28				
29				
30				
37				
38				
46				
51				
52				
53				
54				
A	++	++	++	++
10				
15				
Empty 16				
16				
19				
20				
23				
24				
25				
26				
27				
28				
29				
empty 30				
35				
36				
H			++	++
7	+	++		
8	+	+		
12	—	0		
23	+	+		
24	+	+		
27	+	+		
28	+	+		
29	+	+		
30	+	+	-	

Zelle 6/5

F	EMBLac	EMSMal	EMBXyl	EMBAra
11	++	+↓	++	+↓
15				
16				
19				
20				
21				
22				
25				
28				
29				
30				
35				
36				
37				
38				
53				
54				
55				
J	++	+↓	+↓	++
7				
17				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
37				
38				
K	+↓	+↓	+↓	++
21				
23				
24				
25 empty				
26				
27				
28				
30				
45				
46				
59				
60				





6/5/50

Cut

Dear Josh:

Just to prove I haven't entirely forgotten about the staff, I'm sending the above cuttings. Other will be all for another two weeks as I'm off on another rover mission. I haven't had any time to think out a new route but I'll try to get at it when I get back to town.

If I haven't already given you it, my new home address is;

30 Decatur St
Kensington, Maryland.

I hope the cuttings don't all turn out to be hopeless. I'm sorry about so many uncertain groups of 4 cells but they grew too fast for me, probably due to the humidly warm humid weather we've been having.

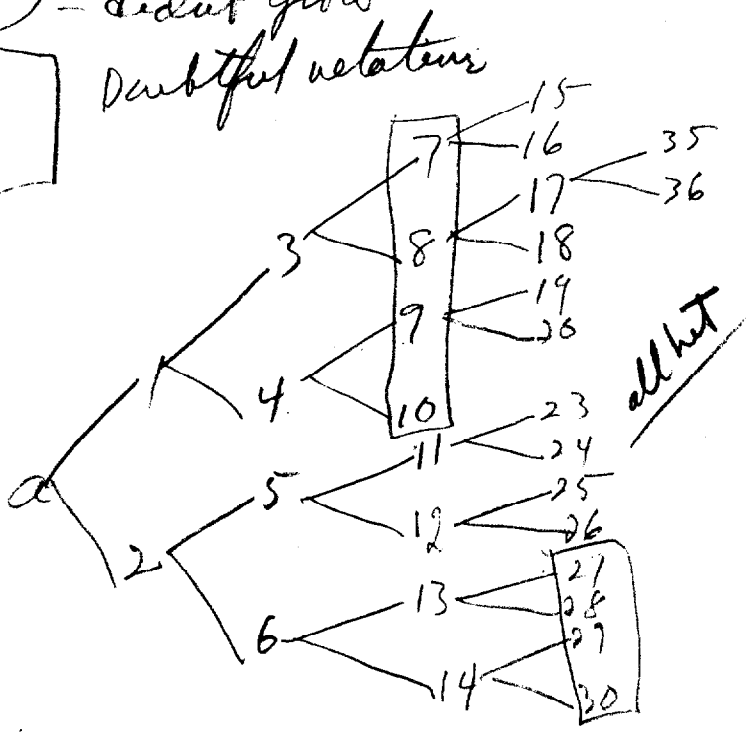
As ever

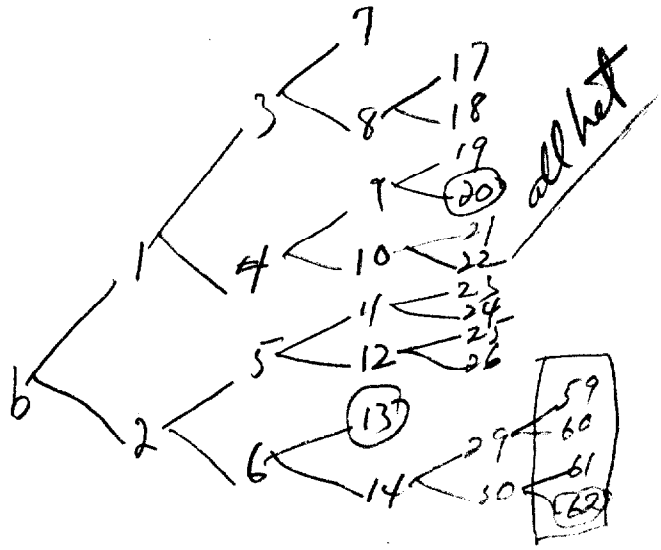
Mary

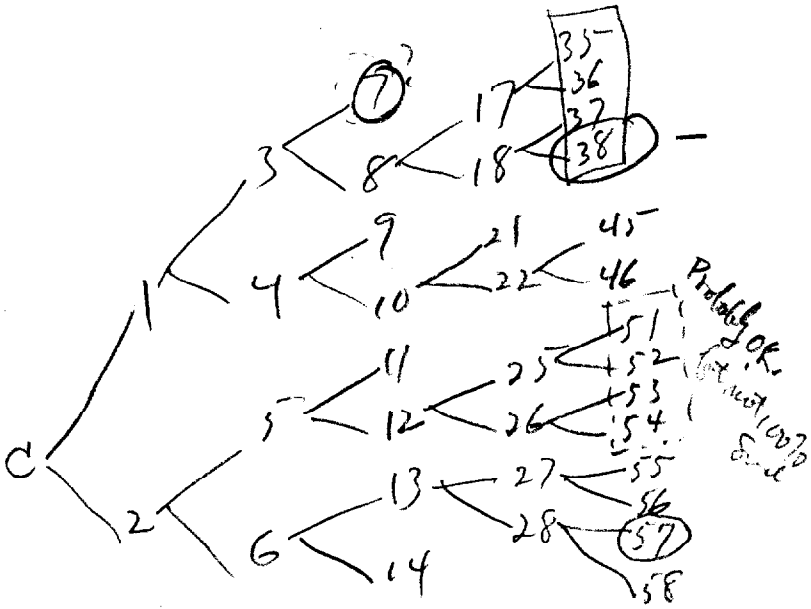
Cultures of 6-5-50.
Some H-226 - EMS colony in Davis synthetic media.

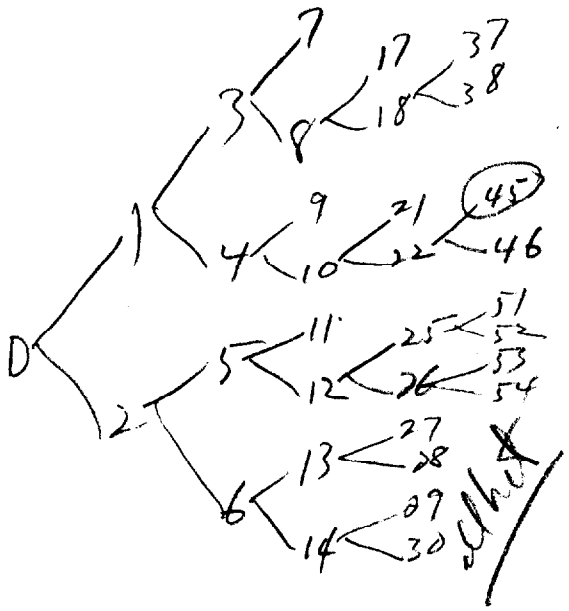
○ = didn't grow

▯ Doubtful colonies



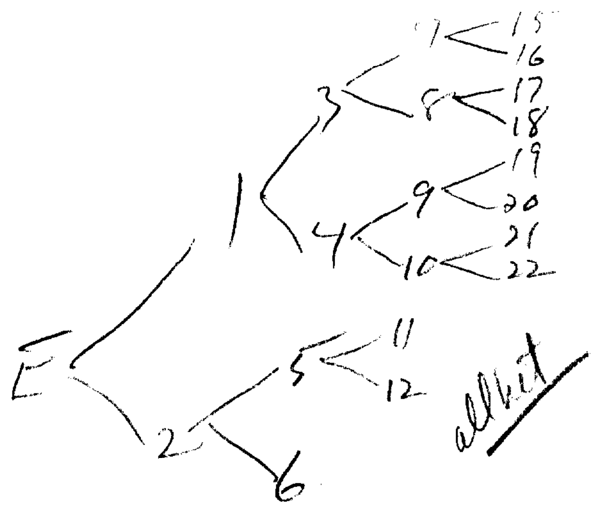


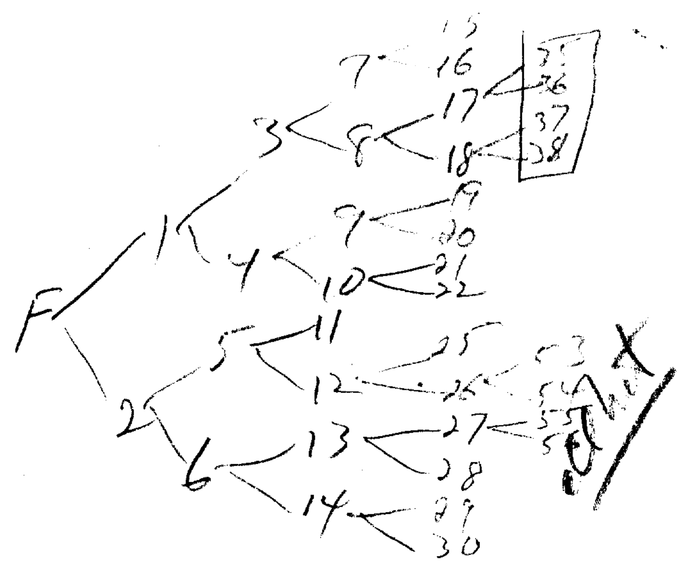




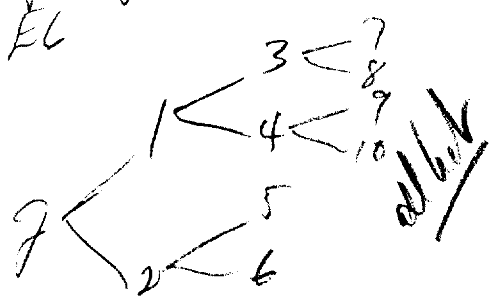
Picture
3 equal cells

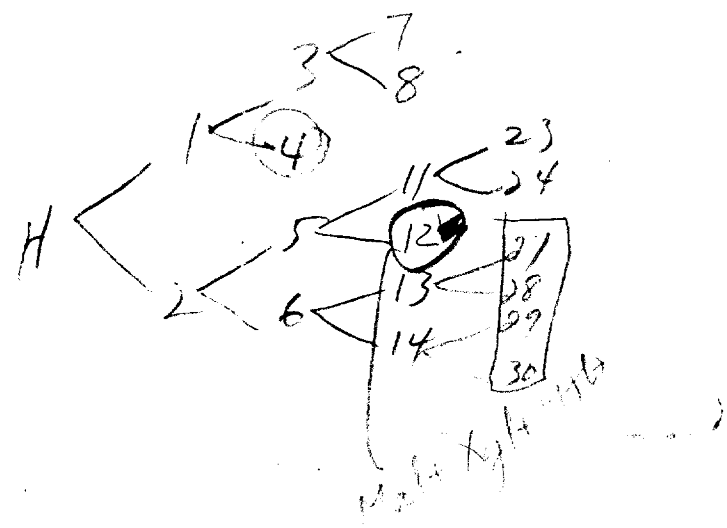
/ = 21
/ = 45
/ = 46



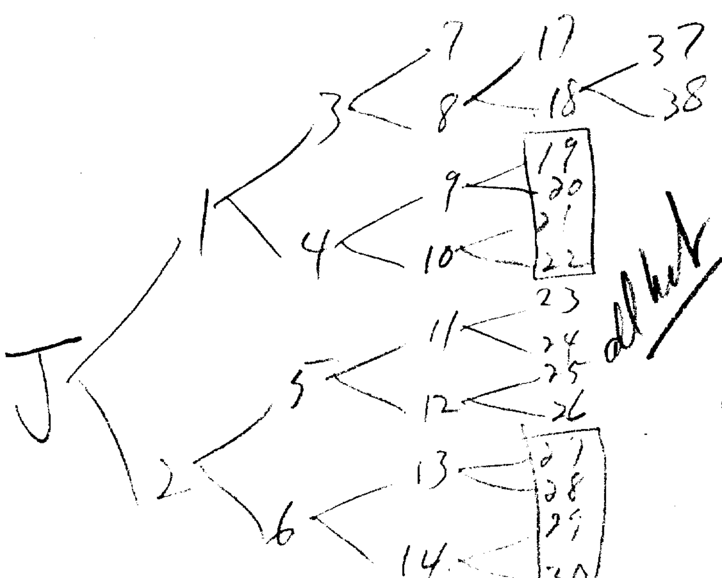


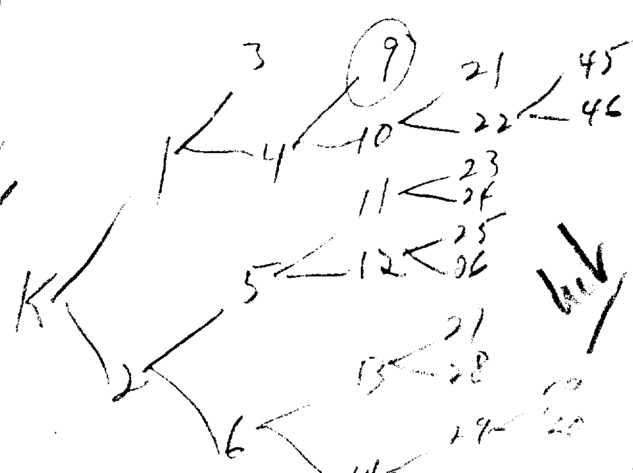
E 6 [illegible] E 13, E 14 [illegible] by the [illegible], & left E 13 or E 6





J. [illegible] cells, 1.0 larger than usual.





	A	B
Q_{CO_2}	40.0 ul/hour mg^{-1}	34.2 /hour mg^{-1}
=	1.78 uM^{CO_2}/hr/mg	1.52 /hr/mg
=	.71 uM^{gluose}/hr/mg	.61 uM^{glu}/hr/mg
=		

Original suspension (A) had $\frac{1}{.01} \times \frac{1.29}{.9} \times .23$ mg/ml bacteria.

$= 33$ mg/ml.

Each vessel therefore contains $33 \times 2 \times .15 = 9.9$ mg.

$\therefore$ ~~q_{CO_2}~~ $q^{lactose} = 1.09$ mg / 9.9 mg / hour

$= .11$ mg / mg / hour.

		glucose	lactose
~~$q_{CO_2}^{CO_2}$~~ q_{CO_2}	= +	396 ul	338
$q_{CO_2}^{CO_2}$	=	40 ul/hr	34 ul/hr
	=	uM 17.8/hr	15.2/hr.
	=	7.1 uM glucose/hr.	6.1 uM glu(lac)/hr
	=	1.27 mg glucose/mg/hour	

MR Zelle: single cell isolations H226

Summary of exceptions:

		Sib
H12	Lac− auxotr.	23-2✓
C38	~~Lac-Mal-Xyl-Mtl−~~ prototroph.	35-37. ✓c7
M 1	prototroph } Lac−	—
M 2	auxotroph } Lac−	

Check samples of each pedigree for heterozygosity

			Lac	Mal	Xyl	Mtl
A	1	A 10	✓	✓	✓	✓
	2	B 7	✓	✓	✓	✓
	3	D 7	✓	✓	✓+	✓
	4	E 6	✓	✓	✓	✓
B	1	F [redacted] 11	✓	✓	✓	✓
	2	G 5	✓	✓	✓	✓
	3	J 7	✓	✓	✓	✓
	4	K 3 28	✓	✓	✓+	✓
	5	L 23	−✓	+✓?	✓	✓
M		7	—	✓	✓	✓
		9	—	✓	✓	✓
		23	—	++	++	++
		30	—	++	++	++
C		35	✓	✓	✓	✓
		36	✓	✓	✓	✓
		37	−✓	−✓	−✓	−✓
		38	✓	✓	✓	✓
		7	−−✓	✓	✓	✓
H		12	−	+	+	+
		23	✓	✓	✓	✓
		24	−,✓	+ (=)	+,(−)	+(−)✓

} prototroph. Restricts on ERS Mal.

∴ H12 is haploid segregant.

C: none heterozygous!

M 1 prototroph partial segregant — No information!

M 2 auxotroph segregant.

Irradiation of H226

June 12, 1950.

Exp. 757 interrupted owing to "Virus Seminar".

Repeat. A = H226 10^{-7} B = H226 10^{-7} UV 20 sec. Plate on EMB lac, and pick Lac v. Spot on EMS lac EMB Mal EMB Xyl

A). 100 suspensions.

nogr. on # 2, 29, 43. Remaining 97 are all Mal+ Xyl+ (v presumed).
" " on EMS: #49 Residuals: Nogrowth

B). 99 ~~100~~ suspensions.

nogr: 36, 44, 56, 91

Xyl − 28, 31, 37, 83

Mal − 37, 39?, 43 83, 89.

~~Auxotr: 37, 39?, 4~~ 6, 9, 10, 13, 17, 18, 25, 26, 27, 29, 32, 34, 35, 39, 43, ~~##~~ 46, 48, 49
Lac-pr.: 5, 12, 30, 45, 57, 63, 72, 74 51, 52, 53, 59, 61, 62, 65, 66, 67, 69, 70, 75, 78, 79, 80, 81, 84, 86, 87, 89, 91, 95, 96, ~~100~~

Exceptions: 7 Fermentative (Mal- or Xyl-)

Residuals on EMB Lac. Examine for non-segregating + colonies.

Lac − 95 None found.
ng ~~100~~ 49 52

Replate lac v for confirmation of EMS character.

This test takes into account only those cases in which no Mal+(e.g.) is produced by the colony. A closer test would involve the fractions of Mal− and Mal **8** which are partial segregants

Retest single lacV colonies

Mal: − : 6, 16,17 22 24 37 40 42 43 51 52 53 56
63 64 71 83 89 90 92 98

Xyl − 4 7 14 18 19 25 28 31 37 38 42 43 58
63 64 68 75 83 92 97

MR − 4 7 14 18 20 24 25 30 36 37 42 43 51 52 58
63 64 68 72 80 83 84

DISCREPANT TYPES. lacV:

Mal	MR	Xyl	
−	−	−	~~37~~ ~~24 42~~ 42, 43, 63, 64, 83
* −	+	+	16, 17, 22, 40, 53, 56, 71, 89, 90, 98
+	−	−	4, 7, 14, 18, 25, 58, 68,
+	−	+	72, 80
+	+	−	~~#~~19, 28, 31, 38
−	−	+	~~24, 42~~, ~~37~~ 24, ~~42~~ 37, 51, 52,
−	+	−	92

Prototrophs	1	11	21	31	41	51	61	71	81	91
1	·	+	+ /	+	+ /	·	· /	+	(+)	·
2	+	· /	·	· /	+ /	·	+ /	· +	+ /	+
3	·	·	+	+ /	· /	· /	·	(+) /	·	+
4	+ /	+ /	·	−	·	+ /	+	* · /	−	(+)
5	+	+ /	−	·	−+	·	* · /	(+)	+	·
6	/ + /	·	− /	+	· /	+	* ·	+	−+	·
7	/ · /	·	· /	+	+	+	+	+	· /	+
8	· /	· /	+ /	+,	+ /	· /	+	+	· /	+
9	+ /	+	+	·+	+	·	* ·	+ /	·	·
10	· /	+ /	(+) /	·	+	+	· · /	· /	+	

lacV

40

	Mal	Xyl	Mtl	Nutrition
4	+	−	−	+
6	−	+	+	+
7	+	−	−	−
14	+	−	−	+
16	−	+	+	−
17	−	+	+	−
18	+	−	−	−
19	+	−	+	+
20	+	+	−	+
22	−	+	+	−
24	−	+	−	−
25	+	−	−	+ (±)
28	+	−	+	+
30	+	+	−	(+)
31	+	−	+	+
36	+	+	−	+
37	−	−	−	+
38	+	−	+	+
40	−	+	+	−
42	−	−	−	+
43	−	−	−	−
51	−	+	−	−
52	−	+	−	−
53	−	+	+	−
56	−	+	+	+
58	+	−	−	−
63	−	−	−	−
64	−	−	−	+
68	+	−	−	+
71	−	+	+	+
72	+	+	−	−
75	+	−	+	+
80	+	+	−	−
83	−	−	−	−
84	+	+	−	−
89	−	+	+	−
90	−	+	+	+
92	−	−	+	+
97	+	−	+	+
98	−	+	+	+

18 −
22 +

Total aberrations (no overlaps)
are 40 fermentative (18 aux + 22 prot)
+ 19 aux (sum +);
59% detected changes!

S^R x Prototroph Method

September 21, 1950.

Inoculate 8 PM 9/20 from young aerated cultures in Penassay. Grow overnight & wash.

Aerated.

1 K-12
2 K-12 + W-1177
~~3 K-12 + W-1246~~
~~4~~ W-1246
5 W-1177

Unaer. (less time)
6 K-12
7 K-12 + W-1177
8 W-1177
~~9 W-1246 + W-1177~~

Results:

9/22 1, 5: 3 plates each. No colonies

2: ca 1000/plate

9/23: 1 colony on 5. (K-12 S^R mutant, check uninoculated medium.

See 763

9/23 6; 8 2 plates each. No colonies

7 ca 400/plate.

Test single colonies on EMB lac

760-2 Either found, on EMB lac

	+	−	?
	42	46	88
	57	41	98
	109	87	196

$\chi^2 =$

Conclusion: (11/12/50): $S^R X^+$ selection is a reliable method for detecting recombinations.

Pseudomonas fluorescens

Preliminary and penicillin rxn.

Sept. 21, 1950.

P. fluorescens. A3.12 received from R. Stanier.

Grow at 30 ±. Aerated cultures gave heavy growth overnight in Penassay or in D(glucose). However D— also supported growth, presumably due to citrate utilization.

a) Test "PF" base: per liter with addn. of 0.1% substrates.

K_2HPO_4	4
KH_2PO_4	4
$MgSO_4$	.5
NH_4NO_3	2

n.g. with benzoate or glucose.

Throw out!

b Dilute cells from dense Penassay culture 1:20. Add varying units penicillin and aerate. 230 PM.

Pen.	4^{30}	6^{30}
0	++++	++++
50/ml	"	++++
100	"	+++
500	"(+++?)	++ lysed
1000	++ lysed? (granular deposit).	

∴ 500 – 1000 units/ml will lyse Pseudomonas fluorescens.

9/22/50. [20-40-60 sec. doses. Dilute in Penassay 1145]

Wash 48 hr. aerated D(Glu) culture and resuspend in $M/_{15}$.

UV at 50cm 10ml samples in Petri dish. Dil. ½ml 1/10 and dilute from this as 10^n for viable counts

UV sec.	Count
0	5.3×10^7; 4.8×10^7 – 5×10^7
20	5.6×10^6
40	5.5×10^6
60	10^7

See over for conclusions

Auxanograms – P. fluorescens

Culture	Responses	(old symbols)	new symbols · TEST	
1	Yx, HC, A1(?), A5 (illegible)	A5	A4	HISTIDINE (±)
2	Yx HC A3	A3	A2	A2. (comprng.)
3	Yx HC		A2	
4	Yx. HC very slight		A2.	
5	A3	A3	A2	A2
6	Yx Vits?	Vit?	V	not VITS.
7	Yx HC A4 A5	A4 A5	A3, A4	ϕalan. HIST (±)
8	A3	A3	A2	A2
9	A4	A4	A3	TRYPT. HC ng
10	A4		A4	
11	Yx HC	A4	A3	TRYPT. HC ng
12	A4			

Further tests

				PF
1			HISTIDINE	1
2	IV	✓		2
3	IV	✓		
4	IV	✓		
5	IV	✓		
6	Reculture	9/30 A2		
7			ϕalanine	3
8			histidine	
9	IV	✓		
10			TRYPT.	4
11	A4	TRY HIST.		
12			TRYPT.	

10/1 Throw out all but PF – x strains.

Notes:

PF-1. Growth on histidine is slow and limited compared to A4.

761-8. After 48 hours, growth on glutamate exceeded histidine. Try hist + glut and hist, glut + vits; + yna.

PF-2 Growth on A2 ~~slightly~~ considerably faster, than on isoleucine-valine. (balance?)

PF-3 and PF-4 probably preferred as mutants for further work

Pseudomonas – penicillinum

Sept. 23, 1950.

P.F. A3.12 irradiated 60 sec. Grown overnight in aerated Penassay. Remoculate into Penassay 9^00 AM for young growth.

2 PM wash and resuspend in D(Glu). Rm. temperature

2^20 aerate A) .5/10 dilution

B) .05 x .2 = .01/10 dilution

2^40 add 1000 u penicillin per tube. (1000 u/ml)

6^15. A strongly lysed cf. non glu – non pen. control

Plate A, B, in D(0); NSA.

9/24 1PM. Comparison of D(0) with NSA impractical owing to failure of subsurface colonies.

A2 → ca 30 surface colonies

B1 → ca 3-4.

Pick from each to water. Test on NSA; D(glu)0 agar.

A: 30 tests. 11 did not grow on D(0).

B: 20 tests 2 did not grow.

lower dilution into penicillin is not more effective. Possibly a longer interval should be used.

Pick presumed mutants to nutrient agar for ~~pass~~ preservation.

Further colonies tested

A 20/27 + 13/19 = 33/46 Store on NSA plate.

B 4/10

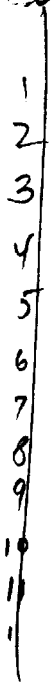
Pentagrams.

Positions 1-10 on periphery
A-D in center

A Yx	1 A12
B HC	2 A3
C YNA	3 AV
D V.t.	4 A5
	5 A6

Picture

Responses



Test remaining stocks of mutants on D-agar + Val, tupt, ~~his~~ IV, his⁺ + glut.
to screen hitherto undetected mutants. Hold only those which show ± response.

	D -- agar
PF 0	++
1	++
2	+
3	++
4	++

Keep ~~14~~ 36 as distinctive mutants. Some ± growth not retained.

Test on plates & random supplements. Allgrow on EMB.

			HC	V,+	YNA	A1	A2	A3	A4	A5	
PF 0			+	++	++	+	+	+	+	+	FINAL TEST IN LIQUID
	1		+	−	−	−	−	−	+	−	
	2		+	−	−	−	+	−	−	−	
	3		+	−	−	−	−	+	−	−	
	(4)		+	++	++	+	+	+	+	+	
PF #6	1	A2	+	−	−	−	+	−	−	−	LEUC
	~~2~~	~~XX~~	+	± +	++	±	+	± +	+	+	
	3	A2	+	−	−	−	+	−	−	−	LEUC GUAN OR AA AD±
7	4	YNA	−	−	+	−	−	−	−	−	
	5	HC	+	−	−	−	−	−	−	−	
8	6	A1	±	−	−	+	−	−	−	−	ARG AD, GU OR wk±
	7	YNA	−	−	+	−	−	−	−	−	
9	8	A1	+	−	−	+	−	−	−	−	METH GUAN OR
	9	A1	+	−	−	+	−	−	−	−	
	10	A1	+	−	−	+	−	−	−	−	
	11	A1	+	−	−	+	−	−	−	−	
	12	YNA	−	−	+	−	−	−	−	−	PUR
	13	YNA	−	−	+	−	−	−	−	−	PUR
	14	??	−	−	−	−	−	−	−	−	

ERROR? CROSSFEED?

4 YNA
2 A2
5 A1
1 XX
1 HC

(see over)

PF5 = PF3 S^R (surface plating - ca 100u/ml effective).

Still to check:

5: AA ok Sujkadd. allo. Sujkcons: (-3 -2) no growth

9 ——— Meth
10 } A1 Meth.
11 } —— Meth

14 Yx. → YNA+V garega.

PF 1

Hist + A1235	→	+++
– Hist (AA)	→	–
Hist + V		+
Hist		+
" + Selit		+
" + YNA		+±

other AA stim.

PF 4 now prototrophic. T. O.

5 Pal + A2 (L, Isol., IV n.g.)

14 YNA + B12 !! Punnia n.g. for YNA

H-1 F2 self. 762

September 23, 1950.

Lac+Mal+ Lac,-Mal,-

W478 × W1178.

2- : 100+

in EMS Lac.

9/25 Pick 100 lac+ colonies and streak on EMB lactose.

All lac++

9/26. 28 addnl. Lac+ 2: Lac v. (762-1, 21.

9/29 ← EMS Lac → Restreak 4 single lac+ from EMS to EMS, EMB lac + Mal.

1. all Mal++, lac v. ~~in EMS lac.~~

2. " " "

Conclusions:

This cross yields too many Mal+ (lac+ and lac v) to be very efficient in testing for Mal- lac v hemizygosity. Renew with other combinations of markers. See W1325 × W588 (767)

E. coli outcrosses

September 23, 1950.

See 760. (W1177 × K-12).

Incor. from overnight cultures W1177 + various prototrophic E. coli isolated by S. Shapiro from chickens. into Penassay 9 BM

Wash + plate mixtures on D(SM) agar.

	#	
1	803	
2	115	no X+ S^R noted
3	110	
4	111	~~3~~ plates each.
5	109	
6	105	

X+ " " prototroph
X " " auxotroph

" 7, 8 " "

Centrifuge, using 2 drops from mixed Penassay culture, overnight, as inoculum, without washing, on D(SM) plates.

W-1177 + Culture #

K-12 3 plates: ca 50 S^R prototrophs/plate

	Ag. Bact. #
7	866
8	114
9	7
11	10
12	23
13	834
14	841
15	865
16	863
17	
18	828
19	
20	833
21	827
22	
23	
24	2 plates 830
25	825
26	
27	839
28	
29	818
30	

other cultures, 1 plate only.

31	111
32	820
33	114
34	
35	
36	816
37	106
38	845
39	838
40	120

111

No X+ S^R found in any of these test crosses

E. coli outcrosses.

9/27/50. Plate mixtures of indicated strains + W1176 on DSM without washing:

W 1115
1113
1114
1117
W1045
W1176
} 2 plates (2-3 drops culture)

W1258 5 plates. No X+ S^R.

No X+ S^R colonies except for

1) 1176 → pure lac −

1 2) 1115. → pure lac −

Pick and streak out on EMB Lac.

9/29/50. Concentrate mixed cultures above from 10 ml YE to ca. 1 ml H_2O and plate .2 ml per plate. W1113, W1114, W1117 gave pellets not readily redispersed. Disperse these as far as possible P30.

B.

(CONCENTRATED SUSPENSIONS)

10/1/50. B: K-12 x gave 10^3 X+ S^R per plate

	1115	1 (2 plates)
	1176	2 (2 plates)
	1045	ca 50 /plate (2 plates)
10/2/50	1258	0
	1113	0
	1114	0
	1117	0

10/3/50. Plate 12 plates, conc. W1117 — no S^R X+.

Stock is also pure − in lac, Mal EMB.

760-5 is mixed on EMB Mal: must be a mixed culture, or somewhere. Not a W1177 X+ lac+ Mal+ !!

Marker tests on various prototrophs

Test on EMB Lac, Mal, etc:

	Culture	Lac	Mal	Xyl	Gal	Sucr.
1	W 1177	−	−	−	−	−
2	W 1176	+	+	+	+	+
3	W 1115	+	+	+	+	+
4	W 1045	+	+	+	+	+
5	760-5	+	− +	−	−	−
6	K-12	+	+	+	+	−
7	K-12 × 1177	+	−	−	−	
8	"	+	+	+	−	
9	"	+	−	−	−	
10	"	+ −	−	−	−	
11	1115 × PUR.	−	−	−	−	−
2	1176 × PUR.	−	−	−	−	−
3	1176 ×	+	+	+	+	+
4	1176 ×	+	+	+	+	+
5	1045 ×	+	+	+	+	
6	"	+	+	+	+	
7	"	+	+	+	+	
8	"	+	+	+	+	
9	"	+	+	+	+	
20	"	+	+	+	+	
1a		−				−
21-30	1045 ×	all +	all +	all	+	all +
31-42	direct from colonies & suspension.					++

! Repeat. See 760

Re: K-12 [illegible]!

1045× very likely S^R mutants. W1115×, 1176× may be either recombinants or very peculiar types like 760-5 which demands explanation.

Inoculate Y2 with stock W-1177. Inoculate heavily into DSM.

H248 : 3 papillae on Mal EMS. (hemizyg. test)
all bac - segr. pure Malt pat in test.

E coli outcrosses

763c

10/8/50 + prev.

W1177 ~~plate~~ cultured with

1 W1176
2 W1115
3 W1258 in ½ broth 24 hours. Wash + plate very heavily (ca 10^9/plate DSM).

Controls: W1177, etc. alone: no colonies

1. ca 200/plate
2. ca 1000/plate
3. 0 colonies. (10 plates)

These colonies on test proved to resemble their prototroph parent: Lac+ Sucr± and Streptomycin-sensitive!!

10/~~10~~9/50. Repeat with fresh cultures:

Sh24x 5 plates ca ~~4~~/
Sh24c 2 plates ca ~~15~~/

W1045x 3 plates 1
c 2 plates 0

Brush ~~streak~~ on EMB Lac, Mal. Result very peculiar:
all slow on Mal. Sh24x; 1045x are Lac+, -
Sh24c Lac+.

Sh24c Lac+ Chief prototrophy! — none prototrophic!
Sh24x (1-8): Lac- S^R
W1045x Lac- S^R

probably S^D mutations.

W1177 x

1	Gaunt +	4.	W1113
2	W1281 (Coli Lisbonne - Cavier)	5	W1115
3.	ML (Monod - Lwoff mutable)	6	W1176

1.	5 ⊗ ; 2 control plates	0	
2.	5 ⊗ 2 " "	0.	
3.	5 ⊗	16	#1-16
	2 c	0	
4	2 ⊗	0	
5	2 ⊗	1	#17
6	2 ⊗	15	#18-32
	1 c	3	#32-34.

Pick all colonies to water. Spot on DSM for preservation.

Streak

1-16 (ML x 1177) : all Lac - mutable; Malt (~~numerous~~ 3^R mutations??)

Peculiar sectoring noted in ~~16~~ colonies on EMB Mal

17 mucoid mutation on EMB lac. mottling of fluid streak.

SD ? { 18-31 weak growth on EMB lac Lac -

32 mg. on EMB lac

DSM plate check.

#1-17 show peculiar mottling of colonies on EMB Mal.
Resemble (from #3), and ML itself. However, ML is definitely a weak MH+; 763-1-16 are all strong MH+. (Maybe effect of S^R mutation)

Clues:

#3 resembles ML in variegation on EMB Mal, from strong to weak Mal+. Not unique.

#1-16 also resemble ML in Lac^{-mut}; Mal+; Xyl+, (V_1^R) and must be regarded as S^R mutations in absence of evidence for recombination of any other nature.

763f- #17 appears to be Lac+ Xyl- MH- V_1^R. v. Poor growth on EMB xylose!
From W1115 x W1177.

Compare 1. W1115
2. W1177
3. #17
4. 1+2

on various media.

EMB:	Glu	Lac	Suc	Mal	MH
1	+	±	+	+	+
2	+	−	−	−	−
3	wk. +	wk+	+	wk+	+ wk
4	+	+, −	+, −	+ −	+, −

#17 is a weak fermenter, but may be merely an S^D or S^R type mutant from W1115. It does not provide evidence for recombination.

S^R/S^S heterozygotes

Sept. 26, 1950

9/29 W67 x W1177 heavily plated on EMS lac 7 plates x ca 200/. ~1400 2 (lac+) prototrophs. Restreak on EMB lac, EMS lac, EMB Mal, DSM.

#1. Mal−; gives a few papillae on D(SM). lac v. grows poorly on EMS lac.

#2. Mal−; grows well on EMS lac. large colonies on ~~D~~ DSM. Streak out several colonies of each on EMB lac for segregants for S^S/S^R test.

---

6 EMS Lac+ colonies to ~~EMS~~ EMS Lac (SM)

3 " lac ± . (noted on streak of 764-2)

all S^R

ca 30 Xyl− S^R (taken from EMB lac segregants).

This culture H248 is evidently pure S^R Mal− Xyl− (coupling of S^R to Mal− is not unexpected). It may be useful in physical comparisons with K-12.

B Repeat W67 x W1177, v. heavy parental inocula, on EMS lac. After 3 hrs., ca 1700 prototrophs. 10 possible lac+ picked for test: streak on EMB lac; spot on EMS lac; streak v. streptomycin on EMS Mal

	lac	EMS	Mal	S				
1	−			R				
2	~~+~~ ++			R				
3	−			S				
4	−			R				
* 5	v			R	1	Pure Mal+	Xyl+	S^R
6	−			R				
* 7	v			R	2	"	−	
8	++			R				
* 9	v			R	3	"	−	
* 10	v			R	4	Pure	−	Xyl− S^R

None of these is segregating S^R/S^S. Repeat cross again (to look for Mal v)

→

~~to~~ S^R dominance?

W67 x W1177

10/11/50.

Cross 67 x 1177, 10/9/50 EMS lac.

C Colonies examined A12. 14 plates ca 100/ = 1400.

3 colonies picked: (2) very doubtful). Test on EMB Mal
#1 + 2 Lac v Mal −; Mal + S^R EMS Lac (S14).
#3 Lac − Mal + S^S

D1 ① W67 + W1177 + 1 added volume ×2 9:30 — to

② Separate until washed.
concentrate each from 20ml combined volume to ca. 1.5.
(Spread .1ml each on EMS lac (± camphor))

Yields in 1 and 2 ca same. (① may be not more than 2 × ②).
2?? lac+ in 19 × 50 prototrophs

1 (Acenaphthene) Lac − Mal + S^S
2 (Camphor). Lac + (pure) Mal − S^R

2da → Neither is lac v.

3da. P15. 2 additional possible lac+

(1. − camphor ① Lac v Mal − Gal v Mtl − Xyl −. Gives many lac − proto
2. − acenaphth. ① Lac − Mal + trophs. (partial segregation?))

P17: P## (camphor; colchicine; controls)

E very poor yield.
20 plates × ca. 25/plate = 500. No lac −.

F 10/19 10 plates. Parental media derived from cultures exposed to camphor on EMBagar for 6 hours.] Ca 160/plate. 12 Lac + segs. (ca 1%!) (Reincubated more)
Is this due to character of parents?
4 additional lac + 10/22. (14-17)

No suitable S^R/S^S heterozygotes recovered.

10/22/50.

F's streaked out on EMBlac (poor batch!), EMSlac; EMSMal.

	Mal	lac	
1	+ ✓	~~+~~	~~v~~ ✓
2	—	~~+~~	v?
3	—		v?
4	—		v?
5	—		v?
6	+ ✓		~~+~~ ✓
7	—		v
8	—		v
9	(✓ mucoid)		v/mucoid
10	—		v
11	—		v
12	+ ?		v ✓
13	—		v
14	—		v
15	—		v
16	+		v
17	—		v

Recheck 1, 6, 9, 12 for Mal, lac v.

Save F1 as example of Mal+ lac v.

all Mal − lac v were strongly mucoid on EMSlac Mal+ were not.

See 771 D

Mal- mutant hunts: W478, W466

9/26/50 ff.

A) W478 2 plates × 300. (600) 1 Mal− (sectorial). Primy and slant as W−1323 4v 7secs.

W466 2 " 0.

10/1/50 W466 14 plates, 150 (2000) 1 sectorial → 1 Mal− { 1324
4 −? → 1 Mal− { 1325
→ 1 Mal slow 1326

10/3/50. W1326 Appears + in thick streak portions. May be highly unstable.

765-4 Many "−" colonies have + wedges. Restreak these

All streak ++.

−+ sectors give a profusion of Mal+ colonies.

Restreak apparent pure "−" and "+" + These remain pure. However, from original plating * 1 a Mal v line was established. Hold in refrigerator.

10/6/50. After 3 days, Mal- colonies show + papillae and sectors. Picks to test for segregation.

10/7 → all streaks show: Mal v (D) and Mal−; a few Mal+. ? E

? W466 → = → − → + → + + (mut) = ; − + → W1327

Hypothesis

A * Streak on EMB Mal as 765-4A. Put on NSA slant (grow mixture).

B + Re " " " 765-4B. Pure Mal+. This turns out pure −

C = W1327

D = W1329

E = W1328

Unstable Mal+ ⇌ Mal−

↓

Stable Mal+

Pseudomonas: double mutant penicillin res.

Sept 30, 1950

PF-3 = ϕ alanine
PF-4 = tryptophane

Aerate overnight at room temperature. Harvest and resuspend in saline. Irradiate 9 ml samples, in Petri dish, 40 secs.
Reinoculate 1 ml samples into Yz, aerate 10 AM —

n.g. Agglutinated too heavily in saline

10/4. Repeat, washing in H_2O. with PF 3.
Irradiate 40 secs. Inoc. in Yz, grow overnight
Regrow 1:1 inoculum 4 hours. Wash, treat with 1000 u pen. for 4 hours in D (ϕ-trypt). Aeration.
Plate out on EMB lac. Platings of 10^{-3} unpickable.

about 60 clones of PF 3 / tested: no mutants. (Poor lysis)

No useful result

~~4~~ 10/4/50.

1.	W1323 × W1177	Mal EMS.	Mal×
2.	W1324 × W1177	"	Mal, ??
3	W1325 × W1177	"	Mal, ✓
a 4.	W1324 × W588	Lac EMS	
b 5.	W1325 × W588	Lac EMS.	

All crosses gave good yield. Parents checked for purity: o×.

1. gave Mal+, ca 2%. 16+/433 total one plate
5/260 total. " "

2. gave Mal− only. o+/ca 1000 (2 plates) Mal,
Later: 1+ noted. Confirmed on EMS Mal. Crossover or Recomb??

3. gave Mal− only o+/ca 1200 (3 plates) Mal,

4.

30− : 18+	
31− : 8+	
39− : 22+	
100− : 48+	/ 148.

In standard cross, excess lac + expected.

5.

119− : 30+	
91− : 32+	
210− : 62+	/ 272

Pick lac+ for test as lac ✓. Spot to EMS Mal. Select "lighter" Lac+
4: 1-11
5: 1-12

Test these crosses in EMS Mal

Het mixosse

10/7/50

#4	Lac	EMB Mal	EMS
1	V	—	—
2	V	—	—
3	+	+	+
4	+	+	+v?
5	+	+	+
6	+	+	+v?
7	v ?	—	—
8	v ?	+	+
9	V	—	—
10	V	—	—
11	+ ?	+	+
12			

13–44: lac v 22, 35, 34?
lac+ ??

All Mal+ on EMS except 14, 22, 31, 35, 46, 47

Reticule likely lac v on EMS Lac for purification

		767-
1	Mal −	1
2	+	
3	−	2
4	−	3
5	+	
a 6	−	4
7	+	
8	−	5
9	−	6
2=H2 54		7
		8 → Mal+ lac v
3		9 = Mal+ lac v
4		
b 5		10 Mal+ lac−, no test.
6		11

Reversions:
2: (3) pure Mal+ lac v (2); + (1) Mal Δ/− → Mal Δ/+.
3: (1) pure Mal+ lac+ no test

Conclude: 767-9 (+ stalls) and 767-2 are clearly Mal−/Δ (hemizygous)
767-8

Het mixoses.

767b

10/7/52

#5	Lac	B Mal S
1	V	—
2	V	—
3	V	+v?
4	V	+v?
5?		+
6?		+
7?		—
8?		+
9		—
10		+v?
11		+v?
12		+

13-36 Lac: V 13 17 19 24 36

\+

? 14 21

Mal −: 21 22 24 29 30 35

Restreak likely lac V on EMS lac for purification; to Mal EMS for Mal+ rev.

H253	1	Mal+
H254	2	—
	3	—
	4	—
	5	—
	6	—
	7	Mal+
	8	Mal+

[1 M+L+ (rocket)].

4 Mal+ → 3 Lac V; Mal+. ; 1 Lac − Mal+. 3 kolt: Mal−/Δ

Het mixoses → Mal−/Δ

PF crosses.

10/9/50.

Cross streak, incubate 48 hours EMB lac. P. dig growth from intersection and spread with loop on D(o). Record #cols. 48 hrs.

PF ×	1	3	6	7	8	9
1	(0)	0	0	0	0	0
3	0	(0)	0	2	0	0
6	5	0	(0)	0	4	2
7	0	1 mm	15 ±	(0) mm	0	0
8	0	0	1	1	(0) ×	3
9	1	±	0	0	3	(0)

Note that parental controls are all negative. 6 × 7 is the most promising. (also 8 × 9)

10/13/50. Inoculate Y≠ from EMB Brushes. Grow still 24 hrs; aerate 24 hours. Wash + conc. ca 5×. Plate. 1 ml per plate D(o).

PF	6	1-2/pl
	7	100/plate
	8	Diffuse growth.
	9	1-2/pl.
	6×7	2-3/plate
	8×9	diffuse or 10^3/plate

No evidence of crossing! Put addtl. markers into PF 6, 9 and attempt these. (e.g. S^R)

Inconclusive. Used additional markers.

10/9/50.

Streak W1327 and 1328 on EMB Lac for reversions. Pick papillae for examination as lac V.

W1327

	Lac	− Mal
1		↓
2		
3	all about pure × no obvious variation	
4		
5		
6		
7		
8		

W1328/9

1	little.	−, V
2		V, −
3		V, −
4		−, V
5		V, −
6		V −
7		V −
8		V, −

This suggests a mutable Mal allel rather than segregation.

For 1327, Select a lac+ and streak out to allow lac analysis; Mal reversions.

B) 1329: Pick a Mal V from each and restreak on Lac; Mal.

C) Pick any possible lac V from reversion plates and restreak.

"W1328" No pure + obtained! Study other reversions of W1327

c1 1 possible lac ±. Restreak single colony → pure lac+.

B: #3, 5, 8 are pure Lac+, Mal V. Others happen to be Lac− Mal V
No pure Mal+ seen. Repropagated lac+ Mal V as W-

D. Papillae from W1327. Streak out individually: are there any Mal++?

W1327 revertants. 769D

8 Mal+ revertants from single Mal− colonies tested for purity. Some − components

	Mal
1	+ −
2	++
3	++
4	+ −
5	v
6	v
7	++
8	++

Restreak − and + components of 2, 3, 4, 7, 8.

Confirm: stable + ✓.

10/19 Repick associated − and + to determine whether a single − type gives both Mal+ and Mal+^{m}. Transfer + to slants as 7 & 8 etc.

D7 is distinctly more powerful Mal+ than the others.
D2− is most papillated

Reversions from 769 D:

2	almost all variegated.
3	occ. Mal v. Some Mal++, Mal+?
7	mostly Mal v " " " ?
8	occ. Mal v.

∴ all Mal− give Mal v revertants; some Mal+??

P 10/21 Restreak single Mal+ or v colonies from above.

2	varying degree of instability
3	2 stable +; 1 stable ± *; 1 variegated +
7	2 stable +; 1 unstable ++ *; 1 variegated + *
8	1 unstable very weak + * 3 variegated ++

Repick interesting types *

10/21. Cross D7+ with Y10 for "suppressor" to 11 1 possible − / ca 1000 proteolytics (& plates)

↳ streaks out: mucoid Mal+ (not Mal+)

∴ W1327 probably differs in only reverse from K12

10/7/50.

Irradiate PF0. (30 sec.) Incubate in D(0) glucose.

A 8: Inoc. into T(mand) to preadapt 3 - 4 hours (heavy inoculum 1:1)

Wash and reinoculate 1:10 into T(mand). After 5 mins add penicillin to 1000 u/ml. After 5 hrs & overnight spread on EMB Lac. Test on EMB Lac; T(mand)agar

P10 55 tests : no mutants

Addnl test

P11 (Polyflis Fuid). 50 tests " ". No mutants.

ca 50 addnl. tests : " "

Query: Are methods suitable for C-source : 1. Penicillin selection
2. Agargrowth tests.

Check 1 by reconstruction with Mand- strains
2. by T(m) agar (+ mandelate).

EMB lac used solely for convenience + for identification.

10/18/50

a. W1303 × W1178
b. W1303 × W1177

10/20 c W1304 × W1303

10/21 d W1304 × W67
e W1304 × W478

10/21 a. very infitile: ca 5-10 small colonies/plate. Reincubate. 8 plates
Meanwhile 2 lac+ seen. Picke to EMSlac, streak on EMBlac, Mal. (lac v ? + ? / Mal −)

b 11 plates ca 40/plate (fruit crop). New small colonies appearing: reinc.
No lac+ seen.

10/22 a. About 2% lac+. Ca 100/pl. 13 lac+ picked.

(b.) ca 200/plate. No lac+. Striking colony dimorphism 0°0
2000/0 apparently both lac−.
Picke to EMBlac to verify lac− of large colonies.

(c) ca 150/pl., small colonies. 14/pl. No+ Reincubate No+. 2000/0

(d) 12 plates, ca. 20/pl. No+. Reinc. 1 unlikely +. No+ 250/0

e ~~Good yield~~.

+	−
~~58~~	21
48	19
3	3
11	7
28	32
148	82
+	−

Repeat:

10/23 b 10 × 50/ 1 mucoid lac? pure lac+ Mal+. An Mal EMS, ca 10% Mal+. 500
c 10 × 150 1 lac+: ~~[illegible]~~ " 2+ : 8− 1500
Mal− lac v 771-C1 See over ↓
d 11 × 50 0+. 550
5 × 0 − Repst at 42° 12 hours after plating. Then 37.

771 C → see C1

Repeat W1303 – 1304 crosses.

10/27. large scale

b‡	1303 × W1177	2 × 30ml → 5ml	.1/plate	Shake mixtures at 37
c‡	1303 × W1304.	2 × 20ml → 5ml	.1/plate.	11:10 – 2:10

b1 repeat 40° 2:30 → ||A>B (B) — 6:30 (A)

↓ b2 37° At 6:45 PM, expose to ~~(seconds)~~ UV 50cm distance

10/30. b. Very low yield. 768/36 plates. Many small colonies.

b1A Ca. 10-15/plate (3 plates)
B 0. 1 plate

b2.

2sec	109	(small colo.)
5 "	146	var. size
10 "	47	" "
15 "	181	" "
20 "	98	" " many small.

1-4 from b; 5 - b1A; 6 = b2-2 7,8 : b2-5 9 b2-10
10,11,12 b2-15
13,14 b2-20.

c. 37 plates very low yield c: 15–38

[28–38 from one plate. Destroyed feature only a possible excess accumulation of MB.]

None except: 2, 6, 7, 8, 10, 11, 12.

Relabel 1–7, 771B

Results of W1303 x crosses

10/23/58.
W1303 x W1178.

a.

	EMS lac	Mal	EMS lac —
1	V	—	+
~~2~~			
3	V	—	+
4	V	—, + ?	v. poor growth — + ?
5	+, v?	—, +	v p.g.
6	V	—, +	v. p.g
~~7~~			v. p.g. mucoid
8	V	—	v. p.g.
9	V	+ mottled	+ + single cols.
10	V	+ —	— + → reisolate
11	V	+ —	— + → reisolate

aar ~~1~~ 2
ag2 ~~8~~ 7

✓ lac v + mottled Mal —

lac v Mal + mottled *
lac v Mal —
lac v Mal —

verifications ruled from EMS lac.

Restreak where possible.

* Results. Pure Mal+ but mottled (modifiers?)

✓ Reisolated cultures No Mal v.

	Lac	Mal (3 days)
1	V	—
2	V	—
3	V	—
4/5	+	—
6	V	—
7	V	—
8	V	—
9	V	+
10	V	—
11	V	—

	Lac	Mal	SM EMS	SM EMB
1	+	—	R	R
2	v ?	—	R	R
3	+ ?	—	R	R
4	v	—	R	R
5	v	v	S ... (S R)	
6	v	—	R	R
7	+ ?	+	R	R

Xgl 1HR ...

Segregating S^R/S^S

Streak Mal v colonies from 5.

Many Mal − and Mal v. Few or No Mal +. Repick possible +.

20 Mal − : S^R

Reisolated cultures, after purification

	Lac	Mal
1	+	—
2	v	—
3	+	—
4	v + ?	—
H257 5	v	v
6	v	—
7	+	+

Check #4 ✓ Lac v. But very stable.

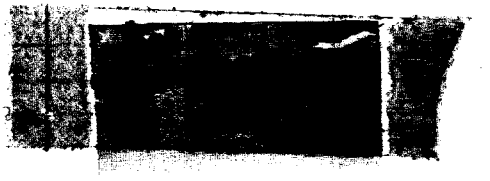
771C

cl see 771. Mal++ Lac$\underline{v}$ extreme "bullseye" type ⊙

Restreak from center of ⊙

95% lac−

Pure Mal+ Sal+ Xyl − Mtl −



EMS lac 48h: colonies are variegated on EMS Lac.

Restreak on EMS Lac; EMB Lac. Streak on EMS Mal.

− Picks from ⊙ center → almost all lac − (some pink, some blue). Very few ⊙

→ ca 10% lac+ on EMS! extremely unstable Store on # D Lac

Attempt to find Mal+ reversants still lac$\underline{v}$.

771C1-1 : all Mtl lac −.

2-5 ditto.

This enterprise is very difficult owing to the extreme instability of 771C1.

10/25/50.

e. 100 lac+ streaked out on EMBlac. Restreak[12] on EMSlac Likely lac v.
3 Additional 24 (small colo.). Take 4 as likely lac v.

	Mal		Lac	Mal.
1	—		√	√	
2	—	nogr.		...	
3	—		√	√	
4	++		√	√	
5	—			+	
6	++			+	
7	—			+	
8	+ —		√	√	—
9	—		√	√	
10	— +		√	√	—
11	—		√	√	
12	+ —			+	—
13	—		√	√	
14	++	no. growth.		...	
15	++			+	
16	—			+	

10/28. Replic EMSlac+ colonies to EMSLac; EMSMal (if Mal- or mixed) and EMBL

10/19/50.

.0001 ml 5% Na tellurite in ~~tube~~ phage plate W67 culture added.
$= 50 \times 10^{-4} / 25 = 2 \times 10^{-4}$ mg/ml = .2 r/ml
inhibited W67, with colonial survivors. Festucoid survivors:
When cross-streaked, showed no greater resistance than W67 per se.
2 r/ml plates and higher remain sterile.
Prepare DNG with
.2
.5
1 r / ml Tellurite.
W-1177 poured with ± 1 r/ml tellurite shows considerable turbidity, no definite resistants in 24 h.

Streaking W67 and "W67/Te" (from above) on surface of .2, .5, 1 r plates:

Te		W67	~~67/Te~~	67/Te
	.2	Heavy background. freq. outgrowths		Lighter background. large coal colonies
	.5	light background. a few res. outgr.		Heavier bckg. many large outgrowths
	1 r	v. light background; a few papillae		light bkg many large colonies * festucoid

10/26. Streak W67 and W67 Te, on DNZD + 1, 5, 10 r Te/ml.

24 h.

	67±	Te,
1	—	+++
5	—	+ declining
10	—	±

Medium grossly contaminated throw out experiments

W1034 × W1177

October 25, 1950.

W1034 × W1177 EMS Lac.

Yields (one. noc.) ca 30/plate. Ca. 98% Lac+

Picks 50 Lac+ and streaks on EMB lac.

49 → pure lac+ streakings 1 → lac+ colonies / colony Restreak.

Repeat 11/5 —

+	−
4	6
2	3
3	4
5	9.

No unusual appearance this time. Note low ratio, however!

f. 590

October 24, 1950.

A. 58-161 × W1177 (677) on EMS Lac.

Pick −, + and streak out on EMB Lac (to use in backcrosses)

	Lac	Mal	Xyl	Mtl	Gal
1	−	−	−	−	
2	−	+	−	−	?
3	−	+	−	−	
4	−	+	−	−	
5	−	−	−	−	
6	−	−	−	−	mucoid
7	−	−	−	−	
8	−	−	−	−	
9	−	−	−	−	
10	−	−	−	−	mucoid
11	−	+	−	−	
12	+	−	−	−	
13	+	−	−	−	
14	+	+	−	−	~~Mal+~~
15	+	−	−	−	?
16	+		−	−	

Cross Lac+ to W1177
Lac− to ~~W1177 Lac+ (W 1372)~~ W1394 - 410/5.

12 × : 47+ : 37−

14 × : 8+ : 39−

1 × 110+ : 31−

B. Also ~~58-161~~ (W478) × W1177 : 1-3: ~~Mal+~~ Mal− lac✓ 3/20 Lac EMS 9/28 Mtl EMS
4-12 #3 is lac+ others lac✓

C. 58-161 × W1022 #9 Mal+ all others Mal−

D. 478 × 677 #6, 10 Lac− all others Lac✓

B: 26 Mal+ tested on S. (Lindegg return)
25 S's 1 S^R.

Check above: 6 Mal− Mtl? lac✓?
10 " Mtl✓ has lac+ (Rw??)

Repurify! H268

11/30/50.

Repeat W478 x [redacted]177 mEMS MH. Isolate possible MH$\underline{v}$ and check. (Plates have ca 30%+ → 26/80.).

a. 12/2 80 tests : Reisolate 22 EMS MH+ from gross streaks on EMB MH.
12/3 26 — 6 ... 1

A = streaks from A+ gross streak on EMS

	MH	lac	Xyl	Mal	lac	
1	v	v	v	—	+	
2	+	—	+	—	—	—
3	v	v	v	+	+	
4	v	—	v	—	—	
5	+	+	—	+	+	
6	v	—	v	— +sap.	—	—
7	v	v	v	—	+	
8	v	v	v	—	+, —	—
9	v	+v? (—)	v	—	+	
10	v	—	v	—	—	
11	v	—	v	—	— +	v
12	v	—	v	—	— +	v
13	v	v	v	—	— +	—
14	v	—	v	—	— +	—
15	v	v	v	—	+	
16	v+	v (—)	+v?	—	+	
17	v	v	v	—	+ —	
18	v	—	v	—	— +	v
19	v	—	v	—	—	
20	v	— v	v	—	+	
21	v	— v	v	—	+	
22	v	— + —	v	—	—	

worthy mixable by 6/21/52 see 951.

... of 20 diploids MH$\underline{v}$, allare Xyl$\underline{v}$.

9 are	lac —
1	lac +
10	Lac $\underline{v}$.

23	v	v or +	v	—	v
24	v	—	v	—	—
25	v	—	v	—	—
26	v	—	v	—	—
27	v	v	v	+	v
28.	v	—	+	—	—

See 799

Lac+,− components: 11, 12, 18: on EMB Lac, these papillae show lac$\underline{v}$. On EMS Lac:

11: + colonies obtained → lac$\underline{v}$ Xyl$\underline{v}$ MH$\underline{v}$

12: − and $\underline{v}$ on EMS lac. → Lac$\underline{v}$.

18: EMSlac +. Lac$\underline{v}$. Xyl+ MH$\underline{v}$

8 Lac −, (+)

13 Lac −, (+)

~~EMSlac: pure+!~~ (Error in picking?) or recording.

H268 Revisions 777E

11/29/ff/50.

(8) Reisolated from single Mtl v colonies streak on EMS Lac; Mal.

12/2/50. #2 shows several papillae on both Lac, Mal. Purify.

#6, 1 isol. pap. on Mal. "

→ 2: All Mtl+, Lac+; Mal+ ...

on EMS Lac, H268 slowly turns very dark (v. slow Lac+ ??)

3 (1) Mtl − Mal+ } no test

4 (2) " Mal+ }

6 (1) Mtl v Mal+ ✓

7 (2) Mtl v Mal+ ✓

Lac – homozygotes 778

October 26, 1950

10/26 A W466 × W1177 BM Lac+ het (Xyl+) × lac – Mal – Xyl – on EMS Xyl

B " × W814 " × lac+ Mal – Xyl – "

10/28. B: EMS Lac

+	–
25	14
40	19
65	33

No yield on EMS Xyl.

10/29. 1 colony A. ca 10/plate B. Streaks on EMB, EMS Xyl.

B. 20 picked: 4 Xyl+ 16 Xyl – (sic!) No V.

Repeat on EMS Mtl.

A) 50 Mtl+ streaked on EMB Mtl; Xyl. No Xyl, .. V.

Repick further colonies

11/7 52 picked, streaked on Mtl: 3 very likely Mtl V 1-3
3 possible 4-6

	Mtl	Xyl	Mal	S	Gal	
1	V	V	–	R	–	H258
2	+ or V?	+	–	R	–	H261
3	+	+	+	S	+*	
4	+	+	+	S	+	
5	+	+	+	S	–	
6	+	+	+	R	–	

778-2: Mtl V verified from EMS → EMB Mtl

258 8 distinct reversions Lac+ each Lac V Mtl V
1: Mal+ Mtl V

REVERSION TESTS: H258, 261 on EMS Lac, Mal
Test segregants from #1: 6 Lac+ → Mtl –
10 Lac – → Mtl – 8; +2
not suitable for linkage phase study!

H261 8 " " each Lac V Mtl V
2 – Mal+ Mal+ (no test).

Lac –/– : Mal – /+

Met outcross (histidinoless)

October 26, 1950.

A	W1325	x	W826	Mist; sulfdy	EMS Lac, Mal
B	"	x	W826	Mist; glut	
C	"	x	W836	Meth; mist	

	Mal	−	+
A		81	26
C		80	38

	Lac	−	+
A		143	8
B		69	27
C		174	25

Pick +, − to EMS Lac for linkage test.

		Lac −	Lac +
A	Mal −	25	3
	Mal +	19	4
C	Mal −	20	2
	Mal +	18	4

no linkage Mal to lac

Ca 70 tests each. Detect likely lac V: (I'ete. from thick streak not single lac EMS col.)

A.
1 Lac+
1' Lac+, −

B
1 Lac+ Mal−
1' Lac+ "
2 Lac+ "
2' Lac+ "

C
1 L+ −
1' + −
2 + +
2' + +
3 +
3' + −
4 + −
4' +

None are heterozygotes!

H might be linked to Δ.

UV Effect on recombination

November 1, 1950.

W67 x W1177. Mix suspensions (20 ml → 1.5)

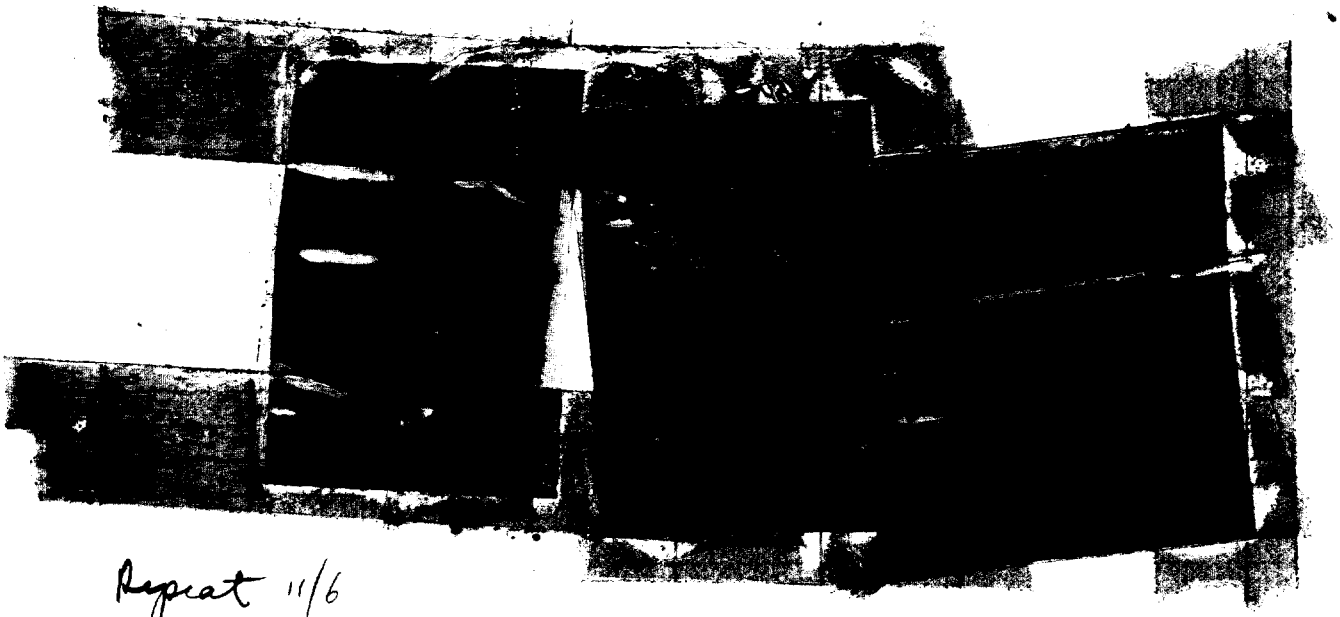
Plate .1 ml / EMS Lac.

a = no treatment

b = 10 secs UV 50 cm.

	~~EMB~~ Lac−	Lac+
a	24	
	29	
	31	
	37	
	25	
	58	
	69	
	38	
ca	40 /	0

	−	+	
b.	17		
	146	1	→ Pure +.
	61	mostly tiny	
	57		
	66		
	66		
	87	many tiny	
	several hundred large. very many small		?
ca	100 /	3?	



Repeat 11/6

Spread mixture (in saline together ca. 2 hrs.) at 3:15 PM.

Irradiate 10 secs. at intervals:

11/1-10. 3^{15} Control: normal. 10 plates

3^{15} 10 secs UV 3 "

5^{10} " 3 "

8^{10} " 3 "

No marked effect of irradiation at any time: sectors.
Probably more small colonies w/ UV series.

In ca. 6000 colonies, 3 likely lac+.

		Lac	Mal	S (EMS)	
#1	~~at~~	V	−, +	S	Mal− is pure S^S / Mal+ S^S probably ~~at~~ but is pure Lac−.
#2	3^{15}	+	−	R	
#3	3^{15}	~~B~~V	V	? (S)	S^R/S^S → 3 Mal+ revertants pure M+ Lac V. ∴ Mal-1Δ

Restreak 1, 3 from EMS lac to EMB lac
EMB Mal
Brush EMS Lac
Test S^R.

Also streak ~~B~~ on EMS Mal for Mal+ component.

1a-d Lac V (rel. stable) Mal− S^S

3 a-8. Lac V. Mal V very sensitive to sm. (system streaks selected or destroyed exc. for segregants).

Include segregants for $S^R//S^S$. For !, use no growth of S test.

Plate H267 on EMB Lac, Mal ± SM. (5×10⁻⁸)

	V	−	+
Lac	46 ~~47~~	~~48~~ 3	~~4~~
Lac SM	~~37~~ 0	~~0~~ 0	0
Mal	37	0	4
Mal SM.	0	1	0

Restreak H267 on Primary

Mal+ S^S may be pure segregant

E. coli outcrosses
Verifications

× W1177 on DSM

All Mal-
1. W1362 a — No yield.
2. W1362 b — ca. 20-30 colonies / plate all lac+ on EMSlac SM. *
3. W1373 — ca 5-9 / EMSlac SM plate. Mostly Lac+ (- maybe strains to grow: Rusic.
4. ~~W776-16~~ W1374
5. W1377 = 23a.

* Check colonies on EMS Mal. 4. original streak on EMB Mal: uniform Mal+

2: 100 Lac+ tested on Mal: 99+ 1− ... Restreak & check:

Streak out mixture as plated on DSM; EMSlac SM. ca 99% Lac+ 1% Lac−.

5: Pick Lac− for test on S. 4. Lac− from 776-23 original cross plate, streaked.

Lac− from mixture 2: S^R
from plate 776-23 {9: S^S } ! (also Lac, Xyl, or Mal −)
{3 S^R }
5 colonies were Lac±. Streak out and compare with Lac− and W1377.

---

2 Pick from streaks on EMS Mal (unpur.) and spot on various sugars, phages.

	Mal	lac	Xyl	M+L	T1	T5	T6	T7	
1	+	+	+	+	S	S	R	S	R −
2	−	−	−	−	S	R	S	S	S +
3	−	−	−	−	S	R	S	S	S +
4	+	±+	−	+	S	S	R	S	R −
5	+	+	±	+	S	S	R	S	R −
6	+	+		+	S	S	R	S	R −
7	+	+	−	+	S	S	R	S	R −
8	+	+	−	+	S	S	R	S	R −
Remaining 14.	+	+or+	#8,9,10	+					
			others +	#10±					

15 purified 781-2. 13 L− M− $T5^R$ $T7^S$ X−
2 L+ M+ $T5^S$ $T7^R$ X+
Check on Xyl; T7; prototrophy; RS app. Parent mix; non-proto rogue

Restreak on EMB Lac. ✓ check of W1362 a & b.

3: 22 streaked on EMB lac — 3 Lac− } Mal− ; Brush to Mal; all Mal− Hold! " "
+8 " " — 6 Lac− }

4 16 " " — 15 L− } Mal−
1 L+ }

-23 clones from 116-23 plate.

Duplex Prototrophs

11/2/50.

58-161 x W-1177.

20ml → 3ml susp. ca .1ml/plate.

EMS Mal
(EMS Lac)
(DSM)

EMS Mal, (Lac)

+, − differentiation very poor.

ca. 200-300/plate. No sectorials noted +. Too crowded.

~~on DSM, 3 colonies were observed. Streaked on EMB Lac~~

[Check on parents.]

	Mal	Lac	(SM)	
1	−+	−	S	1
2	+	−	R	
3	−	+	S	

Repeat 11/6.

11/8/50: 12 plates EMS Mal 1112 prototrophs examined under binocular scope. Picked any colony that might be Mal+/−. Mal+ not always readily scored (thick plates). Where scoreable:

+	−	S	Σ
17	80	1	98
9	64	2	75
33 ?	80	0	113
17	68	2	87
76	292	5	375

or 20%+ probably an overestimate.

Hold x-plates in refrig. for back samples

conc. inocula in EMS Lac SM. (ca 5x) ca 10 colonies/plate.

20: Test for S^R: all S^R, $\lambda+$

11/10 of 15 possible Mals streaked out on EMS Mal, 6 were Mal+/− (4 + 5 −).

#1 also had a sectored colony. Restreaked as 782A1.

Test paired segregants for Lac, Mal, Xyl, MH, Gal, V_1 and S^R

	Mal		S	S	Lac		Mtl		Xyl		V_1		Gal	
1	+	−	R	R	−	−	−	−	−	−	S	S	+	+
2	+	−	S	R	−	−	+	−	+	−	R	R	+	−
3	+	−	S	R	−	−	−	−	−	−	S	S	−	−
4	+	−	S	R	−	+	+	−	+	−	R	S	+	−
5	+	−	S	R	−	−	−	−	−	−	R	R	−	+
6	+	−	S	R	+	+	−	−	−	−	S	S	−	−
1a	±				−		−		−		S		+	

Correlation is best for lac, V_1 (#4 only exception).

C 4/19/50. 58-161 × W1177 m EMS Lac; B_1. Lac, Mal B, were turbid.

15 plates ca 30/plate. No lac ±.

→ +.

Lac ± no data regular occurrence!

Duplex prototrophs

11/11/50. Repeat 58-16 1 x W1177. EMS Mal

30 plates. ca 100/. Mal+/- scoring: optimal

Total Mal+ (incl. ±) 277.

11/13. Ratio of Mal+ : - (sample plates).

+		−	
19		101	
8		47	Pick these; reincubate all plates. 11/14: 7 additional possible Mal+.
9		47	
10		42	
4	(15)	42	
10	(15)	53	Also pick non-selected Mal+ and - to EMS Lac.
60		332	

∴ $60 > \frac{277}{50}$.

Among ca 5 of 50 colonies on EMS Lac, 1 Lac+ noted. Purify as 782L.

Non selected colonies. (to EMS lac; Mal for -) Hold for later analysis

	Lac+	Lac−
Mal+ :	33	34
Mal−	25 +2	44 +.

	Mal		S		R	Lac		Xyl		Mtl		Gal		V_1	R
1	+	−	R	R	−	−	−		−	−	−	−	S	S	
2	+	−	R	S	+	+	−		−	−	−	+	S	S	
3	+	−	S	R	−	−	+		−	−	−	−	R	R	
4	+	−	R	S	−	−	−		−	−	−	−	S	R	
5	+	−	S	S	−	−	+		−	−	−	−	S	S	
6	+	−	S	R	−	−	−		−	−	−	−	R	R	
7	+	−	R	S	−	−	+		−	−	+	−	S	S	
8	+	−	S	R	−	−	−		−	+	−	−	S	S	
9	+	−	R	R	−	+	−		−	−	+	+	S	S	
10	+	−	S	R	−	−	+		−	−	−	−	S	S	
11	+	−	S	R	−	−	+		−	−	+	−	S	S	
12	+	−	R	R	−	−	−		−	−	−	−	S	S	
13	+	−	S	R	+	+	+		−	−	−	−	S	S	
14	+	−	S	R	+	+	−		−	−	−	−	S	S	
15	+	−	S	R	+	−	−		−	−	+	−	S	R	
16	+	−	S	R	+	−	+		−	+	−	−	S	R	
17	+	−	R	R	−	−	−		−	−	−	−	S	S	
18	−	−	S	S	+	−	−		−	−	+	+	R	S	

Punct mutants

of 17 tests, Mal+/− and $S^{R/S}$ accorded in 12

Lac	concorded	14	
Xyl		10	Both − −
Mtl		15	" " − −
Gal		13	
V_1		14	

Lac; V_1 concorded 13 .

#18 was Lac$\underline{s}$. In view of concordance of Mal-S^R probably not an artifact.

~~Reticula 78265~~

M257 S^R/S^S

11/6/50.

Incor P6. into D(Lac). Grow 36 hours aeration.

Plate out at 10^{-7} on EMS Lac } ± SM 12 N 7
EMB Lac }
EMB Mal

Repeat 11/9:

11/8. EMB Lac:

V	—		V	—
174	71	245		
129	62	191		

EMB Lac SM

0	51	51	1000u SM	0	7	
0	57	57		1	4	
0	54	54	A6-7	1	8	
* 3	60	63				

EMB Mal

V	—	+	Σ			
193	52	4	249	170	12	
206	64	3	273	194	11	1+

Presumably, all diploid cells are killed by sm, with 3* exceptions.

Test these for Lac, Mal, S heterozygosity.

M257' segregants tested for S on EMB Xyl.

~~8~~ 8 Mal− : Xyl− S^R (28)
4 Mal+ : Xyl+ S^S (28)

?

Δ S Mal Xyl

Continue or take out verify?

* Exceptions:

	Mal	Lac
1	—	V
2	—	V
3	V	V

Back out 3 for Mal+.

204	1	
210	2	

	Lac+ (ug)	Lac−	
EMS Lac−	196	2	41 small cds. Lac+?

EMS Lac SM 0, 1, 1, 0, 55, 66, 47, 44. Total: 2+/212− 0, 4 (tiny)

	Mal+	Mal −	Mal v! (sic)
EMS Mal	172	55	21

H257

783A, D

See previous page
S^R Lac $\pm$ exceptions.

	Mal	lac
1	—	v
2	—	v
3	v	v
4	v	
5	v	
6	+	?
7	—	

or E.

4 Mal+ segregated : S^R v.

D. Inoc H257 1/100 Penassay. Grow overnight and plate out.

11/16 EMB Lac

v	—
85	26
75	15 + sprinkling −
73	14
81	37

average − : $\frac{92}{4} = 23$

= H257'

5.

EMB Mal

v	—	+
75	11	6
89	14	4

EMB Lac SM+

8
3

Phenotypic lag?
Test Lac− segregants for S^R. — Rather uncertain tests:

	R	S
25	2 Mal+ 23 Mal−	11 Mal−

SM .5u/ml

36
36

EMB Mal SM +

5	1
11	0
10	0
11	1

.5/ml

29	0
33	2

Note: Colonies on EMB + SM .5u may represent late segregation products of Lac± cells and may not reflect phenotypic lag. However, comparison of EMB Lac with Lac + SM (100u) may reflect phenotypic delay. Repeat plating. Also test Lac+ from EMB Lac for SR.

See over

M257'

EMS lac SM

V	—
1	0
2	2 v. sm.
2	1
2	1

Transfer to EMS lac; test on EMS Mal

H257

(EMS) Mal v. Almost all colonies of H257 plating show some signs of Mal aggregation

Streak out 8 Mal v colonies from EMS Mal to same.

Pick Mal+, − patch streaks separately to EMB Lac:

6: Mal+ lac+ / Mal- Lac − 1: Mal+ lac− / Mal- lac −

1: Mal+ lac+ / (Mal- lac+) Restreak on EMB, EMS lac as 183B1 ~ Lac v.

BB. H257' (~~Reg~~ Y2 1:100 . 24h. 37° 48h. Room T.)

EMB lac	v	−	EMB lac SM 100u/	v	−	
	123	43		4 (3... incl.)	82 *	smeared
	129	100		1	71 ±	smeared 4
	116	94		0	60	
	125	107		0	83	smeared.
	127	111		1	56	not smeared.
	620	505		6	349	
m	132	101		1+	70.	

* These counts are likely overcompensated for smearing, i.e. overestimates. Repeat plating, also with H267.

Test Lac− from H257' for S^R/Mal. (Also, see F)

Mal-S^R	22	20	42	42:12 S^R/S^S
Mal-S^S	1	2	3	
Mal+S^R	0	0		
Mal+S^S	IIII	++++	9	

H257 783C

(EMS) Mal–.

Test 20 Mal– colonies on EMS Mal:

a. Brush on EMS Lac /SM
b. " " EMB Mal /SM
c. Streak (few) on EMB Lac. cf. Mal+ colonies.

{ all S^R. ~~Lac–~~ Haploid prototrophs? (~~[illegible]~~)

EMS Lac –

Pick ~~5~~ 3 "small colonies" and streak out on EMB Lac, Mal. ~~Lac EMS Lac~~, ESM.

cf. 1 standard. ✓: Lac– Mal–
control Lac+ Mal+

11/16/50: From 783D

CC. Segregant colonies from H257' on EMB Mal SM. (i.e. S^R only).

		Xyl	Mtl	Xyl	Mtl
Mal+	~~5~~ 4	+	+		
Mal–		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	*	–
		–	– +	–	–
		–	– +	–	–
		–	–	–	–
		+	+	– +	– +
		–	–	–	–
		– +	– +	–	–
		–	–	–	– +
		–	–	–	– +
		–	–	–	–
		–	–	–	–
		–	–	–	–
		–	–	–	–
				–	–
				–	–
				–	–
				–	–
				–	–
				–	–
				– +	–

From EMB Mal.

Mal+ ~~5~~ 4 all S^S; 1 Mtl– Xyl–
4 Mtl+ Xyl+

Mal– 17 all S^R 15 Mtl– Xyl–
1 Mtl+ Xyl+
[1 Mtl– Xyl– +]

31 shown in 8 tm?

Consistent with order:

	R	–	–	–
+	S	Mal	Xyl	Mtl
– Δ	S	+	+	+

Limiting conc. SM. H257. 783DP

Plate H257 on EMB ± SM (.5u/; 100u/ml).

	v and +	—
EMB Lac	149	8
	150	16
EMB Mal	185 (+ mucoid)	10
EMB Lac SM.5	127	19
	44	16
	18	24
EMB Lac SM100	0	3
	1	1
EMB Mal SM.5	63	27
	30	27
	58	18
SM 100	0	2

} Lac v in this series have very luminish faded + var. centers.

very faint

At this concentration of streptomycin, diploid S^S/S^R are not regularly killed but are strongly selected against in favor of S^R segregants. This conc. cannot therefore be used for phenotypic tag as it will produce artificial S^R from S^R/S^S.

M257 / Gradd conc sm 783D

Plate M257 11/10 on indicated media. Read at 40 hours

	+ = v	−	v	E
EMBLac	169	14		183
	184	11		195
EMBMal	+	−	v	
	2	10	149	162
EMSMal	209	4		213
EMBMal SM. (100u)	0	13	0	
	2	5	0	
	1	4	0	
	1	4	0	
	1	3	1	
	5	29	0	
EMS Mal SM (100u)	0	4		
EMS Lac SM (100u)	1	0		
	0	0		
	0	0		
	0	2		
EMBLac SM (st. 100)	+	−	v	
	0	9	0	
	~~0~~ 0	~~1~~ 7	2 1	
1000		4	0	
100		17	3	
50		9	2	
10		9	0	
5		5	0	
2		9	1	
1		18	1	
.5 *		24	45	
.2		13	177	
.1				

Test Mal+ S^R and Mal− S^R on EMBXyl:

	Xyl+	Xyl−
Mal+	5 (5+ 0−)	0
−	3 (3+ 0−)	25 (2+ .23+)

Consistent with:

	R	−	−	−
− Δ	Sm	Mal	Xyl	MH
	S	+	+	+

↑ M+X+ ↑ M−X+

OK = M−X−

Test MH: Distribution of +, − and above.

* Sm app. unevenly distributed as center of plate is virtually sterile. Many v colonies have very faint ± component 2 Lac v have very little −. Disregard as possible crossovers.

M257 partsg. Mal Revertants. 78BE

11/18/50 ...

	Mal	Lac EMB	Lac EMS
1	—	V	
2	—	V	
3	V	V	
4	V	V	
5	V	V	
6	+ ?	V	
7	—	V	
8	—		+
9	—		+
10	—		+
11	V		+
12	—		+
13	—		+
14	V +		+
15	—		+
16	— +		+
17	—		+
18	+		+
19	—	— ?	—
20	—	—	—
21	—	*	+
22	—		—
23	—		—
24	—		—

From EMB Lac SM M257 [redacted]

from EMS Lac SM M257

8 Mal+: 1, 5, 8 are Mal± Lac V
2, 3, 6, 7 Mal + Lac −
V #4 → Mal++, (−) (Malv+ ?)
Lac±.

E7 #4 → Reisolated from single colonies
Malv (+ predominant) Lac±.

 Phenotypic lag. 783F

November 20, 1950.

Plate H257 ; H267 on EMB Lac; ± SM 100. Cf. with °/, s R.

11/19/50: H257.

	Lac		Lac + SM.
V		V	
92	143	0	125
78	97 src	12	99
52	130	0	130
82	132	0	94
84	135	1	126
399	637	3	574
$\bar{m}$ =	80 127	<1	115

Fr. carriers, s's, from streak tests on Lac−:

V	Mal +	Mal −
102	10	109

V	Mal +	SM −
1	3	92

H267

Lac	
V	
43	84
25	78
28	73
34	69
26	97
156	401
$\bar{m}$ =	31 80

Lac SM	
V	
0	6
0	4
0	6
0	5
0	5

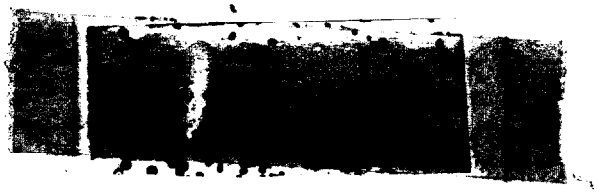
Mal		
V	+	−
28	54	18

Mal SM		
V	+	−
0	0	2

Threshold sus: # S^R/S^1 heterozygotes. 183

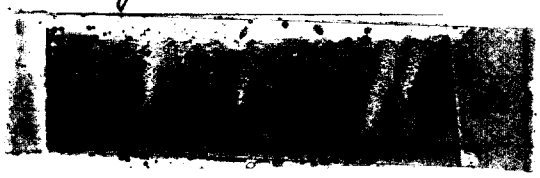
11/20/50.
Plate each of following strains, grown as D(Lac) (except W1177 - YZ) at ca. 10^{-7} on indicated EMB. 8 45 PM. Read at 4: 45 PM 11/21. = 20 hrs.

K12. # Lac
Lac SM 1 — sl inh
Mal SM .5 — sl. inh. turn.
Lac SM 100 — No colonies



0 .5 1

W1177 No differences.

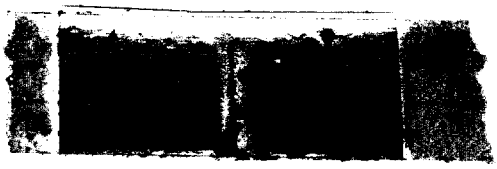


0 .5 1 100

H266 ($S^S/-$) 40 hours.

Lac	typical mosaic colonies ca 400	Mal —	do.
.5	reduced count; smaller Lac —		All Lac — Full ct.
1	19 colonies		21 Lac — colonies
100	No colonies.		No colonies.

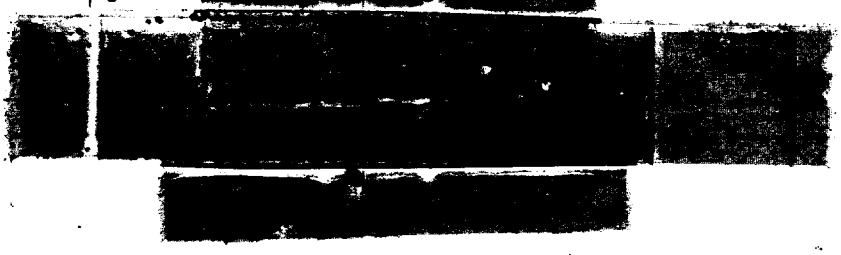
(Segregating partial resistance)



.5 0

H257 Lac typ. (somewhat small) Lac v. ca 400

.5 reduced Lac — Mal — (hint of +)
1 cols.; some normal.
100



0 .5 Mal .5 100 1
Lac

H 267

Lac	typ	lac v	ca 300
Mal. 5	5	large	1-3 v. small cols —
Lac. 5	10	med	1-2 " " —
Lac 1	2	large cols.	lac −
Lac 100	5	large cols.	lac −

H 267 may be more resistant than H 767, or give SR survivors more readily.

Linkage comparisons: SR minimal 78 4

11/7/80.

W1368 x

W677	standard	A
WH78	inv	B
W 1022	inv	C
W 1015	mv	D

			−	+	
A	+ TLB,		12	45	(some ~~unresolvable~~ doubtful microcolonies recorded as +)
			18	40	
			Lac−	+	
B	SM+ TLB,		4	72	
C			1	12	
D			5	15	

~~SM-0.~~ 0 0

Need repetition

11/18...

	+		−	
A	39	53	14	13
B	37	42	0	
C	6	15	5	0
D	20	16	1	0

(1561) Mutant Run 1373 785

10/7/50.

See 786.

A. Sterile.
B. (1): ca 10 colonies. Some lac-?
Pick to water; spot on EMB, D(0).
B: 11 tests: nonegrow on D(0) in 24h.

Light

			A1	A2	A3	A4	A5	YEx	YNA	MC	
785B	1	(A1) 1421	++				+	++	−	+	Cyst
	2	(A3) 1425			±		-	+	−	+	Tyro.
	3	(A3) 1426			+		-	±	−	+	Tryptic
	4	(A2) 1423		+			−	++		+	IV
	5	A3			±		−	±		+	
	6	(A1) 1427					−	±		±	
	7	(A4) 1428				+	−	++		+	No A4 resp
	8	A1	+				−	+		±	
	9	(A3) 1427			±		−	±		+	Trypt on Ø not tyr
	10			+			−	++		+	
	11	A3			+	−	−	+		+	−
	12		++	+			±	++	+	+	
	13	(A4) 1429				+		++		+	Hist
	14	(A2) 1424		+				++		+	IV
	15		+	+	+	+		++		+	
	16	A3			+			+		+	
	17	A1	+					+		+	
785B	0					+		++		+	−
786B	0					+		++		+	
	1	(A4) 1432				+		++		+	− A4−
	2				+			+		+	
	3				+			±		+	
	4				+			±		+	
	5				+			±		+	
	6				+			±		+	
	7				+			±		+	
	8				+			±		+	
	9				+			±		+	
	10				+			±		+	
	11				+			±		+	−
	12				+			±		+	
	13				+			±		+	
	14	A4			+	+		++		+	
	15				+			±		+	
	16	A4 1433			±	+		±		+	A4−
	17	(A3) 1431			++			++		+	A3−
	18				±			±		+	
	19	(A2) 1430		+				++		+	Leucine
	20				+			±		+	

All − unless indicated otherwise

(38-40 = 1431-33)

Mutant Run: W137

10/7/

UV - 30sec - incubated 4 minimum. Penicillin overnight.

A. 1:20 300u P/ml 29 tests: all x+

B 1:1000 100u/ml 40 tests: 2 x+ (24h.)
38 x-.

See 785

			A1	A2	A3	A4	A5	MC	YC	
	21	A3?	−		±	−	−	+	+	No Response to A3.
	22	A2	−	++	−	−		+	+	
	23	A4	−	−	−	+		+	+	PROL (0).
	4	A1	+	−	−	−		+	+	D(0) +
	5		−	−	−	−		+	+	
1421	6	A4	−	−	−	+		+	±	PROL
	7	~~A4~~	−	−	−	−		+	+	
	8	A4	−	−	−	±		±	−	
	9	A2	−	+	−	−		+	+	
	30	A2	−	+	−	−		+	+	
	1	A4	−	−	−	+		+	+	
	2	A4	−	−	−	+		+	+	
		A4?	−	−	± ✓	+ ✓		+	+	
	3		−	−	−	−		+	−	
	4		−	−	−	−		+	−	
	5		−	−	−	−		+	+	
	6		−	−	−	−		+	+	
	7	A4	−	−	−	+		+	+	
1431	8	A2	−	+	#	−		+	+	No resp. #2 IV; 0; +.
1432	9		−	−	−	−	↓	+	−	
1433	40	A2	−	+	# −	−		+	+	as 1431
	0	~~A~~ ++	+	+	# +	+	+	+	+	

None on YNA

A1 : 24.
A2 : 22, 29, 30 ; 1431; 1433
A3 : 21 ?
A4 : 23, 26, 31, 32, 37.

Throw out non W −.

Double mutants:

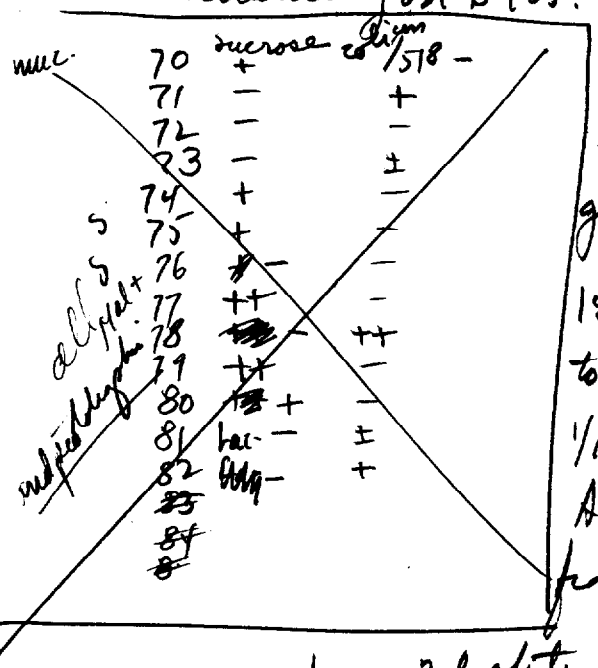
1421	1
1423	5
1429	3
1430	2

11/25/50.

A W1377
B W1395
C W1396
D W1397
[E W1441]

A-D grown in ~~Lactose~~ D(0), E. D(prol).. Inoculate directly (30 sec. UV 50 cm) and innoculate 1:10 in Penassay "A 25. Wash 8P. (C shows very little growth – unusually sensitive to UV ?) Incr. ca 1:500 in D(0) + penicillin [+ prol. for W1441].

A-D give erratic tests on minimal agar as they themselves grow erratically on D(0). Restreak parents on D(0).



W1377 .... 97 "A" isolated as repeated selections on D(0) agar until homogeneous, uniform good-size colonies are obtained.

1st Penicillin Rein: [W1396 is extremely sensitive to UV.] In 6 hr. rein, 100 u/ml., C gave 1/10 mants; A, B, D 0/10. ~~11~~ Overnight, A, B, D were overgrown. 1/20 additional mants from 1st plating of C (= 2/30). Test 50 cols. from 2d plating of C.

12/12 Repeat penicillin rein. using 500 u/ml., 6 + 24 hr. platings C did not grow sufficiently after UV.

12/21 Repeat with A, B, D. Each is resistant to 1000 u/ml penicillin and therefore unamenable to the penicillin method!
4 mutants obtained from C above. 3 A2; 1 A1.

Outcrosses : nutritional

K12 - W1373 - W1374 - 776.44

			24h	48h.	
1	785	B1	Many ++		x
2		B2	-	0	
3		B3	+		x
4	786	B1	+	50+	x
5		B2	-		0
6		B3	-	0	
7	776-44 = W1416		-	0,0	
8	W-1177			0	
9	1+8		++	200+	x
10	2+8		-	(0)	2+ → Mal − lac − + Mal+ lac+ (small colo.)
11	3+8		+	20+	x
12	4+8		+	10+	x
13	5+8		-	(0)	0
14	6+8			(0)	0
15	7+8		-	(0)	0,0
16	1+2		Many ++		x
17	1+3		++		x
18	2+3		+	20+	x
19	1+4		++		x
20	1+5		++		x
21	1+6		++		x
22	4+5		+	40+	x
(23	4+6		+	20+	x
24	5+6		-	(2+)	●

Repeat 10, 13, 14, 15, using growth together + sep. 48hrs.

31	785-2	0	0	
32	786-2	0	0 0	
33	786-3	0	0	
34	W1416		0	
35	W1177	0	0	
36	31+35	5+ 8+8+	+, − ++−	
37	32+35	+	1+ 1+ 1− 3−	
38	33+35	2+ 2−	1+ 2+ 2−	
39	31+32	1+	1+ 1+ 0	
40	31+33	0	0 1±? 0	
41	32+33	1+	0 0 0 0	
42	34+35	0	0 0 0	

36' etc. mixed with washing

36'		0 0 7+ =	40'		
37'		1+	41'	1+	
38'		0	42'	0 0	
39'	0 small v. small	0			

W1416 unscoreable
W1374,75 mutants
remarkably infertile
of sR x+ crosses.

Infection of W518 with λ

11/14/50.

To 5ml washed W518 suspension in saline add
1ml broth lysate of λ (11/7). 2:38 PM Held at R.T. to
Centrifuge 15 mins at 2:58. Resuspend in saline
Resediment, completed 3:43.
Plate 2×10^{-7} dilutions of each on EMB Lac, and on W518

A. washed cells
B supernatant 1
C supernatant 2
D original λ, titrate.
E original 518

A. Ca 300 colonies. No plaqued colonies or nibbling. Test sample for lysogenicity. 100 tested: all λ- ! (slow absorption)

B 18; 6 plaques on W518

C

D 19; 11 " " "

C 20; 18 " " "

D 10^{-5} 45 " "
-6 47
-7 28
-8 64

W836 x K12...

790

Nov. 20, 1950.

A W836 x W1177
B × W1178
C × W1406

2 Mtl S. colonies on EMS Mtl. Mal+ S^S
A: ~~Mal+ S^S~~ Mtl A: <+/− " "
∴ true duplex colonies. B <+/− Mal− S^R / Mal− S^R !

A. Plate on EMS Lac; Mtl. Lac: 60+ = 104− Mtl: 57− 47+ 2s (2 plates)

Test linkage of Lac, Mal.

	Mal +	−		Mal+	Mal −
Lac +	+	+	MH+	++	±
−	+	+	−	±	++

B. Lac: 137+ : 29− C.

C. Lac: 33+ : 60−
34+ : 57−. Mtl: 70+ : 32−

B. 24 tested: 4 possible ± → not ±, but may be segregating mutants!
C. 20 " No ±.

50 addnl. B + C. →
a few lac −; all others Lac++ no ±!
C1 - Mal+.

B 1-4 } apparently pure lac + { Mal− B / Mal+ C
(C1)
↳ self-plaqued (λ). Retests B3 which gives some V_1^R on EMB.

Check parents: W[redacted] proved to be λ^S. W1406 is λ^+ (Rev)

790 B3 is verified lac ±, but very unstable. Test also for λ.
Apparently λ−. H

Dianzini Strains: coli "transformation" (also miscellaneous phage tests).

Obtained various strains & phages.

			T1	T2	T4	T5	T6	T7	λ	518	Lac	Mtl	Xyl	Mal	Suc	
776-66	1	1442	R	↑	R	R	P	R	R	−	+	+	+	+	−	
67	2	1443-6	R		R	R	R	R	R	−	+	+	±	±	−	
68	3	1444	R		R	R	R	R	R	−	+	+	+	+	+	
69	4	1445	R		R	R	R	R	R	−	+	+	±	±	−	
	5	1378	R		R	±	R	R	R	−	+	+	±	+	−	colicin?
	6	1375	R		R	R	R	R	R	−	+	+	+	+	−	col?
	7	1377	R		R	R	R	R	R	−	+	+	+	+	−	
	8	1395	R		R	R	R	R	R	−	+	+	+	+	+	
	9	1396	R		R	R	S	R	R	−	+	+	+	+	−	colicin?
	10	1397	R		R	R	R	R	R	−	+	+	+	+	+	
	11	W1177K	S	R	S	S	S	S	R	λ+	+	+	+	+	−	

All are P^R exc K12 P^P

Dianzini M.U. (1950) Bollettino I.S.M. 29: 161-172. Mutazioni indotte dagli acidi nucleinici batterici.

He claims that 1443-5 are sucrose-positive but deals inadequately with problem of adaptation. Character of growth-agar not clear in his paper.

783: Partial segregants: tests for Mal heterozygosity

11/20/44. '50.

	783E −+	EMB Mal	tran EMB Mal EMB Lac	
1		++	−	
2	8	−	−	
3		−	−	
4		++	−	
5	9	++	−	
6		++	−	
7		v	v	✓ Mal v Lac v
8		++	−	
9	10	++	−	
10		++	−	
11		++	−	
12		++	−	
13		v	v	✓ Mal v Lac v
14		++	−	
15		++	−	
16	12	−	?	Mal− lac+ (~~crossover~~??)
17		++	−	
18		++	−	
19		++	−	
20		++	−	
21	13	++ −	v	Mal v Lac v
22		++	−	
23		++	−	
24		n.gr.	−	
25		++	−	
26		++	−	
27		n.gr.	−	
28	16	++,(−)	−	
29		++	−	
30		++	−	
31		++	−	

Recheck: 7, 13, 16, 21 on EMB Lac; Mal.

~~K12 x K13, K14~~

WG-1 x WG 3, 4

A W1446 x W1435 (WG4 x WG1 Het) → M269
B W1446 x W1177
C W1449 x W1435 WG3 → M270
D W1449 x W1177 (WG3 x WG1)
E W1447 x W1177 WG4
F W1448 x W1177 WG3

G 1451 x 1435 WG3. L−: 2 M+ / 1 M− L+: 30 M+ / 1 M−

A.

lac +	−
1	25
1	8
0	6
0	16
0	7

lac- predominates!
Streaks not lac+. Brush lac− to MalEMS.

B. Rather low yield (3-5/pl.) all lac −

C. Mostly lac+

4	5
7	1
2	3
12	1
8	2
33	12

very variable colony morphology.
Pick 52+ and streak out on EMB lac

D. No prototrophs (4 plates) [Alleles cancelled ??]
E " " " " ? (But cf. B)
F " " " "
G V. Numerous prototrophs. Mostly Lac+. Cf. on EMS Mal.
Ca 1% lac −.

L+ 1. Lac v. (clearly). Restreak a group of single lac v colonies and restreak on EMS lac; EMB Lac, Mal, MacConkey.

L+ 2. Lac ++. Restreak as above.

Lac − : on EMS Mal + 10 − 20 Not easily scored.

Restreak some Mal+.

M269' segregants

Lac − : 25 all Acriflavine R.

Lac + : 15 2 ? S 1 ? ? 12 R.

Restreak representatives for each.

32 lac+ streaked on EMB lac.

#6 Lac^{v}. All others Lac++. Reisolate.

Of others, all are Mal+ except #7. Pick single colonies to EMB-O

Restreak #6. ⟶ Pure lac+! Lac?

Lac− : 4 Mal+ 4 Mal−

M270

Choose weak lac+ for possible lacx.

W1452 x W1262

For M[redacted].

W ~~139~~ 1452 x W1262 on EMS Mal.

Picks Mal+ and brush against S/4 on Lac EMS.

only 16+ among 8 plates (ca 50/plate).

4 Mal± noted. Restreaks on EMS ~~Lac~~ Mal, EMB Mal; Lac.

795:1-3 of 16 tested in first selection, 3 are Mal+ S[s] on EMS. ~~Repeat~~ Streaks on EMB Mal for ± test. 10 reset Mal- on EMS..

After reincubation, additional Mal+ appear Test those as above 8/19 tested 4-11

1 pure mal+
2 ?
3 Mal+.

Retest: Mal+ Lac±.

Held in abeyance.

12/16- 1950s

				Yield m EMS Lac. /plate	
A	1482 × 1451	4-3	5-10	+, -?	
B	1482 × 1435	4-1	20	-	
C	478 1482	1-4	20	- (+)	
D	410 1482	1-4	10-15	-	
E	1455 1451	4-3	10		(contaminants ?)
F	1435 1455	1-4	0, 1	Lac -	
G	1451 1435	3-1	3-4	+, -	
H	1482 1455	4-4	0		

W1455 highly inhibited!

C: 20 colonies streaked out: 16 - 4+ Lac. No v.

E: 4 Lac+

G 8: 6 Lac+ #2-7 | 1 Lac - #1 | 1 Lac v. Resolate.

C: 16: Lac-Mal- # 3 Lac+ Mal- 1 Lac+Mal+.

12/25 E 4: Lac+Mal+ (no sig.) #1 and 4 are R #2, 3 S. Recheck vv

G 1 Lac-Mal- 6 Lac+Mal+ 1 Lac+Mal+/- G 1 Lac v / Mal v / Mal -

6 Lac+ 3 sig. tested all Acr R.
9 Lac- 3 sig. tested all Acr R.

E: v m Acriflavine : 2S 2R OK.
colonies very similar on Tryptone agar.

12/26. Cf. Ck reactions

C' all Acriflavine R. (as parents).

G. 1 S

2 a R b S
3 R
4 R
5 R
6 a R b S
7 a R b S.

2 morphological types noted upon streaking out Resistant Lysis components on N.A.

see over:

Compare various photographs of 7b 6.

	T7	Acr.	λ	Morph.
1	S	R	+ ?	" R
3	S	R ✓	R	R
2a	R	R ✓ ✓	R	R
2b	R	S	R	S
6a	S	R ✓	R	R
6b	R	S ✓	A	S
7a	S	R ✓	R	R
7b	R	S ✓	R	"S's more opaque" pinks diffuse

green diffuse

#1 gives an undoubted Acrif R reaction, but

redisperses very readily to resemble S or RS.

Morphological differentiation probably better on EMB.

M271 — 273.

W1435 x W112 on EMS MM.

Pick MM +, purify on EMS MM. Test for discordant V_6 reaction on EMB, EMS (MM).

Out of ca. 30 such tests, 3 likely cultures segregating V_6^R.

M271 – 273.

M271 is verified as segregating $V_6^{R/S}$. V_6^S predominate.

M272 – Lac ?

$V_6^S \rightarrow$ Lac – stable $V_6^R \rightarrow$ Lac – unstable on EMB Lac.

Streaks (MM)

of EML60 12/28/50. Nonallelism of lac, c – b.

12/23/50.

See 777B.

Ca 1/2 Mtl$^{\vee}$ isolates are ~~#~~ Lac − 1/2 Lac$^{\vee}$.

Some Lac − EMS may have come from duplex, whence Lac + might be isolated. All original 1-22 are on D(Mtl) or D(Lac) segs.

Lac+ isolated: 11, 12, 18. All appear to be stable Lac+.

11/23 In course of isolation 8, 13.

To be isolated 14, 17.

#8^{+} is ~~[redacted]~~ Lac+, apparently pure, but restreak.

By EMS Lac → both Lac+ and −. Restreak + to verify, and to provide Lac − for further testing.

#13 → both + and − colonies. Restreak Lac+.

#14 → pure + EMS Lac. (mislabeling ?). Isolate to slant. ✓ as EMS. cf. "14 −"

#17 + and −

Note: since Lac$^{\vee}$ components of #8, 13, and 17 have already been isolated, attend to Mtl$^{\vee}$ character of Lac − "segregants".

12/27. "14+" is pure Lac+ Mtl − 14− : pure Lac − Mtl + (? Rev).

~~#~~8+ Lac$^{\vee}$ OK.

17− : 3 Mtl + 1 Mtl − No$^{\vee}$.

8 − 4 Mtl + no$^{\vee}$.

13 − 4 Mtl − no$^{\vee}$.

Tentative conclusion: These cultures which give Lac − prototrophs from Lac$^{\vee}$ isolations are throwing prototroph segregants, not partial segregants. Restreak from original slants. This does not explain 11, 12, 18 which are apparently duplex.

Comparison of Lac$_1$ – homozygotes hybrids and parents

12/24.../50.

Lac (EMB) 364..

1	H271	Bright red centres (confluent papillae ?)	
2	H258	type – papillae in streak	
3	H268	type – no " "	
4	H273	as 1	
5	H261	as 2	
6	799-11	as 3	
7	W1435	–	pap.
8	466	–	pap.
9	112	–	stable
10	1177	–	stable!!!

H271 and 273 may show very slight + reaction; more likely frequent crossovers lead to lac+ segregants.

W-1177 appears to have become lac– stable. Therefore lac– types such as H268 are unsuitable for homozygosity analysis. Review strains for lac– mutability. Resuscitate W660 for new set of diploids carrying mutable Lac$_1$–.

see 803

Lwoff Effect.

January 1, 1951.

A = K12
B = 1485.

Young cultures from YZ. Irradiate 2 ml samples in 10cm plates.

1. Pre-irradiation control.
2. After 120 seconds uv.
3. After 80 minutes incubation in YZ.

Incub 1.10 in YZ
No lysis observed.

A	1	ca 2×10^9 cells.	10^{-7} λ. (1 plaque)	B1	ca 2×10^9 cells
	2	ca 3×10^6 cells	10^{-7} λ (1 plaque)	2	ca 3×10^7 cells
	3	2×10^5 cells	No λ at 10^{-7}	3	

Although K-12 liquid survival appears diminished, no marked lysis or release of λ was detected. Aeration may be minimal, or medium may be unsuitable.

≡ Inconclusive ≡

Test for release of λ on EMB agar
Dilute fresh K12 10^{-6}, spread .1 ml on EMB Lac.
Irradiate uv 50cm. 0, 5 sec, 10, 20, 30, 40, 60, 120., dupl. plates.
A) Just add drop W578 immediately & spread.
B) " incubate ____. spread W578.

Count K-12 as papillae
λ as plaques.

	λ	0 K12	10	20	40	60	180 sec.
A.	11	ca 1000 202	± 1000 322	351	421	342 109	198	14 ± 0	5
B	21	"	255	± 1000 342	392	ca " do.	118	7 ± 0	7

January 3, 1951.

Compare sensitivity of λ+ and λ^s strains: All loses 30 secs.
~~1.~~ Dilute to 2×10^{-7} each. Mix 1:1.

A K12
B 1485
C 518.

Immediate mixtures

	UV 0		UV 30 s.	
	Lac+	−	+	−
A+C	109	109	10	61
A+B	186		30	
B+C.	135	108	29	49

$5\lambda^{(+)}_R$ $15\lambda^s$.

K12 and W1485 are both more sensitive to UV than W518.
Test A+B UV for proportion of λ^s.

Repeat 801. K12 40 sec. 2×10^{-7}

	λ	UV 0 K	λ	UV 40. K.
K12	1	204	54	10±
delay 90 mins before spreading	2	271	58	12±

90 minutes in agar not long enough for burst!

Conditions of uv-λ burst. 8016.

January 4, 1951.

Effect of old culture; D(Lac); on uv λ effect.

A. = K12 36 hour culture in Yt Dilute to ~~2~~ × 10^{-7}

B = " overnight " " D(Lac). 2 × 10^{-7}.

B: Immediate plating with W518

	K	λ (numb)
	126	5
20 sec uv	96	13
40 "	17	45

	K	λ
uv 0. 12~~00~~	100	2
uv 30 (smear) 12~~00~~	18	70±

uv 30 no 518: 146. 16 24 17

uv 30 2~~30~~: 179.

Increase in λ? Uncertain.

Should use ~~yng~~ young cells in Yt + assay super. from time to time.

Crossing sun-inactivated cells.

Jan. 4, 1951.

W1269 grown overnight (+) in 50 ml Y2. 12 Yo PM Add sun to 9 ml samples of both culture and incubate at 37.C. Expose 2 hours. To estimate survival: standard loopful:

a. loopful. = L.

b. L/1ml. L.

c. L/1ml : L/10ml : L.

Wash cells 2x. Add 1ml to pellets of 1269; 2ml to pellet of W1177. Mix W1177 .4ml with 1ml 1269. Plate .1ml samples on EMS Lac. also streak out mixtures on EMB Lac.

1000 units give only ca 1-10% survival! Results indeterminate except that sun has poor killing power under these conditions!

Prototrophs seen in each series.

Standard heterozygotes.

January 3, 1957.

W1490 x W660. on EMS Lac.

1st Rem: 28 colonies picked. (Poor diffuntiation on EMS MM
Many mucoid).

A) 12 X 13, MM v

6 ace MM –

18, 20 v. pu[illegible] MM – Very flat on EMS Lac.

others MM +

λ in 19 ?

B) MM EMS poor diffuntiation.

Lac: 38 tests: 8 likely Lac v. 25, 33, 35, 24, 32, 13, 18, 12

Reisolate on EMS Lac.

MM: 100 tests (not necessarily + !)

1-12	9 MM –	# 5 v ?
13-100	47 –	# 26, 33, 47, 66, 71, 72, 77, 85, 87, 98.

(12 on Xyl rather than MM).

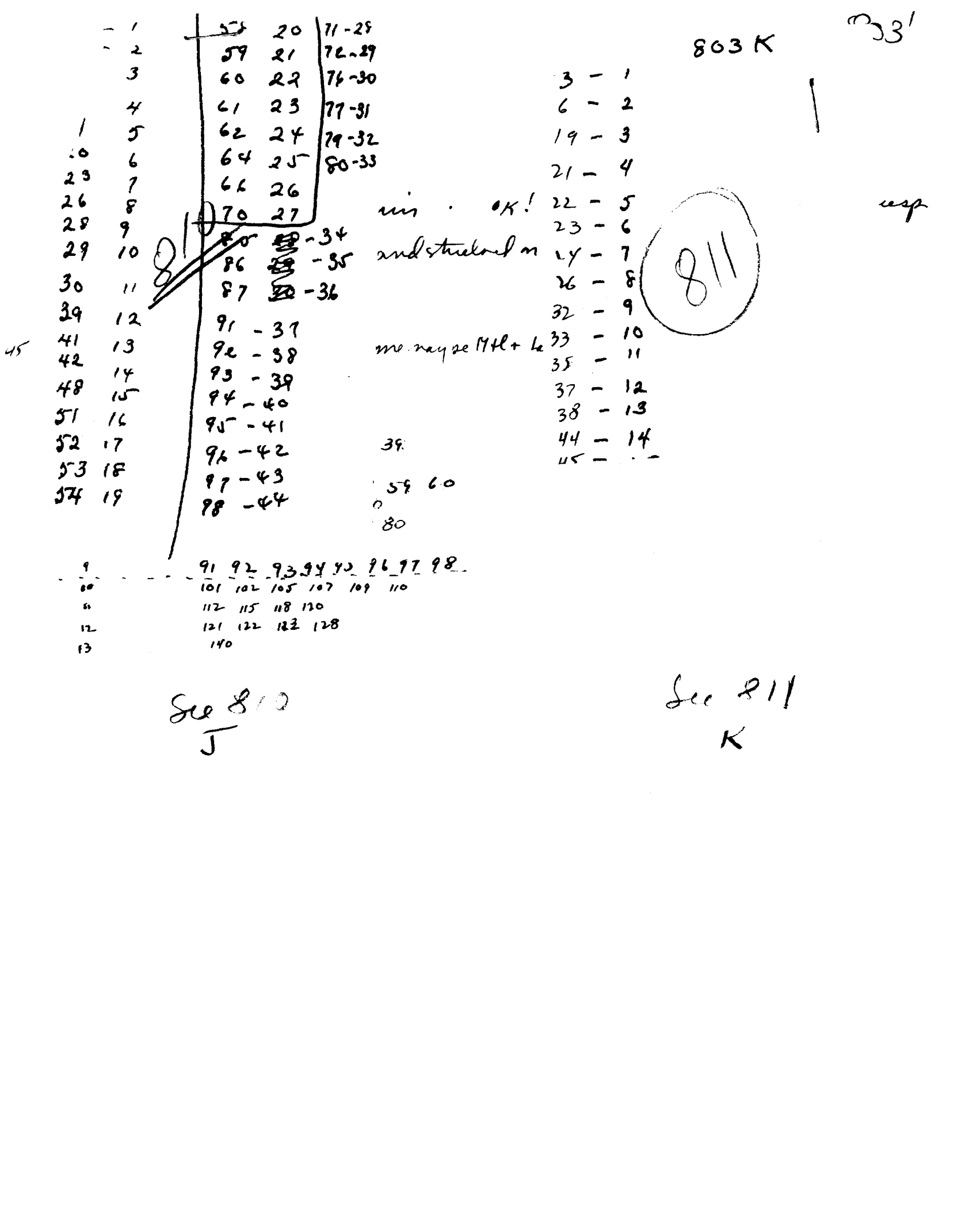
	MM	Lac	Xyl.	
1	–	+	–	
2	–	+	+	
3	v	v	v ?	–
4	– +	– +	– +	
5	–	–	–	
6	+ λ?	–	+	
7	–	–	v	–
8	+	+	+	
9	–	+	+	
10	v	v ?	v	–
11	+ λ?	–	?	

C 1490 x 660 100 isolates from EMS MM. Poor diffuntiation! 17 MM+ 6 MM v ? Remainder –

D 478 x 660 Diffuntiation v. poor. on MM or Lac.

Clones tested on EMB, EMS MM before streaking. 27/50. MM+. of these: 7 MM v.

49/100. +


803 K
33'
3 - 1
6 - 2
19 - 3
21 - 4
22 - 5
23 - 6
24 - 7
26 - 8
32 - 9
33 - 10
35 - 11
37 - 12
38 - 13
44 - 14
45 - 15
811
esp
min . OK!
and stacked m
me. may be MHL + L
39
54 60
80
1 2 3 4 5 6 7 8 9 10 11 12 13
55 20 71-28
59 21 72-29
60 22 74-30
61 23 77-31
62 24 79-32
64 25 80-33
66 26
70 27
80 -34
86 -35
87 -36
91 -37
92 -38
93 -39
94 -40
95 -41
96 -42
97 -43
98 -44
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
91 92 93 94 95 96 97 98
101 102 105 107 109 110
112 115 118 120
121 122 123 128
140
See 810
J
See 811
K

Heterozygotes:

1804

January 3, 1951

A	1435 ×	1446
B		1449
C		1451

1/6/51.

Lac−	Lac+
22, 35	27 0, 3, 1
5, 10	1, 0.
11, 10, 7, 53, 1, 0, 3	
1,	
0, 1, 0, 0	2, 4, 4, 1

Pick + streak lac+ on EMB, S, Lac.

A). 5 tests. #2 v. ?

B) 12 tests. No v. Some Lac+ are λ^s.

C) #2, 10, 11.

Restreak from D(0) to EMS, EMB lac.

lost on faulty EMS!

1/29/51. Repeat A, C.

A: ca 1% +. 100/plate.

4 Lac+

But #4 apparently Mal v

(mostly Mal−, one clear ✱)

→ This colony → ?! Mal v. Lac+ MH+

From EMS, allypure +. Retest.

C. Mostly +. Lac v: ? 2, 53.

130 Lac+ tested

Restreak a, C1, C2

	MH	Mal	Lac
1	−	−	
2	+	+	

WT

Differential uv sensitivity — λ

W811 x W1274

January 7, 1958.

			Gal+ = λ^-	Gal− = λ^+	Adj. Ratio λ^-	Adj. Ratio λ^+
A	Control	(.05)	45	80	1.	1.
	uv	(.1)	116	155		
B	10	(.05)	48	53	.9	.64
		(.1)	105	99		
	20		80, 84	72, 68	.71	.45
	30		73, 57	34, 57	[illegible]	[illegible]
	40		69, 56	24, 19	[illegible]	[illegible]
	60		22, 13	0, 2	[illegible]	[illegible]
	80.		1, 2	0, 0	[illegible]	0

Young cultures of W811 and W1274 grown in Penassay, diluted ca 10^{-6}; mixed; .1 ml spread on EMB-Gal. Expose plates to uv lamp.

. Effect maximal at 40-60 sec. exposure. Effect may not be so pronounced on EMB agar. Cf. nutrient broth.

Dilute H226, H232 to 10^{-7}, spread .1 ml on EMBlac, radiale plates (50 cm.).

H226.

	·	–
0	41	38
10	5	59
~~20~~	0	14
30	0	3
40	0	1

H232

	✓	+	–
0	58	1	4
10 s	70		20
20	50		12
30	19	1	8
40 s.	17	1	9

λ^+ seems to be more sensitive than λ^s. However, similar, but less dramatic haploidization effect noted. These effects may be residual, and the experiment should be supplemented by comparison of H232 and unfused H232. See 808

Reinfection of λ^S lysogen

1/16/51. ff.

H232. (= W518 λ^R × W588) cross streaked with λ on D(0) & EMS lac.
Single colonies picked and tested against λ on EMS lac.
Ca 12/25 were /λ. Restreak these on EMB, EMS lac, saving latter.
Single EMB colonies restreaked on EMB lac; tested against λ^S (W1321) on EMB-0.

	lac	λ	(518) λ
1	V	R	+
2	V - plagued.	R?	plagued
3	V	R	+
4	V	R	+
5	V very small!	R	+
6	V	R	− or ±
7	V very little	R	+
8	?+ V	R	+
9	+	R	+
10	V	R	+
11	?	R	+
12.	V	R	+

In fact streaking may be ~~bad~~ segregating? λ^S and λ^+.

all λ+. No sign of λ^S segregants.

(K-12)

From uv experiment, 801 one rather clear plaque noted, indistinguishable from λ on W518. Isolate single plaques.
Attempt to induce lysogenicity. Many autolytic colonies.
Streak one of these out: Test non-autolytic colonies on ~~W518~~ 1371.
~~In this streak of W518λ~~ numerous clear plaques. Basis?

30 tests. 7 autolytic 32 non-lysogenic 1 lysogenic.

Restreak as W 1516 Lost

λ' OK.

Analysis of 803 J

EMB

EMS

Replicate ← single col to EMS MH

Strain		MH	Lac	Mal	Xyl	MH	
D(MH)pJ							
D(Lac)							
M	1	v	v	–	v		
M	2	v	v	–	+v v		
M	3	v	–	–	v		
M	4	v	v	–	v		
M	5	v	v	+v +	+v +		
M	6	+	+	+	+		
M	7	v	–	–	*v		
M	8	v	–	–	v		
M	9	v	v	–	v		
M	10	+v v	v	–	–		
M	11	+v +	–	+v +	+v + mucoid		
M	12	v	–	–	v		
M	13	v	v	–	–v v?		
M	14	v	v	–	– v	+ – → MH– Lac+ MH+ Lac v.	++ –
M	15	v	v	+mot +	+v +		
M	16	v	v	–	v		
M	17	v	v	–	v		
M	18	+ ? +	+	+ ? +	+		
M	19	v ? v	–	–	+ ?		
M	20	+	+		–		
L	21	v	v	–	v	+ – MH+ Lac v MH– Lac – all +	
M	22	v	+– v? v	–	v	Lac+ are MH v ; Lac – MH+ or – ∴ sibling pairing.	
L	23	v	v	–	v		
L	24	? +v	v	v ? +	v		
M	25	v	–	–	v	+ – MH+ Xyl v MH– Xyl –	++, –
L	26	v	+	+mot +	+		
L	27	v	v+	v –	v		
M	28	v	–	+mot +	+v		
	29	+	+	+	+		
L	30	v	v	–	v		
L	31	v	v	+v +	+v +		
L	32	v	v	–	v		
L	33	v	v	–	v		
M	34	v	–	–	–v		
L	35	v	v	–	v!		
L	36	v	v	–	– *		
L	37	v	v	–	v)		
L	38	v v	v	+v +	+v +		
	39	– v	–	–	–v v		
M	11 40	v	v	–	–v v	+ –	
M	20 41	v	v	–	v	+ – MH+ Lac v MH– (Lac –, +) replate original	++, –
	29 42	v	v	–	v		
	43	v	–	–	v	+ – MH– : Xyl – MH+ Xyl v	++, –
	44	+	+	+	+		

No evidence of sequelae

all three pure +

Test – for another sing.

{ MH– Lac –
MH+ Lac v

810 B - refuse to Lac+ revisions

810 C - refuse to Mal+ revisions

2/15ff.

Tests on Lac reversions of J Lac – diploids. From papillae on EMS Lac. Essentially all from separate colonies.

Lac

(6, 7, 8, 12, 19, 20, 28, 34, 39).

	Repun.	Pune
6: Reisolate	4	0
7: "	4	0
8: 2 ✓ 2"	2	2
12	2	2
19	2	2
20	1	3
28	0	4
34	3	1
39	2	2

Lac	Mtl
; ; ; ;	
; ; ; ✓	; ; ; ;
✓ ✓ ✓ ✓	✓ ✓ ✓ ✓
✓ ; ✓ ✓	✓ ; ✓ ✓
✓ ; ✓ +	✓ – ✓ –
; ; + ✓	; ; + ✓
; + + ✓	– – – ✓
✓ ✓ ✓ ✓	✓ ✓ ✓ ✓
+ ; – +	– – – –
; ; ✓ ✓	– + ✓ ✓

; indeterm. owing to virginity

Mal

		Mtl	Mal
1	1	–	+
	1	–	+
	2	–	+
	2	–	+
	3	+	+
	4	–	+
	4	–	+
	4	–	+
	6	–	+
	10	✓ + ✓	+ m + ✓ (1)
11	12	–	+
	12	–	+
	11	–	+
	14	–	+
	14	–	✓ ? or –
	14	–	✓ ? or –
	14	–	+
	19	✓ + ✓	+ mut + ✓ (2)
	19	✓ ✓	+ " + ✓ (3)
	19	✓ + ✓	+ " + ✓ (4)
21	20	–	+, –
	20	–	+
	20	–	+, –
	20	–	–
	30	–	+
	30	–	–
	30	–	+
	30	–	+
	35	–	+
	35	–	+

		Mtl	Mal
31	36	✓ ✓	+ m + ✓ (5)
	36	–	+
	37	+	+
	37	+	+
	32	–	+
	33	✓ ✓	✓ ? + ✓ (6)
	33	✓ ✓	✓ ? + (7)
	39	–	+
	~~39~~		

Most Mals already segregated, unfortunately.

Lac ✓ preliminary reading on appearance of colony. Should be confirmed.

2/#17/51. Reisolate single EMS lac colonies to EMB lac; Mtl for verification of Lac ✓

EMS Mal spots to EMB Mal to verify Mal +

Reisolate all Mtl ✓ or Melibiose ✓ or Lac from single EMS colonies.

2/18/51.

Lac. Plates from 810 a., single colonies from EMS lac.
Tests showing Lac = → Lac +

	Lac	Mtl	
6	– – – –	– – – –	
7	– – – –	v v v v	
8	v + v v		3
12	– + v +	– – v –	1
19	v – + v	v v + v	2
25	v	v	1
28	v v v v	v v v v	4
39	+ v +	+ v –	1

Lac v designations based upon presence of ± and – colonies from single EMS +.

EMS plates faded (owing to storage during Chicago trip), and therefore some were "mispicked".

Test Lac+ for pail s.g. Lac.

Recessives. (Mal

810C

2/28/51 ff.

M+.	
1-13	39.
14-15	12
16-21	1
21-31	35
32	10
33	8
34-37	3
38-39	7
40-42	34
43-51	25
52-60	6

Check stock cultures from O'(MH) to ♂MB MH.

Lac −:

(3): Lacv Mdlv ✓
6 OK
7 ca ½ Mdlv ½
8 OK.
12 OK
19 Monthly MH+, same ✓
25 " " "
28
34 OK
39 OK

	Lac	Mal
1	−	+
2	−	+ − wo v
3	−	+
4	−	+
5	−	|
6	−	|
7	−	|
8	−	
9	−	|
10	−	|
11	−	|
12	−	|
13	−	+
14	−	+
15	−	+
16	−	+
17	−	+
18	−	+
19	−	+
20	−	+
21	−	|
22	+	|
23	+	|
24	+	|
25	+	|
26	+	|
27	+	|
28	+	
29	+	|
30	+	|

	Lac	Mal
31	+	|
32	+	
33	−	|
34	−	|
35	−	|
36	−	
37	−	+
38	−	+
39	−	0
40	−	+
	−	|
	−	|
	−	|
	−	
	−	|
	−	|
	−	|
50	−	|
	−	|
	−	|
	−	|
	−	|
	−	|
	−	
	−	|
	−	|
60	−	

Completely n.g. owing to segregation! Note pattern 1.

22-32!

See 823

all Mannitol V.

		Lac	T6	Mal	Xyl	Sm	Tip	Repeat T6 on further growth	Tests for homozygosity Lac = (8:10B)	Mal −/+ (8:10C)
1		−	V SR°	−	V	S	A	R		
2		V	SR	−	V	S	A	R		
3	H283	V	SR°	−	V	S	A	R	• Lac ✓ !	
4		V	SR	−	V	S	A	R		
5	V ~ + V	V	SR	+	+ *	R	C	R		
6		−	S	−	V	S	B	R	• III	
7		−	S ✓	−	V	S	B	S	• IIII	
8	H282	−	S	−	V	S	B	R	IHH III	
9		V	SR	−	V	S	A	R		
10		V	SR	−	−	S	E	R		1
11		V	V'	−	V	S	A	R		
12		−	S	−	V	S	B	R	II	
13		V	V	−	V	S	A	R		
14		V	V	−	V	S	A	R		
15	V	V	V	+	+ *	R	C	R		
16		V	V	−	V	S	A	SR		
17		V	V	−	V	S	A	R		
18		?	R			R	F	R		
19		−	S	−	V + *	S	B	S	II III	III
20		V	V	−	V	S	A	R		
21		V	V	−		S	A	R		
22		V	V	−	V	S	A	R		
23		V	V	−	V	S	A	R		
24		V	V	+	V	R	I	R		
25		−	V	−	V	S	G	R	I	
26		+	R	+	+ *	R	J	R		
27		V	V	+	V + *	S	H	R		
28		−	V	+	+ V *	R	D	R	IIII	
29		V	V	−	V	S	A	R		
30		V	?*	−	V	S	A	R		
31		V	V	+	+	S	H	R		
32		V	V	−	V	S	A	R		
33		V	V	−	V	S	A	R		II
34		−	S	−	V	S	B	R		
35		V	R	−	V	S	J	R		
36		V	V	−	−	S	E	R		1
37		V	V	−	V	S	A	R		
38		V	V	+	+	R	C	R		
39	H284	−	V V	−	V	S	G	R	IHH III •	

See 833

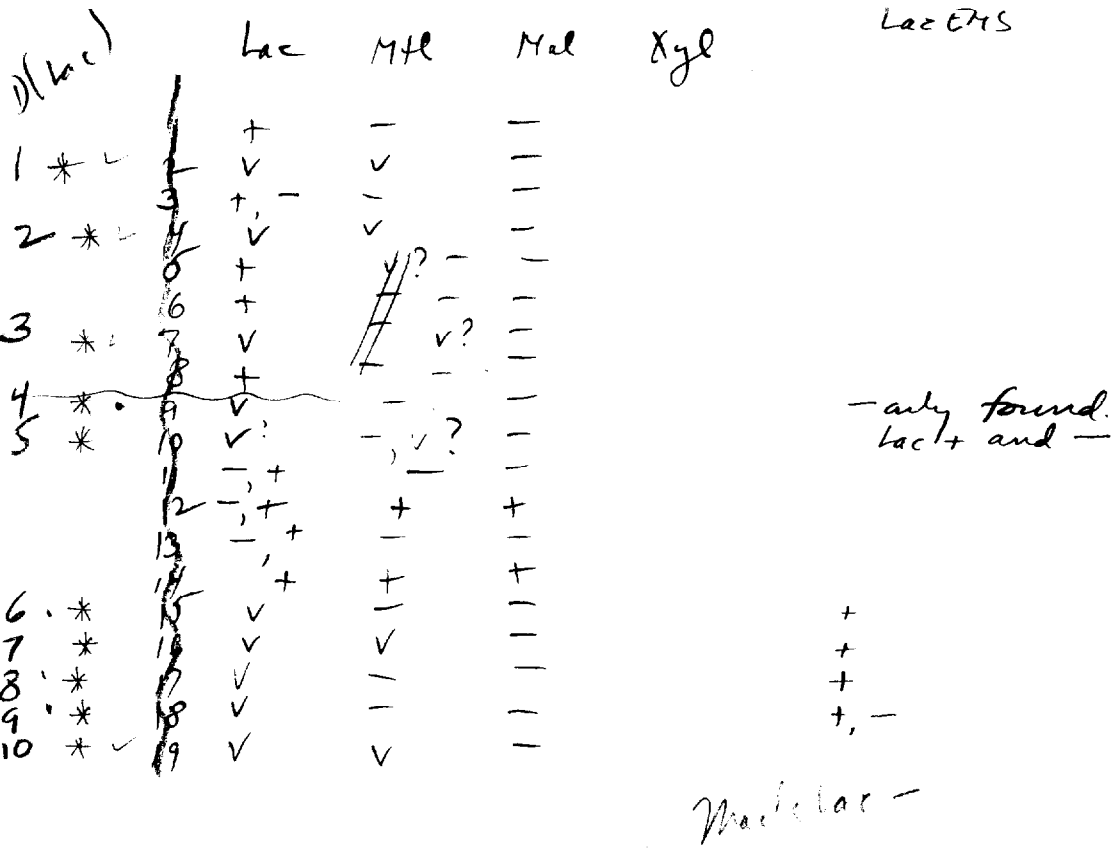
1 Lac V Mtl V Rev.

• Lac+ Mtl−(ort)[?].

* V_6^R m EMS
V_6^{RS} m EMB.
S ? V

V° might be V_6^S ; mixed lysis.

Analysis of 803K



8/1
K

	Lac v	Mal	Mtl	Xyl	Sm
1	+	—	v	+	s
2	✓	—	v	v	s
3	+	—	v	—	s
4					
5	+	—	v	—	s
6	v	—	—	—	s
7	+	—	v	+	s
8	v	—	—	—	s
9	+	—	—	+, —	s
10	v	—	v	v	s

2/9/51.

A. W1490 × W660 on EMS Mal ; EMS lac (for sec.)

B. W1490 × W1177 on EMS Mal (for S^R allelism)

C W1435 × W1446 } See 804.

D " × W1451 }

A. About 1% survivors are sectored on EMS Mal or Lac.

1-4 Mal
5-8 Lac

A&B test Mal+ on >14. 2/21 doubtful S^S. Retest for Mal +/-.
3/20 all S^S on EMS; #3 and #7 S^R on EMB. These are
4/20 otherwise Mal++ mixtures of Mal and Mal- on EMB. Restreak on EMS Mal
LA 8/2 AB: 1, 2

B Pick Mal+ and brush on. 12 tests: 3 sensitive!
∴ not allelic?? Purify & streak out; test parents! all 3 are Mal —

D 58 tests: no diploids found. (#38?, 24

C Most colonies appear lac – for first two days on EMS plate, then turn +. 62 tests: #7 only diploid. #16?

	Mal	Lac	Isolate to D(Lac) Mel
C1	+	+, v? ✓	+
C2	+	+	+
D1	+	+, − v? ✗	+
D2	−	+ (slow)	+

A 1-49 tested: 7 steady Lac v. Restreak on EMB Lac

Duplex protoplasts. 812A

2/4/51.

Incubator difficulties made scoring on EMB very doubtful

	Lac EMS	Mal EMS	Lac EMB	Mal EMB	Mtl EMB	Xyl EMB	+ − see if any
1		+, −, v?	++, −	+ −	+	+ −	Mal+, Mal−, Mal+ and − Lac+
2		++, −, v?	++, −	+ −	+	+	Mal+ Mal− Lac+ Lac−
3		+, − v?	−	+ −	− +	+ −	Mal+, − + + Lac−
4		+, − v? muc?	−	+ −	+	+	Mal+ Mal− Lac−
5	+ −	+, −	+ −		+, −	−	Lac− Lac+ +, − (v?) Mal−
6	(+) −	+, −	+ −			+	Lac− Lac+ Xyl−
7	++	+, −	++			−	Lac− Lac+, +, +, − v?
8	++, −, v?		+, − v?	++	+?		Mal+

Repute from EMS.

No diploids represented.

812 Asm

Test Mal+ prototrophs from W1490 x W660 for $S^{r/s}$. Retest S^S Mal+ as possible Mal+.

61 tests: 9 S^S. 2 of these S^R on EMB. Retrack as 812A sm 1-2.

160 tests. 22 apparent Mal+ $S^{\&S}$.

Retrack: 3 are S^R
19 S^S Mal+. But concordant on EMS, EMB Mal.

Retrack by streaking out on EMB Mal

Of these 22,
#7: "mostly +" ; #14 "+ and v?" → not +.

See 817

2/2/51.

Hemolytic colonies from W-1 irradiated on blood agar.
9 colonies picked as hemolytic. Streak out; test for λ from single colonies.

	Hem	λ	
1a	+	−	R
b	+	+	
2a+b	+	+	
3 ~~2a~~	+	+	
4	±	+	
5	−	+	
6	−	+	
7	+	+	
8	+	+	
9	−	+	

Peculiar appearance on EMB Lac.

Restreak single colonies of 1a, b. 2. on EMB lac; Hem; .... remainder on blood agar.
1b is mixed in morphology. Some colonies flat; others mucoid.

Restreaks. 1a: each of 10 colonies hemolytic, λ^{-R}.
1b.: flat λ^{-R} hemolytic.
normal λ+ " , possibly less so.

Picks 1a to slant as W-1529 ~~2 as W-1530~~

2nd run:

	Hem.		Lac EMB
1	strongly hemolytic	not coli.	flat mucoid
2	"		" non "
3	"		normal colony
4	v. sl.		" and discolored colonies
5	"		" " " "
6	"		" " " "

None λ^S.

Restreak 2 and 3.
Test against λ.

Hemolysis evidently not necessarily correlated with λ reaction

February 12, 1951.

C 1435 × 1446
E " × 1449

C	21	no $\underline{v}$	(lac)
E	150	"	"

2/16/51

W478 Mal− mutants: Test for Mal$_1$ alleleism
(× W1177). Reisolate from viable: check for purity and mutability.

			Papillation	Prototrophs (2 plates) Mal+
	1	W 960	±	2/300?
	2	961	+	3/200
	3	965	±	0/200
A	4	966	+ gummy	0/100
	5	968	+	×
	6	969	+	×
	7	970	+	×
	8	971	±	1 − col
	9	972	−	0/100

2/19/51 Repeat where doubtful:

	1	W 965	0/60	1/100	3+/100	1/100.	Mal+ ✓	all S^S ‖ EMS and EMB
B	2	966	0/50	0/50	0/100			
	3	970	0/10	0/3	1/5		+ on // ✓	S^R ‖
	4	971	2− 0, 0					
	5	972	0/150, 0/50	1?/100	0/200		+ on // ✓	loc − S^R ‖ EMS + EMB. Mal+ not ✓.

Repeats possible # to verify.
W966 is most likely Mal$_1$−. W971 is infertile.
Recheck # 5 for Mal ✓ (tightly linked allele!)

2/21/51 EMS Mal.

C 1. W966 × W660 Not 2? in 12 plates × 250 cols. = 1800 tests.
C 2. W972 × W1177 Picks+, streak out on EMB Mal for Mal ✓ spot on EMS Mal.

C 2:	+	−
	9	99
	10	140
	7	79
	2	69
	9	97

C1. 2 colonies are clear Mal+ (not Mal ✓)
C2. 100 picked: all Mal+ (no Mal ✓)

See 815'

2/26/51

W1177x on EMS Mal for Mal v.

A W960
B W965
C W966
D W972

① No special treatment ② Expose uni plates to UV 50cm. 10secs.

A. 1 2 × 200 3 Mal+ ?
2 6 × 300 15 "

B 1 3 × 200 6 "
2 4 × 300 12 "

} all Mal+ no Mal v ! scoring on EMS Mal very difficult

C 1 } 3 × 200 } 1200
C 2 4 × 150 }

No Mal+ seen.

D1 2 × 300 No Mal+ seen. Scoring ?

Picks to MalEMS: 60, all Mal −.

Contradictory results?

Repeat D1. Ca. 1% Mal+ formed. ∴ W972 is not allelic, but close. Scoring of these Mal+ arrangements because of Mal is not satisfactory. However, W1532 (from W966 Mal −) should serve the same purpose, if it is truly allelic to Mal).

Suppressor: Lac_3 Lac_1 8/6

February 12, 1951.

W1531 $\frac{A}{B}$ x W1490 on EMS Lac. Look for Lac − to verify persistence of Lac_1 −.

A ca 600 all Lac+.

B. ca. 700 prototrophs, all Lac+.

Do $SLac_3$+ are allele of Lac_1 ?
If so, it should be detectable among Lac_1 − revertants.
Check for a) x Lac_3 −
b) Constitutive release

10 recombinants from A seem to have constitutive lactase! (from DNP Glu plates; spot test)
Recheck from synthetic D(o).:

K-12 Prototrophs
1531 A + B
W1490
} are Cst −

W1301 Cst +.

∴ none of these lac+ suppressors are Cst + like SL_3 previously examined!

See 822

2/10/51

Ace : 812B, where $S^R \times S^R \rightarrow S^S$.

Reciprocals parent stocks.

A. W1490 single clones 105 tests : all S^R

B. W1177 " " 100 tests : all S^R

C. Cross again. 160 tests all SR

812B might represent a mixup with A.

W1490 × W660 ctd.

2/18/51

Cross "A" is W1490 × W660

A. Lac EMS 100 + streaked out; 33 retained for recheck.

B Mal EMS 100 Mal+ picked + streaked out. Recheck: 50, 53, 98, 100.

2/20/51. 30 added: all +
2/21/51 16 " " "

	Mtl	Mal	Lac
1	+	+, −?	+, − v?
2	−	++ −?	+
3	+	++ − v?	+
4	+	+ − v?	+

} No Mal v. Repeat Cross:

λ in 2°
mMal

Rechecks from 2 single EMS colonies

C (= 812A 2cm). from EMS Mal single + cols

1	Mal ++
2	"

B

1	"
2	"
3	"
4	"

} no Mal v found > 100 tests

A.

	Lac	Mtl	Xyl	Mal
1	V	−	V	−
2	V	V+?	+	−
3	V	V	V	−
4	V	V	V	−
(5)	− V, V	+?	+	− + (hem)
? 6	+ V	V(+?)	+ V	+ V
? 7	+ V	V	V	−
8	V	V	V	−
9	V	−	−	−
10	V	+	+	−
11	V	+	+	−
12	V,	+ V	V	+ V
13	V	+	+ V	+
(14)	V? V	VV	V+	−
15	−+	−+	−+	−
16	+ V	−V	−	−
= 17	V	V	+V	−
18	+	−	−	−
19	V?	+	+	+
20	− V	−V	−V	−

	Lac	Mtl	Xyl	Mal
21	V	+ V	+ (V?)	+
22	V	−	+	+
23	V	−	−	−
24	+	−	−	−
25	V	V	V+	−
26	V+?	V	−V	−
27	V	V	V	−
28	V	+ V	+	−
29	+ V	+ V	−	−
30	V	V	V	−
31	+	+	+	+
32	V	−	−	−
33	+	+	+	+

5: 4 Lac v are Xyl v
14: 8 Lac v are Mtl v, Xyl v

Diploid : Reverse cross; Mal

2/18/51

W1532 (B14 ~~Lac~~ Mal$_1$- het) × Y53 (TL6, Lac$_1$-)
on EMS Lac
(and Mal for +:- ratio).

Mal: + 103 — 17 (Reversed ratio, as expected).

Lac. : 100 picked as "v+" and streaked out on EMB Lac, spot on EMS Lac
About 57 of these scored as probable Lac v. Retreak on EMB Mal, EMS
Lac to detect a) possible Mal v, b) Mal- Lac v for hemizygosity test,
and to purify for further study.

(814a)
Conclusion : by reverse cross (i.e. B11 × TL6, Lac$_1$-)
the Mal- is also hemizygous, to the + to the
that mal g, & the Lac- hemizygous.

Diploid : as Lac- also hemizygous in reverse cross.
~~W1532~~ W466 × W660 Lac+ on EMS Mal.
(W478 Lac-)

See 829.

	Lac V.	Mal-	
1	2	—	
2	3	+	
3	4	+	
4	5	+	
5	6	+	
6	7	+	
7	8	+	#
8	10	+	#
9	12	—	
10	13	+	
11	14	—	
12	17	+	V
13	18	+	
14	19	+	
15	20	+	
16	21	+	
17	22	+	
18	24	+	
19	24	+	
20	25	—	
21	26	—	
22	27	—	
23	28	+	
24	29	+	
25	30	—	+
26	31	+	
27	32	+	
28	34	+	
29	35	+	
30	39	—	
31	41	+	
32	42	—	
33	45	—	
34	46	+	
35	49	—+	
36	50	+	
37	52	+	
38	53	+	
39	54	+	
40	56	+	
41	57	+	
42	58	—	
43	62	+	
44	72	+	
45	73	+	
46	74	+	
47	76	+	
48	78	—	
49	79	+	
50	80		

51	80	+
	84	+v
	86	—
4	87	+
5	90	+
6	91	+
7	92	+

Note: 15 ~~16~~ Mal −
41 Mal +.

a) Recheck Mal v possibilities } from EMS lac Bursts.
b) Recheck lac v / Mal −.

Mal Reversion test for hemizygosity

		lac v ✓	Mal EMB	
1	1	✓	—	1 Lv M+
2	~~#~~9	✓	—	(1M+L+) 2 Lv M+
3	11	—	—	
4	20	✓	—	
5	21	✓	—	
6	22	✓	—	
7	25	✓	—	(1) 1 Lv M+
8	30	✓	—	3 Lv M+
9	32	✓	—	2 Lv M+
10	35 33	+	—	
11	42 35	✓	—	1M+Lv. (3)
12	48 42	✓	—	1M+Lv
13	43 48	✓	+	
14	~~(15)~~ 53	+v ?		

Total
11 Mal Reversions
still deplaid: each
Mal++
(not Mal v)

Recheck 819-78! Mal − Lac ■
probably mis picked.
But do not pursue!

n. 92. ~~[illegible]~~
~~16~~

~~15~~ 16	12	Mal++
16	52	Mal++

Those which ✓ above are available for further work as lac v Mal − Reversions on Mal tested: ↗

Save 819-58 as lacv Mal −
and 819-15 as lacv Mal +

Irradiation of λ+ and λs diploids

February 20, 1951.

A) H232   B) H278.

Dilute 10^{-6}. Irradiate at 50 cm. Plate .1 ml on EMB lac, EMS lac.
0, 20, 40 sec.

For 60 sec, dilute 10^{-4}, plate. 1 ml.

	UV	Lac +	−	v	Gal +	−	v	Σ
A:	0	2 4 7	7 8 4	65 70 59	2	7	55	64
EMB Lac	20	3 mc-v 17 6		6	1 (14-v)	8	3	18
		24	3					
	40	3? 1?	6 10	01	3	6	0	9
	60 (100x)	35 48 121	49 134 25	4 13 16	14	101	mc-v 46	161
B	0	1 3?	18 21 22	189 146 112	1	11	129	141
		170	0					
	20	1 5 66	70 83 3	18 22	6	34	57	97
	40	2 19	23 6	11	2	20	8	30
	60	~	~	60 Σ 291	14	187	119	3.32

Gal-lac- simulates lac+ exp. Gal-lac+

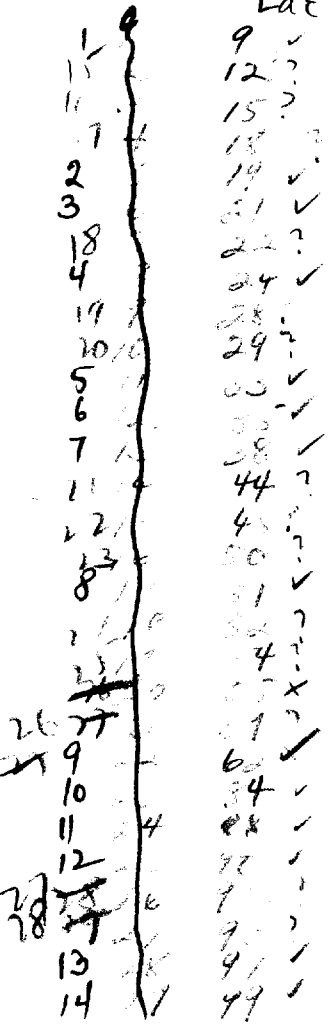
If anything, λ+ seems more sensitive than λs!
Recheck! (use 30 sec)

Hypogamety 1 ea. A, B.

February 20, 1951.

W1502 (478 λ^s) × W660

100 Lac+ picked from EMS Lac and streaked on EMB Lac for Lac v.



2/23/51. 1-14: all λ^+ λ^R.

~~Replica~~ Repeat 2/26/51. 100 Lac+ streaked EMB Lac. no λ^s diploids.

Cotton provided H285 - but this proved not to be heterozygous, although peculiar mottling was observed on Mtl and Xyl.

Slac 2

February 23, 1951.

See 816.

W1510 (gramm NB + Kgma) × W1301 on EMS Lac 14 tl
Cat+ lac,+
= lac,- Glu-

to verify: purity of lac,-
suppression by Cat+.

W1510 checked on Lac, Glu OK
from suspension used
in cross.

a) Picks singly from plates. (may be impure)

Lac	Glu	L	S	L	S	L	S
±	-+	-+	-+	±	+	-	-+
±	-	-+	-+	±	+	-	-+
+	+	±	-+	±	+	-	+
++	+	±	-+	+..	-	-	-
-	+	±	-+	-	-	-	-
+	+	±	-+	-	-	-	-
..+	+	+	+.	±	-	-	-
-	+	±	-+	±	-	-	-
+	+	±	-+	-	-	-	-
±	-+	±	-+	±	-	-	-

(2)

±	-+	±	-	-	-	-	-
±	+	±	-	-	-	-	-
+	+	±	-	-	-	-	+
+	+	±	-	-	-	-	# -
+	+	+	+	-	-	-	-
+	+	±	+	±	-	-	-
+	+	+	+	-	-	+	+
+	+	±	-	-	-	-	# -
±	-	+	-+	-	-	-	-
+	+	+	+	-	+ -	-	-+

(1)

+	+	+	+	# -	+	-	+
+	+	±	-	-	-	-	-
+	+	±	-	-	-	-	-
±	-+	-	-	-	-	-	-
±	-+	-	-	-	+	-?	+
±	-+	±	-	-	+	-	-
+	+-	-	+	-	+	-	-
±	-+	+	+	-	-	-	-
±	-+	-	-+	-	-	-	-
±	+					-	-

Pick possible +- (A) and -+ (B) types and retest on E 4/5 Glu or lac resp.

(See over)

Y87 and Y53 Lac+ revertants selected to test for constitutive lactase:

1-8 Y87	#	5	9	M	15	maybe Cat+ ??
9-15 Y53.			7	11	M2	— .

Recheck!

Photographs picked at random & purified. Stroke test on EMB-Glu, Lac.

G	L	G	L	G	L	G	L	G	L	G	L
−	+	−	−	−	+	−	+	−	−	−	+
−	−	−	−	−	−	+	++	−	−	−	+
−	−	−	−	−	−	−	−	−	−	+	++
−	+	+	+?	+	÷	−	−	−	−	−	−
−	÷	−	+	−	÷	−	+	−	−	−	+
−	÷	−	−	−	÷	+	−	−	−	−	−
−	÷	−	+	−	+	−	+	−	+	+	++
+	++	−	+	−	+	−	−	+	++	−	+
−	−	−	+	−	−	+	++	−	−	−	−
+	++	−	−	+	−	−	−	−	−	+	++

Mostly G-L- ; G+ L+. Pick possible well marked recombinations.
Restreak G-L+ on EMB glu; G+L- on EMB Lac
Streak Lac+Glu+ on D(0)

Partial segregation and coupling of
lac v reversions from Lac- (810B)

March 2, 1951.

a). 810B cultures purified & grown in D/Lac), stored at 37° C. ca 10 hr.
Plate out on EMS lac to look for Lac – MH v partial segregants.

	lac	
B8 – 1	Few cols: repeat:	6/200+
B12 – 1	Ca ½ lac –	54 : all MH – ! Also 24 dunkers: do → 78 : all –
B19 – 1	no cols	
B25 – 1	49+ : 3 –	6 Lac – all MH –
B28 – 1	0 – : 50+	
B39 – 1	0 – : 100+	

Results summarized on 810.

(H282 L+)

b) Coupling: Struck out 810B81 and compare c H283.

Struck out 8 Lac v, test +, – segregants on T6. All are T6^S ! Release Lac – cultures!

	V_6
7	S
34	S
39	SR
17	S
6	S
19	S
8	S

= H284

∴ (39) is sole lac – culture warranting use in coupling – repulsion tests of Lac + reversions. ~~Verify by [illegible] and Lac~~ (Also include #1). All these diploids should be tested against T6

March 10, 1951.

+S = cis
+R = trans

M284 independent lac+ reversals.

(# 5 is lac++). Streak out single Lac V colonies. Test on V_6.

	R	Lac+ S	R	Lac- S		Type
1	0	4	3	0	/	cis
2	3	1	0	4	/	trans
3	4	0	0	4	/	"
4	0	4	3	0	/	cis
~~5~~						
6	0	4	3	0	/	cis
7	4	0	0	4	/	trans
8	4	0	0	4	/	trans.

TOTALS

cis	TRANS
~~HHH~~	~~HHH~~
~~HHH HHH~~	~~HHH~~
/	~~HHH~~
	IIII
15	20

Save 823-1 and 823-2 as type cis; trans respectively: M286 · M287

9 (3)	4	0	0	4	/	trans
10 (10)	0	4	3	0	/	cis
~~11~~ (12)	0	4	2	0	/	cis

L+ : MH- V_6^R 3- 1+ V_1 ; 3MH- 1 MH+ V_1^P L- 1 MH+ V_1^P 2 MH- V_1^R 1 " V_1 MH- V_1^R 2 MH- V_1^R =

(9 others were Lac+ (1 Lac-) MH-)

almost all MH- !

12 (8) 16 others were Lac+ Xyl- gave a 2 2 2 2 arrangement.

Reisolate single EMS lac+ and retest.

cis cultures regardless. Lac+ Lac-	12A	0	4	3	0	/	} cis	Lac+ predominant
	12B	0	3	4	0		} "	
trans	13	3	1	0	4	/	trans	Lac- "
	14	4	0	0	4	/	trans	Lac- "
	15	0	4	2	0	/	cis	Lac+
	16	3	0	0	4	/	trans	-
	17	0	4	2	0	/	cis	+
	18	0	4	4	0	/	cis	?
	19	2	0	0	4	/	trans	-
	~~20~~	3	0	0	4	/	trans	-
	21	4	0	0	3	/	trans	+ (-)
	22	0	4	4	0	/	cis	+
	23	2	0	0	3	/	trans	- (+)
	~~24~~	~~0~~	~~4~~	~~2~~	0	/	cis	+
	25	3	1	0	4	/	trans	-
	26	4	0	0	4	/	trans	-
	27	~~4~~	0	0	4	/	trans	-
	28	~~0~~	4	~~2~~	0	/	cis	+
	29	~~4~~	3	~~2~~	1	/	~~cis~~ trans	+ -
	30	1	3	3	0	/	cis	+ -
	31	3	1	3	0	/	trans	- +
	32	3	0	0	4	/	"	-
	33	4	0	0	4	/	"	-
	34	0	4	2	0	/	cis	+
	35	2	2	0	4	/	trans	-
	36	0	4	1	1	/	cis	+

20 reversions 5 Lac V

37 reversions 8 Lac V MH- others Lac+ MH+

R | V_6 | S
1 | 1

Reisolate + S and test

Reisolate + S and test

Recheck 29, 30

April 2, 1951

Resolate lac v from streakings of 29, and 30 for verification. Score lac +, − from individual lac v.

29.

b	4 − S	3 + R	(1 lac v
a	3 − S	2 + R	2 lac v)
c	4 − S	2 + R	2 lac v
d	4 − S	3 + R	1 lac v
	15 − S	10 + R	

(poor destruction of lac +, lac v → lac + less frequent.)

30

a	4 − S	3 + R	1 v
b	4 − S	0	2 v
c	4 − S	2 + R	2 v
d	4 − S	4 + R	0
	16 − S	9 + R	

both are trans

Resolate and compare with 1, 2.

13

Totals 15 cis

20 trans

$\chi^2 = \frac{25}{35} = 5/7$ $\quad p = .4$

March 7, 1951.

W466 × W1577 mEMS Xyl, Mtl for Lac⁻ (mucoid)

~~36~~ Mtl; 48 Xyl+.

84 tested all streaked on Mtl.

a: 10 possible Mtl v. Restreak from EMB:

a	Mtl	Xyl	Lac	Mal
1	++	++	+	++
2	v	v	v	++
	++	+ v	+	++
3	v	v	+	-+ v
4	v	+ v	v	−
	v	v	+ v	−
	v	v	+ v	−
7	v	+ v	+ v	+
8	~~+~~ (v?)	+ (v?)	−	~~+~~
	−	+	−	−

~~Robust~~ ~~test~~. v Mal v

H 288

Restreak possible v from EMS.

3) addnl. tests from Xyl (17) and Mtl (20)

9	−	−	−	−
11	v	-+ v?	v	−
12	v	v	v	+ v
	v	−	v	−
	v	~~+~~ v	v	−
	v	~~+~~ v	v	−
	v	~~+~~ + v	v	+ v
	v	~~+~~ v	v	−
17	+ v	v	v	−

not to confuse with Mal, Xyl.

Restreak: "3", "8". 824-8 still mucoid. Colonies have somewhat mottled character EMB Mtl. Restreak on Mtl: Mtl v Xyl+ Lac− Mal−

H 288

3: although apparently clean single cols. from EMB Mal gave +, − and ?v, colonies from EMS Mtl gave pure Mal+ and Mal− (Xyl v)

Replate

~~Restreak~~ from gross streak to EMB, EMS Mal.

18 Mtl Xyl Lac v Mal −

~~19~~ 20 " " " "

	Lac	Mtl	Xyl	Mal
1-	V	V	V	—
2	V	V	V	—
3	+	V	V	—
4	V	V	V	—
5	+	V	V	—
6	+	V	V	—
7	+	V	V	—
8	V	V	V	—
9.	V	V	V	—

65

no lac – like as required!

3/19/51. ~~42~~ addnl. tests. 2 possible MHv? : 824-30-31

100 addnl. " 30 " "

?

Typing mouse coli pairs (CP Miller) 825

March 8, 1951 HK

(All numbers represent 776- designations.) q.v.

1	A+B	H F 22-23	Both suc± Ck-	
2	A+B	F H 335-36	Suc±Ck+ ; Suc++ Ck-	"
3	A+B	H F 346-47	Suc ++ Ck-	(3b maybe Ck+).
4	A+B	H N ! 348-49	Suc- ; Suc-^{P} CK+	

a. Streak out on EMB Sucrose.

3/8/51. 1. A+B indistinguishable on EMB, Sucrose. Both Suc±.

2. A. Suc ± (dist. from 1A+B - somewhat lighter). B Suc ++ Obviously distinct

3. A } Indistinguishable Not separable from 2A.
   B } Suc ++. a few slow colonies.

4. A Suc ± with ± wedges
   B " one. " colonies.
   Restreak single ± colonies to compare stability.

Each of these is resistant to phages T1 - T7, and P, except for indecisive reaction to T4, T2 Also Ck^{R} (Ck_{V}, Ck_{B}).
also all Ck^{R} (DAEFCHI) 3A?/Ck G

Typing coli

8234

April 2, 1951.

" Rolenhoff # 41-46 A B 49-50 C 61-62 for comparisons.

= 776-457-8
776-468-9.

A 1-6 Identical on EMB sucrose, faint ± colo with darker centers.

B 1-2 1: Su− Cl± 2: Su ± Cl−

C 1-2 Su − Cl+++

Recheck Cl, Su, character, and response to Q, Cl.

A: 1-6 show identical Su± Cl+. character on W618.

B 1 resembles A 1-6. B2 Cl? Su±
but # Cl ÷

C Inhibited on EMB Mannitol!
Cl++

Newly lysogenic phage: 776-370; 373

March 12, 1951.

(A) (B)

776-370 and -373 were found to be lysogenic for W518. Also for W811.
Picks plaques on W518 to produce a W518 [ ].
Although supplied as separate cultures from NIH, 370 and 373 may well be identical.
They were the sole S^R in their groups and resemble each other culturally.

None of 8 W518 recovered each from plaques from A and B.
Inoculate A + B together with W518 for preliminary growth of the phages.

c) High titer stock obtained on W518. W518 survivors were λ^{370} 18/20. S^R 2.
None lysogenic.

Attempt to induce or modify lysogenicity re λ^{370}.

d) Inoculate suspensions + λ^{370} stocks into Penassay.

1	W1248	P^R	λ^-	
2	1027	S	−	
3	1177	R	+	ca 100 none lysogenic against W1177
4	677	S!	+	
5	660	S	+	
6	58-161			} 40 each tested
7	W518 + λ + λ^{370}		+ λ^{370}	} none lysogenic against W1177

anti - K12 Lactose ›

827

	A	B	C
Antigen .5 ml	1:10	1:20	1:40
Antiserum .5 ml undil. (serum 117).			

Incubate at 37°. Then centrifugate

Sediment the precipitate.

a). Supernatants: Dilute A 1:4 B 1:2 C ~~1:4~~ no dil.

Take .1 ml samples to 5 ml H_2O; 1 ml npg M/200 in M/20 buffer

Inc 37° 10 mins. Add M/1 Na_2CO_3.

npg: A > B > C.

b) Wash ppts twice. Resuspend in 1 ml saline. Assay .1 ml samples as above, 20 mins. Add Na_2CO_3.

npg A > B > C. ca 1/5 as active as supernatants

A	.5 ml antigen	.5 ml antiserum.	to 1 ml volume
B	.05 "	+	
C	.005 "		
D	A' (	with .5 ml NaCl rather than serum	"
E	B'		
F	C')		
G	= 3x washed ppts. of ABC.		
H			
I			

~~For control ppt. washing, also add antigen to boiled serum ppt~~

Assay .5 ml samples, equivalent to 1:100 dilution (C).

A	0.8	227
B	0.2	620 sic!
C	128	142
D	0.0	1
E	0.0	
F	0.0	0
G	0.2	1
H	0.8	
I		

Protection of Ab ??? of serum!!!

Assay antigen

(over)

827:) Assay antigen:
dilute 1:100. 1:1 with saline, then as
in previous assays.
ca 500.

824 - 3
H281

828

3/19/57 H289 is MtlvXylv Lac+ Mal−,+ v?

Inoc D(Mal) 10 ml with mixed growth from original EMS Mtl streaking, incubate in air 24h. Plate out at 10^{-7} on EMS Mal, EMB Mal, Mtl.

EMB Mtl plating shows 90% Mtlv. On EMB Mal, no clear Mal v colonies are seen, but very numerous mottled Mal+, which might be Mal+/− ... v.

↪ ca 30: all Mal++ 20 all ++ Mostly Mtlv.

This "culture" is probably a duplex pair Mal+, − resp. Check Mal− for hemizygosity.

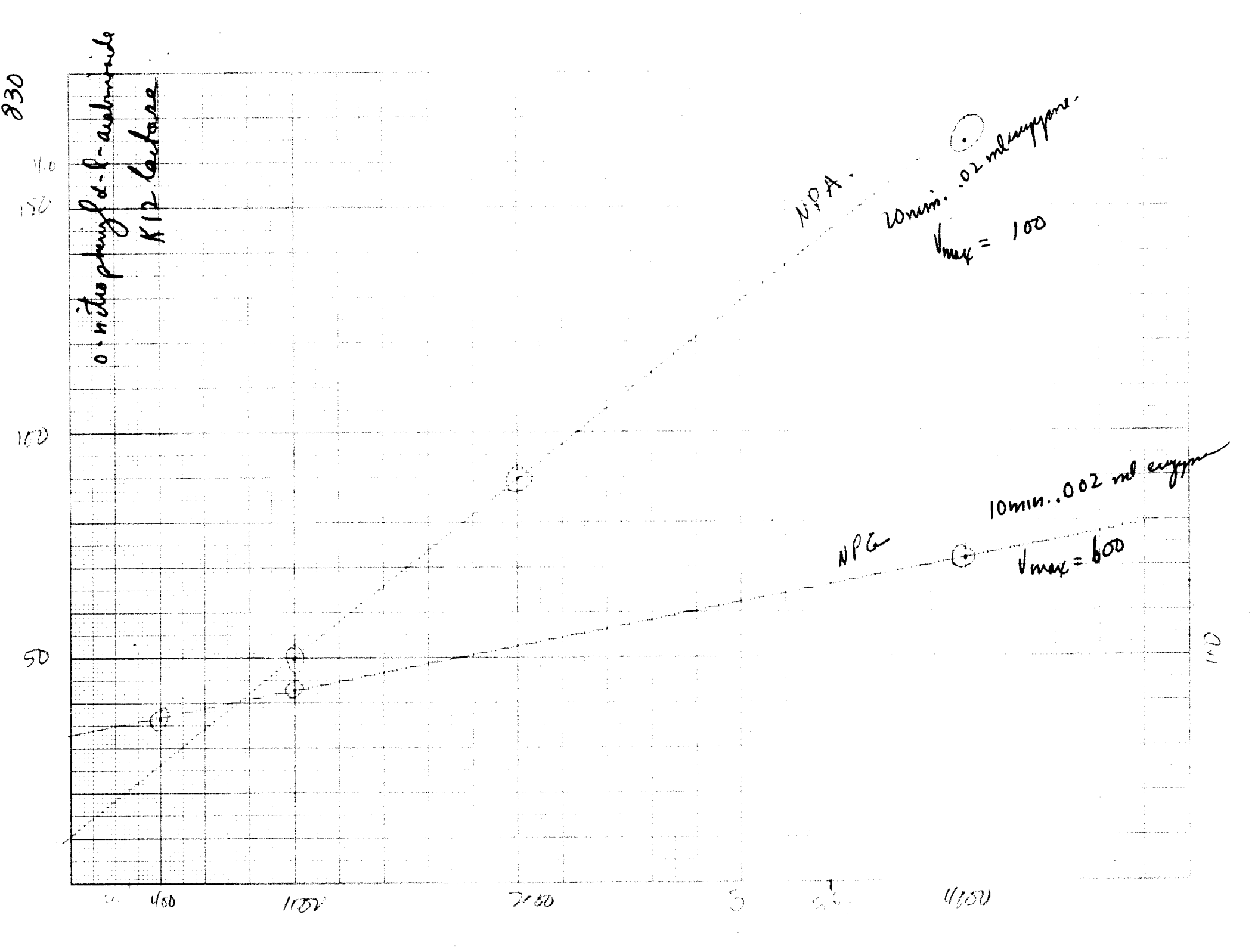
824-8
H288

3/17/44. /50.

H288 is Lac- MHv Xyl+ Mal- from $\overset{BMLac-}{W466} \times \overset{W660Lac+}{W1577}$
$T5^{s}(?)$ V_1^R.

Each of 16 Lac+ reversions on EMS Lac was found to be Lac$\underline{v}$ MH$\underline{v}$ but the latter character is difficult to score.

∴ Lac- derived from reverse cross is also homozygous.
From this one might argue that the corresponding Lac+ found in the cross W478 x W1490, etc., are also homozygous.
Compare 819 for similar data on Mal.


830
0-nitrophenyl-β-D-galactopyranoside
K12 Lactose
150
100
50
NPA.
20min. .02 ml enzyme.
V_{max} = 100
NPG
10min. .002 ml enzyme
V_{max} = 600
400
1100
2450
4(150)

			1/V	1/S
		D_f		
1		098	102	
2		150	67	
3		470		
4		880		
5		128		

	Enz.	Sub M/200				
20min 1	.02	NPA	.5	061	164	4000
20min 2	"	"	1	112	89.3	2000
20min 3	"	"	2	201	49.7	1000
20min 4	"	"	5			
10min 5	.002	NPG	.5	139	71.9	4000
10min 6	"	"	2	234	42.7	1000
10min 7	"	"	5	276	36.2	400

A 259 256

B 279 271 still different

V_{max} 20min NPG 606 K_s M/3400 $= 2.9 \times 10^{-4}$

NPA. 1000 K_s M/250 4×10^{-3}

Place new markers

March 22, 1958.

A W1490 x W1511 (W-1 Mtl) on EMS Lac.

B " x 1578 (W-1 Xyl -)

C " x 1519 (")

A 40 Lac "+" picked, streak on EMB Lac. Lac v : 1-4 1-6 (~~[illegible]~~).

B. 100 Lac "+" (light) picked (Miss Lem) and streaked on EMB Lac. Mostly Lac -!

C. 40 Lac + picked.

Segregation ratios : B among Lac - 13 + : 78 -
5 + : 15 -
9 Xyl+ : 51 Xyl -

C " " 14 + : 46 -

~~A~~ ..

Lac + : C : 7 + : 22 -

B : 3 + : 30 -

Some correlation with Mal+ recheck.

	Mal+ Mtl+	Mal+ Mtl-	Mal- Mtl-	Mal- Mtl+
A. Lac+	7	0	13	1
Lac -	3	0	18	0

very close linkage of Mtl to Mal. Also seen in heterozygote behavior.

March 26, 1951.

1, 2, 3 : Mal − Mal − lac v. Growing poorly on EMS Lac.

There heterozygotes give almost a − reaction on EMS Lac, requiring 48 hours to give a full + reaction. (Modifier or pleiotropic effect?)

Each gave a Mal+ Lac v reversion with stronger lac+ reaction.
∴ Mal− is hemizygous.

	Lac	Mal	Xyl	Isolated EMS lac +	
1	V	−	V	V+	Xyl V
2	V	−	V	V	H
3	V	−	−	V	✓
4	V	+	−	V	✓
5	V	−	−	V	?
6	V	−	V+	+	Xyl +

In B, C 1 EMS lac+ colony was picked as purified haploid stock.

3, 4, 5 each gave Xyl ± reversions after storage on EMS Xyl

∴ Xyl − here is Xyl =

	lac	Mal	Xyl		Isolated EMS+ lac	
1	?	−	−	.	v?	— mottled but not lac v. May be lac+/lac+
2	?	−	−	.	+	xyl-
3	v	−	v		v	
4	v	−	v		+	xyl v
5	v	−	v		v	
6	v	−	v		v	
7	v	−	v		v	
8	v	−	v		v	
9	v	−	v		v	
10	v	−	−	. .	v	—
11	v	+	+		v	
12	v	−	v		v	
13	v	−	v		v	
14	v	−	−	. .	~~+~~ v+/v	
15	v	+	+		v	
16	v	−	v		v	
17	v	+	+	(v?)	v	
18	v	+	+		v	
19	v ?	−	v		v+ (?)	
20	v	+	+	(v?)	v	

Note conclusion has also between xyl+ and Mal+. Would it be possible to arrange to have xyl marker screened to verify the homozygosity of xyl "+" in this case?

March 30 ff 1951

478

(~~58~~ × W660) × W1394 (Y10 S^R)

		lac+		lac−		% [PR]	
A. lac− Mal−	A	1	78 *	18 *	19		96
		2	82	17	18		99
		3	98	30	23		128
		4	130	23	28		153
		5	151	46	23		197
		6	127	30	19		157
		7	96	25	21		121
		8	120	47	28		167
		9	123	38	24		161
		10	159	25	(13)		184
		11	106	45	(30)		151
		12	59	22	27		81
		13	125	43	22		168
		14	139	44	24		183
		15	143	45	24		188
		16	122	32	21		154
			~~458~~	~~530~~	21%		2488

91: 23
83: 17 17

× 1585

B lac+ Mal−		1	10 *	41	(20)		51
		2	69	113	(38)		182
	B	3	30	105	22		135
		4	29	112	21		141
A.		5	56	101	36		157
		6	31	68	32		99
		7	80	134	37		214

25: 95
32: 64

~~B~~	2L−		88	31 *	24		129
	4L+		36 *	77	31		113
A	C	5L−	103	21 *	17		124
		6L+	~~3~~	~~16~~			19
			7 27	54			

.... 28: 78
82: 14

1394
1177
1394

F, 3 wire stuck

Linkage Aberrations Backcross prototrophs e32

March 26 thru 29, 1951

A. Isolate prototrophs from W478 x W660

B " " " 58-161 x "

C " x 1022 ca 5% Lac+, Mal-

Cross x W-1177 with SRP selection (plating on to streptomycin EMB to select SR prototrophs)

D 478 x 1022.

Isolate various prototrophs for crosses. ca 5% Lac+ Mal-

3/29/51.

	Lac	SRP
K12 x W1177	9+	/41-
"777+" x " 1	8+	/45-
777+ " 2	1+	/10-

K x 1590 Lac+ 8 22

K-12 x W1589 (= BM Lac-Mal-SR) . All of 41 Mal+ . (conflict with S-Mal linkage? streaks out on EMB Mal! W1589 is Mal+ not pigm.

lac: 0+37-
4+28-.

A)

A	Lac-	x	1394
B	Lac+	x	1585
C	Mal+	Lac- 1394 / Lac+ 1177	

1,2,3,5 Lac- 4,6,7 Lac+ 4,6,7 not prot.

B) Mostly Mal-.

C)+D. Mostly Lac+ Mal+. Lac- not correlated with Mal-

April 21, 1951.

Lac+ Mal+ from B, C, D × W1177 · EMS lac, Mal⁻

B (++) 4 plates 1 Lac⁻

C (++) 3 plates 1 Lac⁻

D ++ 4 plates 0.

Nearly sterile!

Repeat of K-12.

Exp W1367, W1177 as parents.

		(×1367 ×W1177) Lac+	−	W1367	
K	1	2	52		
	2	2	26		
A	1	38	150		
	2	26	246		
B	1	0	3		
	2	~~20~~	~~812~~		
C	1	10	10		
	2	2	27		
D	1	17	89		
	2	20	186		

nuclear pattern should be repeated.

Repeat A: (extreme ratios)

	L+	L−	% +
A10	97	23 *	
A11	83	17 *	
B1	25 *	95	
B2	"32" *	"64"	
C4	28 *	78	
C5	82	14 *	

of K ×	W1177 Lac+	−	1015	1022
	44	90	+ sterile −	+ sterile!

[Not surprising since 1015 and 1022 are S⁵!!]

Auxotroph partial zygotes. (diploids) 833

March 1945

			Recover in Lac, via D(Lac) + suppr.
1	754A2	M- Lac^v Mal-	-, + Pro^p Lac^v or +?
2	754A1	M " "	" " Recover Lac^v
3	754B5	TL- Lac^v Mal-	—
4	H244	M- Lac^v Mal-	—
5	754B6	nogr. TL Lac^v Mal-	—
6	754B11	TLB$_1$ Lac^v Mal-	ng
7	754B3	L- Lac^v Mal- Xyl-	—
8	~~H245~~	~~H - Lac^v Mal Xyl MTL^v~~	Lac^v TL
9	H244M+	M Lac^v Mal^v	+ —
10	~~H245~~	~~TL Lac^v Mal-~~	Lac^v Prototrophic

H245 and 246 reisolated.

Grow in D(Lac) + BM or TLB$_1$. Cross H245 × W1177
H246 × W1387

Recheck nutrition:

H245 TL
246 prototrophic!

833-1 M$^-$ Pure Lac+? (same mutant)
833-2 M$^-$ Lac^v

= H290
= 754A1

Diploid crosses.

April 4, 1951.

H245 TL- Lac Mal Xyl Mtl v H290 M- Lac v Xyl- Mal-

A. H245 x H290 EMS Lac, Mal

B H245 x W1367

TL	BM	Lac	Mtl	Mal
−	+	+	+	+
−	(−)	− s	−	−
+	−	− m	+	+

C H290 x W1585 (= W1177 Mal+)

+	−	+	−	−
(−)	−	−	−	−
−	+	−	−	+

A. (Lac): Mostly Lac+ occasional Lac−; wide range of Lac+ types. Picks 40: All are lac v except #6, 8, 30, which are Lac−. The absence of Lac+ is easily understood as the parents are each doubly heterozygous. lac 1-4 +/+

(Mal). Mostly Mal+, as above. Clones, m EMB Mal are difficult to interpret as there may be admixture. They are either Mal v or Mal+ v (except 62 +?, 43 Mal−). = A41-80

Hold, if necessary, for analysis of A. Best test for Lac− Mal v.

B As A (Lac)

C Mostly −? →

+	−	v
1	++++	111
	++++ ~~++++~~ (+,−)	
	++++	
	++++ ++++	
	++++ 1	

several +, − streaks noted!

(see over)

833A. Nal status not clear.

4/20/51. Probably +/−

Maybe ++/− ?

April 7, 1951.

Restreak 1-40 on EMB Mal (4/7), EMS Lac (4/8) / EMB Lac 4/7 (Hillman) repeated

Lac v except 6, 8, 30. 28 may be +, −

#6, 8, 30 appear Mal v or +v, like others. 28 is Mal +, −

(41-80: 41, 50, 52, 61, 64, 67, 69, 77 are Lac −, others Lac v.)

4/10. Repeat single EMS Lac + 28 → Lac +, Lac −
6, 8, 30 Lac −

	Lac	Mal	Mtl
1	v	+v	+v
2	v	+v	+v
3	v	v	+v
4	v	+v	+v
5	v	+v	+v
6	−	+v	+v
7	v	+v	+v
8	−	+v	+v
9	v	+v	+v
10	v	+v	+v
11	v	+v	+v
12	v	+v	+v
13	v	+v	+v
14	v	v	+v
15	v	+v	+v
16	v	+v	+v
17	v	v	+v
18	v	+v	+v
19	v	v? +v	+v
20	v	+v	+v
21	v	+v	+v
22	v	+v	+v
23	v	+v	+v
24	v	+v	+v
25	v	+v	+v
26	v	v	+v
27	v	+v	+v
28	v	v	+v
29	v	+v	+v
30	−	+v	−
31	v	+v	+v
32	v	+v	+v

Definitely → occasional Mal −, Mtl −.

+v ? ?

Mal v
Mal v
Mal v
Mal v
Mal v

v − compare original and derived.

As previously, it is difficult to distinguish Mal and Mtl v from + modified by segregation of other factors. But most or all appear to be Mal + / −

	Lac	Mal	Mfl
33	+	+	+
34	+	+	+
35	+	+	+
36	+	+	+
37	+	+	+
38	+	+	+
39	+	+	+
40	+	+	+

Compare B3A (original) and BBB (denied)

April 7, 1951.

M245 × W1367 S^R Malt Lac^{-m} × T-L- Lac 1/4 v Malv ....

Restricted 24 spots on EMS Lac.

#15 is Mal−, others +^{v} or v (from spots only).

4/9/51. Replik single EMS lac colonies and test:

	Lac	Mal		Mtl		Sm
1	v	v−		v	S	v
2	v	v		"	S	v
3	v	+^{v}		"	S	v
4	v	+^{v}		"	S	v
5	v	+^{v}		"	S	v
6	v	v		"	S	
7	v	v		"	S	
8	v	v	v?	"	S	v
9	v	v		v	S	v
10	v	v		v	S	v
11	v	+^{v}		+^{v}	S	
12	v	+^{v}		+^{v}	S	
13	v	+^{v}		+^{v}	S	
14	v	v		+^{v}	S	
15	v	−		+^{v}	S	
16	v	v		v	S	
17	v	v		v	S	
18	v	v		v	S	
19	v	v		v	S	
20	v	v		v	R?	
21	v	v		v	R?	
22	v	v		v	S	
23	v	v		v	S	
24	v	v		v	S	

Mal +^{v} → Lac− ^{s}

Mal v− → Lac− ^{m}

Mal v (→ Malv with much higher Mal− component than original)

Mal v − derivatives

Malv −

→ Malv in reversion Lac− ^{s} Lac− ^{s}

two few initial segregants for critical determination as +^{mottled} or v on Mal, Mtl.

v 1367 S^R v.

A number of types are probably represented. Mal− should be specifically tested for hemizygosity. Study for distributions of Lac−^{m} / Lac−^{s}.

Assume lac$_4$ − lac$_1$+ to be present

Plate out B1, 2, 15, 16 from D(Lac) to EMB Lac, Mal, EMS..

1. Lac v, relatively stable +

   Mal Mottled, no −.

2. Lac v (less stable +). → Lac − m. Mal highly variegated, mostly −. ca. 24 Mal+ and Mal− segregants: each lac− each colony partial segregation in stipe. Pure +, − about =.

EMS Mal : Pure +

15 Lac v (like 1. Mal: pure − Plate on EMS Mal

16 Lac v (like 1) Mal like 1.

Mal +v are apparently Mal+, with segregating modifiers. These should perhaps be studied as stable triploids.

B2 should be studied for interdependence of Mal and lac segregations.

M290 × W1585⁻ 833C

BM Lac v⁻ TLB, Lac⁻ Mal⁺ S^R D

April 7, 1951.

Recover from EMS Lac. 4 Lac+ or Lac v.

also test 12 other Lac⁻ for $S^{R/S}$ (EMB vs EMS)

(have were S^S on EMS Mal.)

	Lac	Mal	(sm)	
1	v	v v	S	
2	v	+	R	mottled
3	v	v	S	
4	+	+	R	

This illustrates that Mal is not eliminated in this 2n × 1n cross (unless #2 is hemizygous). It should perhaps be repeated to look for Mal⁻

D: W1490 × M245⁻

BM lac+ $S^R V_6^R$ TL Lac v... V_6^S ?

	Lac	Mal	sm	EMS	EMB
1	v	v	s		s
2	v	v	s		s
3	v	v(+)	s		s
4	v	v(+)	s		s
5	v	v+	s		s
6	v	v	s		R
7	v	v	s		s
8	v	v	s		s
9	v	v	s		s
10	v	v	s		s
11	v	v	s		s
12	v	v	s		s
13	v	—	s		s
14	v	v	s, (R)		R
15	+	+	s		s
16	v	?	s		R
17	v	v	s		s
18	v	v	s		s
19	v	?	s		s
20	+	?	s		R

EMB Mal scoring imperfect

S^D/S^s heterozygotes

833E

April 14, 1951.
H245 × W1606

TL LacvMalv BM S^D
Lac+

These are uniformly Malt or Malv carrying the Mal factors from the diploid parent!
Study 833E1 for Mal – homozygotes

	Lac		Mal		S EMB
1	v	–	–	-?	S
2	v	+	+		S
3	v	+	+		S
4	v	+	+	–	S
5	v	+	+	–	S
6	v	+	+		S
7	v	+	+	–	S
8	v	+	+	–	S
9	v	+	+		S
10	v	+	+		S
11	v	v	+		S
12	v	v	+		S
13	v	v	+		S
14	v	v	+		S
15	v	+ v	+		S
16	v	+ v	+		S
17	v	vv	+		S
18	v	v	+		S
19	v	v	+		S
20	v	v	+		S
21	v	v			
22	v	+ v			
23	v	v v			q
24	v	v			
25	v	v			
26	v	v			
27	v	v			
28	v	v			
29	v	v			
30	v	v			s
31	v	+ v			
32	v	+ v			all S
33	v	+ v			
34	v	v			
35	v	+ v			
36	v	+ v			
37	v	v			
38	v	v			
39	v	+ v			
40	v	+ v			v

16 others all S^s

M245 x W1602. Purified mEMS Lac.

	Lac	Mal	Mtl	S
1	v	+v	v	
2	v	+v	v	
3	v	+v	v	
4	v	+v	v	
5	v	+v	v	
6	v	+v	v	
7	v	+v	v	
8	v	+v	v	
9	v	+v	v	
10	v	+v	v	
11	v	+v	v	
12	v	+v	v	
13	v	+v	v	
14	v	+v	v	
15	v	+v	v	
16	v	+v	v	
17	v	+v	v	
18	v	+v	v	
19	v	+v	v	
20	v	+v	v	
21	v	+v	v	
22	v	+v	v	
23	v	+v	v	
24	v	+v	v	
25	v	v	v	
26	v	v	v	
27	v	v	v	
28	v	v	v	
29	v	v	v	
30	v	v	v	
31	v	v	v	
32	v	v	v	
33	v	v	+v, v	
34	v	v	+v	
35	v	v	v	
36	v	v	v	
37	v	v	v	
38	v	v	v	
39	v	v	v	
40	v	v		

all S^S on EMB Hyl

TL Lac^r Mal^r x DM Lac$^-$ mal$^-$ S^R

These diploids resemble those of 833B and 833D.

Evidently, the BM parents do not contribute to the Mal-S factors of these heterozygotes. The possibility that these are 3n-2n aneuploids remains open. (cf. B or D).

when M245 is one parent.

Restreak +^{v} colonies from certain Mal plates. All but 10 and 23 showed apparently only Mal+, these also had rare Mal$^-$. It is possible that these are all Mal^v but that segregants occur rarely. Appropriate S^R markers would facilitate the characterization of these diploids.

Compare original and derived Lac v Mal v 833 Ca

April 21, 1951.

Compare original & derived (selected as Mal v) from 833:

1 A9 – σ Lac v
2 – d

Plaques noted in thick streak. Mostly lac –; + on background.

3 B3 – σ Lac v
4 – d Lac – only

Restreak 1 & 2 on EMS Lac to recover heterozygote. Source of λ^S?!

✓ Plaques may be unique phage, rather than λ^S

This is confirmed. The phage attacks all λ^+, λ^R types and resistant mutants are not attacked to λ (E.M.L.)

May be merely a contaminant see EML 163

conc/4 3/10 4 3.

		BM	TL
A	H245 x H290	‖ Lac^{v} Mal^{-}	‖ Lac^{v} Mal^{v}
B	H245 x W1367	≠ Lac^{-m} Mal^{+} S^{R}	‖
C	H290 x W1595	‖ Lac^{v} Mal^{-}	‖ Lac^{-} Mal^{+} S^{R}
D	H245 x W1490	‖ Lac^{+} V$_{1}^{R}$ S^{R}	‖ Lac^{v} Mal^{v}
E	H245 x W1606.	S^{D}	‖ Lac^{v} Mal^{v}
F	~~H290~~ H245? x W1602	‖ ~~S^{R} Mal^{-} Lac^{-}~~	‖ ~~Lac^{v} Mal^{v}~~

A. No Mal− seen. Many are clearly Mal^{v}, but with scarce Mal− segregants.
?? Are Lac− haploid or diploid segregants??

B. Majority are Mal+, probably not Mal^{v}. Also, seemingly S^{S}, including #2 Mal^{v}.
Some would be expected to be S^{R}/S^{S}.

C. Many Lac−. Of Lac+, (mostly) Mal^{v} S^{R}^{v}.

D. Mostly Lac^{v} Mal^{v}. ~~S?~~

E ditto no S^{D}

F. ditto no S^{R}. Mal^{v+}.

The Mal^{v} complex of H245 is retained intact in crosses with BM. Review H290 behavior.

H245 = TL Lac^{v} Mal^{v}
H290 = BM Lac^{v} Mal^{-}

Diploid SRP xx.

March 30, 1951.

			mEMS Lac sm.	
A	H283	× W1177	Streak	
B	"	W1490	ca 20-30 Lac+	→ pure Lac+
C	"	W1387	1 Lac?	→ Lac−

Should be repeated if a reason to carry out this experiment can be thought of.

Linkage aberration study

April 2, 1951.

a) Inheritance through addnl. crossing → 2 Lac^v / 40 tests. = 835 C1 – C2

b) Linkage relationships – preliminary survey.

A. 58-161 × W1022 ± B$_1$ } on EMS Lac, Mal, Mtl

~~B W1490 × W1022 ± B$_1$~~ }

C 58-161 × W1178 EMS Lac to isolate Lac^v.

		+	−
A:	Lac	49 61	2? 2?
	Mal	29 2	2 0 ?? 0 0 1
	Mtl	65	34 21 10
	Xgl	62	6 – (>?)
A (+B$_1$):			
	Lac	110	49 7 7
	Mal	2 1	0 0 0
	Mtl	19	16 4 12
C	Lac	35	23 3 2
	Mal		
	Mtl		
C +B$_1$	Lac		
	Mal	41	46 2 5
	Mtl		

C1, C2 are two Lac^v isolated from 40 tests. Both are Mal − Purify and segregate

L	M	Mtl	
+	−	−	
+	−	−	
−	−	−	A
−	+	−	Mtl −
+	+	−	
+	+	−	
+	−	+	A Mal −
+	+	−	
+	+	−	
−	−	−	
+	+	−	A' Mtl −
−	−	−	
−	−	−	
+	−	−	
+	−	−	
−	−	−	
−	−	−	
−	−	−	A' L −
−	−	−	
−	−	−	
−	−	−	
−	−	−	
−	−	−	
−	−	−	

L	M	Mtl	
−	−	−	
−	−	−	A Mtl − ↓
−	−	−	
+	+	−	
+	+	−	
+	+	−	
+	−	−	
+	−	−	
−	−	−	
−	−	−	
+	+	−	
−	−	−	
−	−	−	
−	−	−	
−	−	−	
−	−	−	
+	−	−	
−	−	−	
+	−	−	
−	−	−	
+	+	−	
+	+	−	
−	−	−	
+	+	−	
−	+	−	
−	−	−	
+	+	−	
+	−	−	
+	−	−	
−	−	−	

Mtl – occurs relatively frequently, not necessarily associated with Lac −, Mal −

	Lac	Mal	Mtl	Xyl	TS	TS
1		+	+	+	S	S ?
2		+	+	+	S	S
3		+	+	+	S	S
4		+	+	+	R	S
5		+	+	+	S	S
6	+	+	+	+	S	S
7		+	+	+	S	S
8		—	+	+	S	S
9		+	+	+	R	S
10		+	—	—	S	S
11			+	+	S	S
12			+	+	S	S
13			+	+	S	S
14			+	+	S	S
15			+	+	S	S
16	+		+	+	S	S
17			+	+	S	S
18			+	+	S	S
19			+	+	S	S
20			+	— ✓	S	S
21			+	+	S	S
22			+	+	S	S
23			+	+	S	S
24			+	+	S	S
25			+	+	S	S
26	+		+	+	S	S
27			+	+	S	S
28			+	+	S	S
29			+	+	S	S
30			—	—	S	S
31			+	+	R	S
32			+	+	S	S
33			+	+	S	S
34			+	+	S	S
35			+	+	S	S
36	+		—	—	S	S
37			+	+	S	S
38			+	+	S	S
39			+	—	S	S
40			+	+	S	S
Lac− 1	—	+	—	—	S	S
2	—	+	+	+	S	S
3	—	+	+	+	S	S

Lac+

See 845

~~Lactata TS keto.~~

~~Xyl~~ — ~~Mtl~~.

No Lac− V, linkage sem.

Lac — I (Xyl, Mtl) — Mal

V,

?

58-161 x W1022

Picked random from D(0). Benshan EMS Lac

9- : 111+.

Pool with lac- from EMS lac cross plates
Streak out on EMS lac to purify and complete
characterization:

	Lac	Mtl	Xyl	Mal	TS
1	-	-	-	+	S
2	-	+	-	+	S
3	-	+	+	+	S
4	-	+	+	-	S
5	-	+	+	-	S
6	-	-	-	-	S
7	-	+	+	+	S
8	-	+	+	+	R
9	-	-	-	+	S
10	-	-	+	+	R
11	-	+	-	+	R
12	-	+	+	+	S
13	-	-	-	+	S
14	-	+	+	+	S
15	-	-	-	-	S
16	-	-	-	-	S
17	-	+	+	-	R
18	-	+	+	+	S
19	-	-	-	+	R
20	-	+	+	+	S
21	-	-	-	+	S
22	-	+	+	+	S
23	-	+	+	+	S
24	-	-	-	-	R
25	+	-	-	-	R
26	+	-	-	-	S
27	+	+	+	-	S
28	+	+	-	-	R

Mal- from EMS Mal?

% 1022 parent among

	Lac	Mal	Mtl	Xyl	TS
Lac+	1/90 ca.	1/40	3/40	5/40	3/40
Lac-		7/24	10/24	11/24	6/24

Lac+ not greatly different from Lac- except for slight increase in

835 C

	B⁻	M⁻	T⁻	L⁻	+	hOL	
1	+	+	−	+	+	+	T⁻
2	+	+	+	+	+	−	
3	+	+	−	+	+	+	T⁻
4	+	−	+	+	+	−	M⁻
5	+	+	−	+	+	+	T⁻
6	+	+	−	+	+	−	T⁻
7	+	+	+	+	+	+	
8	+	−	−	−	+	−	M⁻T⁻L⁻
9	−	+	+	+	+	+	
10	+	−	−	−	+	−	M⁻T⁻L⁻
11	+	−	−	−	+	+	M⁻T⁻L⁻
12	+	−	+	+	+	−	M⁻
13	+	−	−	−	+	+	M⁻T⁻L⁻
14	+	+	+	+	+	−	

Aberrant Linkage

[redacted] 835 a.

April 26, 1951

58·161 × W1022

EMS Lac:

+	−
55	1
48	1
57	1
20	0
30	0
210	3

1½% − !

EMS Mal

+	−	
46	0	
22	0	
38	1	(see) s.o.
22	0	
128	1	

Stockout 70 EMSlac+ → EMSlac.

3 −

s. sectored col.

Place new markers MH —
W1509 — 13.

April 2, 1951.
W1490 x

A 1508 → lac v : MH −, v, +.
B 1511
C 1512
D 1513.
E

Most tests taken on defective EMB! Repick likely lac v from EMS and Retest!

A 1-8 lac v 1 MH+ v 2 MH v 3, 4, +v 5 v 6, 7 − 8+
~~9 10 11 12 15 lac v))~~ 9+ v 10 11 12 v ~~13 MH −~~
B No peculiarity this cross (cf. 831 A) 35-40 are lac v.

1 MH+ lac v ?
2 " "
3 " "
4 MH − lac v ?
5 MH − lac +
6 MH − "

C 1512 } No clear lac v. Repeat cross on
D 1513 } EMS lac.

B. 4 single colonies / prototroph testers. EMS lac →

	abcd	Lac	MH+		all Mal+
1		v	+	(+ mottled)	" " −
2		v	+		+
3		+	−	(Lac somewhat faded)	−
4		+ faded	−		−
5		+ faded	−		−
6					

W1511 MH+ has some epistatic effect on lac+. cf 831A

C : Repeat cross 4/8/51. EMS lac.
Ponyield.

D :

April 4, 1951. ff

W1508 × W1490.

16 picked and tested as lac v from 40 initial tests.

1-10 lac v Mtl v (or +). 11-13 Lac v Mtl − ✓ Apply for hemizygosity tests.

Check single EMS Lac colony reisolates:

	Lac	Mtl	Mal	
1	v	~~v~~ ?	+ ✓	Mtl+ ✓
2	v	v	−	
3	v	v+	−	
4	v	v	−	
5	+	v	−	
6	v	v	+ ✓	
7	v	v	−	
~~8~~	~~+~~	~~+~~	~~−~~	
9	v	v +?	+	✓ Mtl v
10	v+	~~v~~ v	−	
11	v	−	−	
12	v	−	−	
13	v	−	−	

Reisolates 1, 9, ~~10~~ on Mtl, Mal

∴ of 3 Mal+, 2 are Mtl +

Linkage data

	Mtl +	−
Lac +	2	18
+	1	19

no linkage to Lac — very low ratio.

Strike out 11-13 on EMS Mtl for reversions.

Reversions apparently pure Mtl+ ! ✓ Mtl+ Lac v.

The Mtl+ may well be a suppressor mutation.

April 5, 1951.

C W1490 x W1512.

- ① Check linkage Mtl/lac (dwit transfer Lac+, Lac- colonies to EMS Mtl

Mtl	lac	+	−
	+	5	2
	−	15	18

no dwit interaction.

Picks 40 colonies, streaks on EMB Lac for V. (~~15 −~~ 25 +) (0 4 / 26 9)

Lac+, smears of lighter tint.

No Lac V !!

Check Mtl character for further linkage tests to EMS Mtl

D W1590 x W1513 36 colonies for Lac V.

			lac	Mtl	Mal
	1	2?	+	−	−
?	2	3	V	~~+~~ +	−
.	3	5	V	+	−
repeat	4	22	+	+	−
tests	5	28	V	~~+~~ +	−
	6	33	+	−	−

Restreak these on EMS Lac

V on EMB Mtl.

Dwit transfer: linkage tests as above.

	+	−
+	13	7
−	4	14
	16	6
	1	0

clear linkage to Lac (probably to right

Bm V_6 Lac Mtl V_1 TL....

(over)

under suboptimal conditions

Lac	MH
+	−
+	−
+	+
+	−
−	−
−	−
−	−
+	−
+	−
−	−
+	+
−	−
+	−
−	−
+	+
−	+
+	−
+	−
+	−
−	−
+	−
−	−
+	+
+	−
+	+
+	+
+	+
+	+
+	+
+	+
−	+
−	+
−	+
+	−
−	−
+	+
−	+

MH \ Lac	+	−
+	11	4
−	11	14

Lac	Mtl
−	+
+	+
+	+
+	+
−	+
+	+
+	+
−	+
−	+
+	+

Lac	Mtl
−	−
+	+
−	+
−	+
+	−
−	−
−	+
−	−
−	+
+	+

Lac	Mtl
−	+
−	+
−	+
+	+
−	+
−	−
+	+
−	+
−	+
−	+

Lac	Mtl
+	+
−	+
−	+
+	+
−	+
−	+
+	+
−	+
+	+
−	.

Lac	Mtl
+	+
−	+
−	+
−	+
−	+
−	+
+	−
+	+
+	+

Lac	Mtl
−	+
+	+
+	+
+	+
−	+
−	−
+	

Auxotroph Lepil par exp. 837

April 12, 1951

A. H257 suspension from D(lac) streaked out on EMB Lac, Mal on and possible S^R Lac v or Mal v epicked for tests auxotrophs. 2 apparent Lac v (from several hundred S^R) recovered; both auxotrophic 837A-1 and A-2. Check nutrition; S^R, etc. Both are Mal ~~v~~ -

1. eventually grows on D(0), but fertest on D(TLB,) or D(BM)
   b-? or prototrophic?

2. D(0) -
   D(BM,) -
   D(TLB,) -
   D(BMTLB,) +.

B. H261, through Penassay. Plated → ca 10% Lac v.
Test colonies from EMB to D(0).

30 Lac v.	# all prototrophic	#16 ?v Mal S Xyl MM Lac v
20 MM v		#16.
6 Mal v		# 3?

C. H257

35 Lac v.	#17 auxotr.	others x+	Lac v Mal v
31 Mal v	#7 ? "	" "	Lac - Mal +, -

B1 (L)T - Mal Xyl MM Lac 1/4 S^{R1} v. n.g. for crosses.
C1 Mal Lac M+ T+ L+ ... ~~Prototroph~~. not heterozygous

H257 - 267 partial segregants

April 20 ff. 1951.

Irradiate H257, 24, 30 secs. UV 50 cm. ca 20% survival.
Pick lac⁻ centers and streak with ~ 1/3 lac⁻.
Replica lac⁻ on + (?) and ~~streak~~ spot on D(o); ~ 1/3 lac⁻ (or back segregation).
Partial plating gave a 2% S^R lac⁻. Incidentally after UV, this number ~ 6%.
Some "lac+" gave very scarce-lac⁻ - S^R segregants! (Why is ??)

B Check for prototrophy:

H267.

	lac	Sm	D(o)		lac	Sm	D(o)
1	V	R	+	11	V	S	+
2	V	R	−	12	V	R	
3	V	R	+	13	V	R	+
4	V	R	+	14	V	R	+
5	+	R		15	V	R	−
6	+	S	+	16	V	R	+
7	+	R	±	17	V	S	
8	V	S	−	18	V	R	+
9	V	R	−	19	V	R	−
10	V	R	+	20	V		

~~From~~ i.e., ca 9/19 mixed types.] 9 S (maybe either S/S or S/R latter.)
4 S^R
8 S^{RS}
else added L. 4/8.

Note very high frequency of "rearrangements" here (original H267 was S^{RS})

A H257. 8 x⁻ / 38 tests.

4/24 Restreak centers of 40 lac⁻ each (usually ⊙) from H257, 267 UV on EMB lac. Pick possible lac+ (stable lac⁻) for test with Sm
D) UV - on Sm medium. Resolute single lac⁻.

(over)

Natural and tests on auxotrophs.

A	1	T−	S_V
	2	M−T−	S_V
	3	MTL+	S_V
	4	MTL+	S_V
			~~2~~ S^S
			S^R
			S^S
			S^R
			?

B	1	M−	S^S
	2	M−L−	S^R
	3	MTL+	S_V
			S^S
			V
			R
			V

W64 S^{RP} tester

April 16, 1951.

A series of S^R mutants isolated from W1483. (A - F)
(Leu Trp Lac- Mal- S^R)

(1) Grown separately

A K12
B. A
C A+K12 } EMS sm

D 50-161 x A on EMS Lac.

(2) Grown together K12+

A ... F

3/20/51. 1 A 0
B 0
C 1 Lac+ Some very tiny papillae.
D ca 40 small clones, mostly lac+ or very small.

2 A 2+ 3-?
B 2 tiny+; papillae in background. → W1611
C 6+ 1-
~~B~~ E 1+
D 12+ 1-
F 6+ 1-

Heat-stable "gametes"? : K-12

April 17, 1951.

"B" "C"

Grow 68-161 and W-1177 together overnight.

AM. Heat shocks → "A". Into Permeasay 2:15 PM

1. A .5 mL (broth, could not be effectively sedimented)
2. B .5
3. C .5
4. B + A .5 each
5. C + A .5 each
6. B + C .5 each.
7. 1 + 2
8. 1 + 3

Heating inadequate. A was not sterile.

A. (1) No prototrophs, but lac− (W1177) only recovered.

B. }
C. } − gave prototrophs. W1177 contaminated. Expt. worthless.

$S^D \times S^R / S^S$ 840

April 20, 1951.

~~W1606~~ W1606 x 837B1 ($WH78\ A_2{}^R S^D$ x T(L) - Lac v $S^{R/S}$ Mal v...)

Plate on EMS Lac, EMS Lac + sm.

837B1. EMS Lac heavily treated

+ sm: faint clear background; 2 Lac +

control also gave Lac+ prototrophs. e.g. for crosses

Better stock needed

2 Lac − 1 Lac+ grew out. Test on Sm; streak out on EMB Mal.

April 21, 1951.
W1394 x M290

EMS lac. (see 833C).

Mostly lac+. 11 — (ca 1-2%)

lac − 2 of these are Mal+ — no EMS Mal (= 4 total).
2 " " Mal −
7 " Mal+.

✓ for S^V

---

20 lac+ : all lac+ S^R. (not V)
of lac − : 8 Mal+ S^R 4 Mal− S^S 1 Mal− S^S (paired Mal+ S^S).

Most of these are ~~~~ evidently not diploids.

~~Repeat in form~~

Balanced ~~bbt~~ lethals from H267?? 842

See ~~840~~ 837B.

(1). At least one lac "+" noted which gave lac − in cross back with sm. Restrials: Apparently pure lac +. Test single colonies against sm. → ~~Here~~ No lac − formed in 8 streaks.

(previous lac − may have been spattered!)

Repicks from boundary of sm inhibition and plate on EMB lac sm.

If these are heterolac+, UV must greatly increase rate of crossing over.

9 additional H257 lac+ } 5 S^V* 3 S^S 1 S^R

4 " H267 } 5 S^V 2 S^S 2 S^R.

(2) * 1 gave relatively few S^R Restricts + compare with H257. → not more stable!

(1) auxotrophs from H26 / 4v.

843

Repick auxotrophs of series B. Streak out and prepare for nutritional characterization

A S^R
B S^S
C S^{RS}

Final set:

							Kup:
1	H-	S^R	Mal-	✓	Mal-	S: R	
2	H-	S^R	Mal+		V	R	✓
3	H-	S^R	Mal V?		—	R	✓
4	H-	S^V	Mal V		V?	V	✓
5	TL-	S^R	Mal-		—	R	Lac->Mal+.
6	TL-	S^R	Mal+		+	R	✓
7	TL-	S^V	Mal V		V	V	✓

UV effect on M267

April 27, 1951.

Fresh D(Lac) suspension. Dilute 10^{-6}, irradiate 30 sec.

A. Control ● predominates. Plate .1 ml on EMB lac.

B. UV 30 s. ca 50-60% survival. ⊙ predominates

Isolate lac V from centers.

From one set on EMB lac Sm: 843-1 and 843 isolated as M − Lac V S^R.

Isolate lac V from centers of 40 cols. Test for auxotrophy, S^R.

	Lac	S	Nutrition		Lac	S	Nutrition
1	V	R RS		11	V	SR	
2	V	R		12	V	SR	+
3	V	R S		13	V	SR	
4	V	R S		14	V	R	+
5	V	R SRS	+	15	V	R	+
6	— ?	S		16	V	SR	
7	V	R RS	ns	17	V	SR	+
8	—	RS		18	V	R	+
9	—	S		19	V ?	S	+
10	—	S	+	20	V ?	S	

21	+	R		31	−?	R	+	
22	V	R	+	32	V	R ?		
23	V	R		33	O	O	+	sic
24	V	RS	+	34	V	R	+	
25	V	RS		35	V	RS		
26	−?	S		36	−	S		
27	V	R	+	37	V	RS ?	+	
28	V	RS		38	V	RS		
29	V	S		39	V	RS		
30	V	S		40	~~V~~	~~S~~	—	

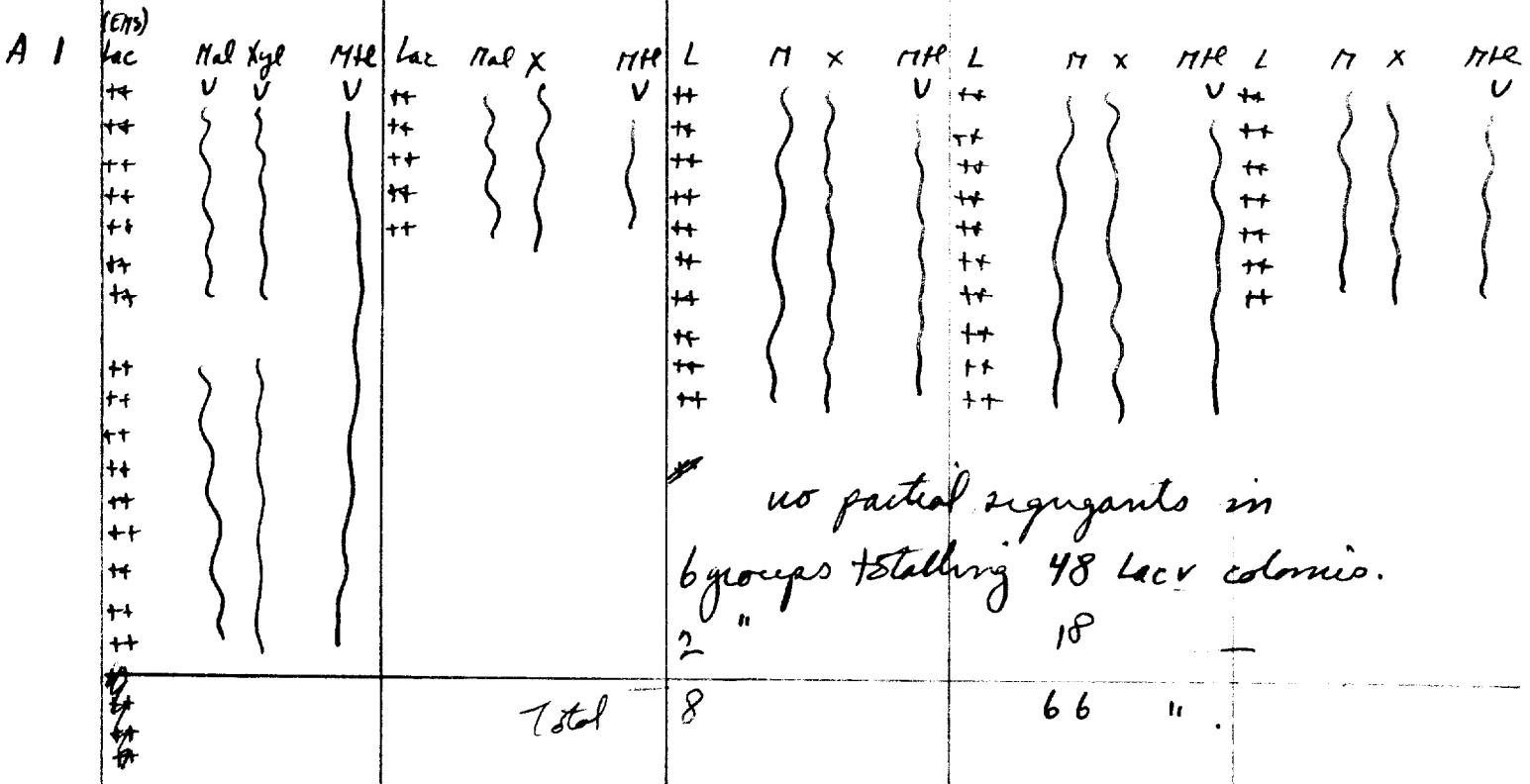
: nonparental — mixed
: parental

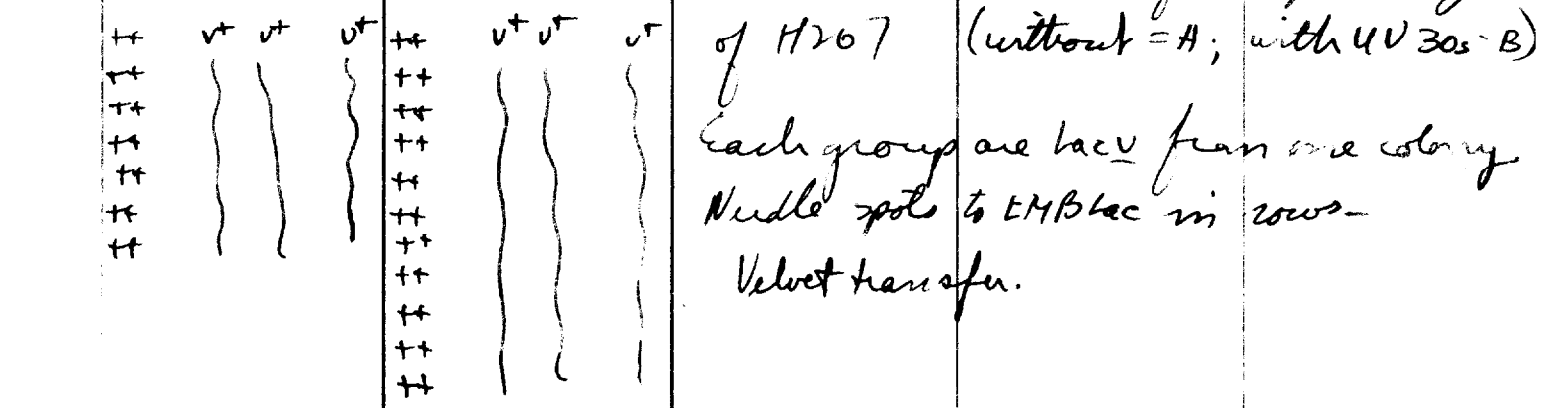
Most surviving hybrids are nonparental. 27 : (8−)

5 : most are changed!

UV sensitivity of UV-surviving hybrids?

(2) UV - diff. I - control





(2) UV effects

1.

B1-

	Lac S	Mal	X	MTL
(1)	−	V	+	−
	−	V	+	−
	−	V	+	−
	−	V	+	−
	−	V	+	−
	−	✓	V (11)	V
	−	−!	V (12)	−?
	−	V	V	V
	−	V	V	V
	−	V	V	V
	−	V	V	V
	−	V	V	V
	−	V	V	V

	Lac S	Mal	X	MTL
(2)	−	V+	+	+W
	−	+	+	+
	−	+	+	+
	−	+	+	+

	Lac S	Mal	X	MTL
(3)	+	+	−?	−?
	−	+	−	−
	−	+	−	−
	−	+	−	−
	−	+	−	−
	−	+	−	−
	−	+	−	−

	Lac S	Mal	X	MTL
	+	+	V	W
	+	+	V	V
	+	+	V	V
	+	+	V	V
	+	+	V	V
	+	+	V	V
	+	+	V	V
	+	+	V	V

	Lac S	Mal	X	MTL
	V	±	−	V (4)
		−	−	V
		−	V (5)	V
		−	−	V
		−	V	V
		−	V	V
		−	−	V
		−	−	V
		−	−	V
		−	+ − (6)	V?
		−	−	V
		−	−	V

B2.

+	+	V (7)	V	
+	+	V	V	
+	+	V	V	
+	+	V	V	
+	+	V	V	
+	+	− (8)	−	
+	+	−	−	
+	+	V	V	
+	+	−	−	

+	V	V (9)	V
+	V	V	V
+	−	− (10)	−
+	−	−	−
+	V	V	V
+	V	V	V
+	−	−	−
+	V	V	V
+	V	V	V
+	V	V	V

see 843 A. Velvet transfer.

Cultures should be checked to verify lac ±.

Check possible discrepancies within groups. Note incidence and uniformity of auxotrophy.

Restricts on EMB Lac

	LAC	D(o)	MAL	XYL	MTL
1	+	−	+	+	+
2	+	−	+	+	+
3	+	−	+	−	+
4	−	−	−	?	+
5	+		−	+	+
6	+		−	+	+
7	+	+	+ −	−	−
8	+	+	+	−	−
9	+	+	+ −	+	+
10	+	±	−	−	−
11	+	−	+	+	+
12	+	−	+	+	+

(1) – Isolation of amino-type diploids

Nutritional tests by decalomania (rose velvet)

		−M	−T	−L	+			
A	1	−	+	+	+	(B)M		1
	2	+	+	+	+			
	3	−	+	+	+	(B) M		2
	BM	−	+	+	+			
B	1	−	+	+	+	M	S^S	3
	2	−	+	+	+	M	S	4
	3	+	+	+	+			
	4	+	+	−	+	L	S^s	
	TLB,	+	+	−	+			
C	1	+	±	−	+	L	S^{RS}	5
	2	−	+		+	M	S^{RS}	
	3	−	+	±	+	ML	S^{RS}	
	4	+	+	+	+			
	5	+	+	−	+	L	S^{RS}	6
	6	+	+		+			
	7	+	+	±	+	L	S^{RS}	7
	8	+	+	+	+			
	9	+	±	+	+			
	10	−	+	+	+	M	S^{RS}	

Salmonella. – F, Hrate activity

May 4, 1951

		5/5 5PM	5/6	5/8
5PM	streaked on EMBlac			
A.	Tetrathionate Broth.			
(2 tubes) 1.	Filtrate .5 ml	no colonies on sh.	1 tube turbid	0 0
2.	K-12 .5 ml	Lac++		++
3.	Filtrate + K12 "	Lac++		++
4.	T2	~~Lac–~~		Lac–
B.	Permeassay 10 ml			
1.	Filtrate .5 ml	0	0	0
2.	" + Boorie serum 1 ml	0	0	0
3	Serum (sterility control	0	0	0
4.	+ Serum + loopful T2 (Toxicity control)	++++		
C.	SS-Agar.			
1-2	streaks K-12, T2	~~Turbid~~		SS- does not inhibit K-12 markedly
3	Plate Filtrate .1 ml	0	0	
~~4~~	Filtrate + K12.	"		
D	D(O)			
1.	T2 Filtrate	0	0	streaked EMB MR ⇒ MR– Xyl– only no recovery of salmonella
2	" + W~~677~~ 677	0	Numerous minute cols. + background + pinpoints	
3	T2 cells	~~+++~~ +++		
4	W677	0	Few pinpoints.	
E-F	EMB			
1	~~T2~~ Filtrate			
2	Filtrate + 677			
	~~" + K12 (Lac)~~			

E: MR, 1 1 col. → Lac– MR+, 2 T, 0 pop, Xyl

F: 0, 2 T, 0 pop.

presumably Salmonella (does not agg. in O Serum)

papillae Mal slow Xyl – not ~~Salmonella~~! but spont. mucoid

G = E 6/6 | 2+ + W677

Salmonella - E. coli combinations 844a.

ctd.

A. Grow W1577 ± T2F in .5ml Penassay overnight. Plate 10ml washed cells: all sterile

B. Incr. W1577 .1ml + ~~T2F~~ S414F .1ml on EMS Lac, D(0).

a. 1577 control } no colonies
b. S414F " }

c. ~~mixture~~ mixture: ca 2 very tiny "lac+" per plate. Replica to EMB lac

↓

only Lac+

D. T2F } + SW 435 → } prototrophs on D(0)
S414F } SW 414 → no prototrophs

No interaction of Salmonella with E coli could be [illegible]

May 8 ff. 1951.

58-161 × W1619 EMS.

	+	−
Lac	141	3
	111	3
	81	8
Mal	40	5

A Pick 60 Lac+ } ± EMS. Test by decal. transfer to Lac, Mal, MH, EMB Xyl

B 40 Mal+ }

C. EMS Mal plate. 8 Mal − 40+. Decal to EMS Lac Mal MH:

MH: 4− Mal+ Lac+

Lac: 3− 2 + MH+
1 − MH+

Mal: 7− 1
6 + +.

∴ of 47 tests by transfer, 34 Lac+ Mal+ MH+

4	+	+	−
1	−	−	+
2	−	+	+
6	+	−	+

No evidence of linkage of Mal, MH. of below & !!

B. Transfer test: 40 all Mal+
#6, 19, 37 Lac−
#12 MH−
Nme Xyl−

somewhat smeared.

	MH	MH	K	60	3 Lac +	9	5	6	
1	+	+	+	+	+	−	−	+	+
2	+	+	+			+	+	−	−
3	+	+	+	−	+	−	+	−	−
4	+	+	+	+	+	+	−	−	−
5	+	+	+	−	−	+	+	+	+
6	+	+	+	+	+	+	+	−	−
7	−	−	−	−	+	+	+	+	+
8	−	−	−	−	+	+	−	−	+
9	+	+	+	+	−	−	+	−	+
10	−	−	−	−	+	−	−	+	−

Lac and Mal –

Mal–	LAC	[redacted] GAL	XYL	MTL	[redacted] MAL	EMS	SM
1	+	+	+	+	–		
2	+	+	–	–			
3	+	+	+	+			
4	+	+	–	–			
5	+	+	–	–			
6	+	+	+	+			
7	+	+	+	+			
8	+	+	–	–			
9	+	+	+	+			
10	+	–	–	–			
11	+	+	+	+			

Lac –

1	–	+	+	+	+
2			–	–	–
3			–	–	+
4			+	+	+
5			+	+	+
6			+	+	+
7	+		–	–	–
8			+	+	+
9			–	–	+
10			–	+	+
11			–	–	–
12			+	+	+
13			+	+	+
14	+		+	+	+
15			–	–	+
16			+	+	+
17			?	–	+
18			+	+	+
19			+	+	+
20			+	+	+
21			+	+	+
22			+	+	+
23			–	–	–
24			+	+	+
25			+	+	+
26	+		–	–	–
27	+		+	+	+
28			+	–	+
29		+	–	–	+
30		+	+	+	+
31		+	+	+	+

A. Ratios: lac− : Lac+ (Frequency)

14 333 5.3%

Mal− Lac+

5 40 11%

Tentative Conclusion: Lac 3− 44+ Mal 7− 40+ Mtl 4− Mtl (Linkage)

B. Lac+ (60)

Mal	Mtl	
+	+	35
+	−	6
−	+	6
−	−	11
		60

Mal−: + 17 : 43

Mtl−: + 19 : 43

Mal+ (40)

3 Lac + + 3

37 Lac+ + − 1

+ + 36

C Lac−

+	+	20
+	−	6
−	+	0
−	−	5

Mal−

6 Mtl+

5 Mtl−

Some Mtl [illegible]

Attempts at λ⁻ diploids

H291 x W1027 on EMS lac.

A) 20 isolated to EMS lac. - Retest on W578 streak EMS lac) all lysogenic

B) 20 addnl. lac+ . Purify. all lysogenic.

May 15, ff. 1951.

A. W1606 x 843-6 EMS Lac 5 plates $S^R S^R / S^D$; Mal+, +

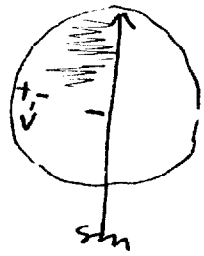
B " x 843-7 " " . S^{RS} / S^D ; Mal +, +

A. 13 Lac v. all S^R. #12 shows some apparent sensitivity to sm. Recheck.

Broz all to Pennassay for later v on S^D.

B. 20 Lac v. All S^S on EMS Lac. with sporadic S^R Lac+, Lac-.

On EMB Mal plates:



sm "bleaches" colonies in its vicinity.

after 36 hours, Mal+, and papilla are seen.

A W1177 x ~~1632~~ 1632 D(o), EMS Lac.
B W1619 x 1632

A. 1 ~~From D(o)~~

A R A 1

B 1.

Lac	Mal	Mtl				
+	+	+	6	15	4	2
+	+	−	4	1	~~1~~	1
+	−	+	2	6	~~2~~	1
+	−	−	1	1	5	4
−	+	+		1		3
−	+	−	1		1	
−	−	+			1	
−	−	−			10	15

A1

COLONY NUMBER	LAC		MTL	MAL	XYL	NaC Sm	T1	T6
1	−	✓	−	−	−	−	S	S
2	+	✓	+	−	+	0	S	R
3	−	✓	−	0 +	−	0	S	R
4	−	✓	−	−	−	−	S	S
5	+	✓	+	−	+	−	S	R
6	+	✓	−	−	−	−	S	R
7	+	✓	+	−	+	−	S	R
8	+	✓	−	−	−	−	S	R
9	+	✓	+	+	+	0	S	R
10	−	✓	−	−	−	0	S	R
11	(+ −) −		+	−	−	−	R	R
12	+	−	−	−	−	−	S	R
13	−	✓	−	−	−	0	S	R
14	+	+ −	−	−	−	−	S	R
15	−		−	−	−	0	S	R
16	−	✓	−	−	−	−	S	S
17	+	✓	−	−	−	−	S	R
18	+	✓	−	−	−	−	S	R
19	+	✓	−	−	−	0	S	R
20	+	✓	0	−	0	0	S	R
21	−	✓	−	+ ? −	−	0	S	R
22	0	−	0	−	0	0		

B1

1	+		+	+	+	0	S	R
2	+		+	+	+		S	R
3	+		0	0 +	0		S	R
4	+		0	0 +	+		S	R
5	+		+	+	+		S	R
6	−	✓	+	+	+		S	S
7	+ (−)	✓	+	−	−		S	S
8	+		−	−	−		S	R
9	+		+	+	+		S	R
10	+		+	0 +	+		S	R
11	+	✓	+	+	+		S	R
12	+	✓	+	+	+		S	R
13	+	✓	+	+	+		S	R
14	−	✓	+	+	+		S	R
15	0	+	+	0 +	0		S	R
16	+	✓	+	+	+		S	R
17	0	+	+	0 +	0		S	R
18	0	+	+	0 +	0		S	R
19	+	✓	+	+	+		S	R
20	+	✓	+	+	+		S	R
21	0	+	0	0 +	+		S	R
22	0	+	0	0 +	+		S	R
23	+	✓	[− (+) +]	+	+		S	R
24	+	✓	[− (+) +]	+	+		S	R
25	+	✓	+	+	+		S	R
26	0	+	+	0 +	0		S	R
27	+	✓	+	+	+		S	R
28	+	✓	+	+	+		S	R

848A Selected as Mal+ or Lac+.

	LAC	GAL	MTL	XYL	MAL	Sm
1	+	+	+	+	+	
2	−	−	−	−	−	R
3	+	+	+	+	+ (−) −	R
4	−	−	−	−	−	R
5	+	+	+	+	+	
6	+	+	+	+	+	R
7	+	+	+	+	+	
8	+	+	+	+	+	
9	+	+	−	−	+	
10	+	+	−	+	+	
11	+	+	−	+	+	
12	+	−	−	−	−	R
13	+	−	−	−	−	
14	+	−	−	−	−	R
15	+	−	− (+)+	+	+	
16	+	+	−	+	+	
17	+	−	−	−	−	R
18	+	−	+	−	−	
19	+	−	−	−	−	R
20	−	−	−	−	−	R
21	+	+	−	+	−	R
22	+	+	−	+	+	
23	−	−	−	−	−	R
24	+	+ (−)	−	−	−	R
25	+	−	−	−	−	R
26	+	−	−	+	−	R
27	+	+	−	−	+	
28	+	−	−	−	−	R
29		−	−	−	−	R
30	−	−	−	−	−	R
31	+	−	−	−	−	R
32	+	−	−	−	−	R
33	+	+	−	+	+	
34	+	+	−	+	+	
35	+	−	−	−	−	R
36	+	−	−	−	−	R
37	+	+	−	−	+	
38	+	−	−	−	−	R

848B. Selected as Lac −

#	MAL	LAC	MTL	GAL	XYL
1	+	−	−	+	+
2	+	−	−	+	+
3	+	−	−	+	+
4	+	−	−	+	+

Selected as Mtl −

#	MAL	LAC	MTL	GAL	XYL
1	+	+	−	+	−
2	+	+	−	+	−
3	+	+	−	+	−
4	+	+	−	+	−
5	+	+	−	−	+
6	+	+	−	+	+
7	+	+	−	+	+
8	+	+	−	+	+
9	+−	+	−	+	−
10	−	+	−	−	−
11	+	+	−	+	−

B2

COLONY NUMBER	LAC		MTL	MAL		XYL	Sm	T1	T6
1	−	✓	0	+		0	0	R	S
2	−	✓	0	+		0		R	S
3	0	+-	0	−		0		S	R
4	0	+	+	0	+	0		R	R
5	+	✓	+	−(+)		+		S	R
6	+	✓	0	+		0		S	R
7	0	+	0	0	−	+		S	R
8	0	−	0	+		0		R	S
9	0	−	0	+		0		R	S
10	+	✓	0	−		0		S	R
11	−	✓	+	0	+	0		R	S
12	−	✓	+	+		+		S	S
13	−	✓	0	0	+	+		S	S
14	+	✓	+	+		+		S	R
15	+	✓	+	+		+		S	S
16	0	+	0	−		0		S	R
17	+	✓	+	+		+		S	R
18	0	+	+	0	+	0		S	R
19	0	−	+	0	+	0		R	S
20	0	+	+	0	+	0		S	R
21	+	✓	+	+		+		S	R
22	+	✓	+	+		−		S	R
23	+	✓	+	+		+		S	R
24	+	✓	−	−		−		S	R
25	0	+	+	0	+	3		S	R
26	+	✓	−	+		+		S	R
27	+	✓	+	+		+		S	R
28	+	✓	+	0	−	0		S	R

A2

1	0	−	0	0	
2	−	−	−	−	R
3	−	−	+	−(+)	
4	−	−	+	−	
5	−	−	−	−	R
6	+	+	+	+	
7	−	−	−	−	R
8	+	+	+	+	
9	0	−	−	0	
10	+(−)	+	+	+(−)	
11	−	−	−	+	R
12	−	−	−	−	R
13	−	−	−	−	
14	−	−	−	−	
15	0	−	0	−(+)	
16	−	−	−	−	R
17	−	−	−	−	R
18	−	−	−	−	R
19	−	−	−	−	
20	−(+)	−	−	−	R
21	−	−	+	−	R
22	+	+	−	+	R
23	0	−		0	
24	0	−		0	
25	0	−	−	−	R

Segregation of H267.

849

May 14, 1951

(from 843A. Mal+)

	LAc	MAL	XYL	MTL	Sm	ENS	BAL	V_1	
1	−	+	−	−		−	−		T−L−
2	−		−	−					
3	−		+	+					T−L−
4	−		−	−					
5	−		+	+					
6	−		−	−					
7	−		+	+			+−		
8	−		+	+					
9	−		−	−					
10	−		−	−					
11	+		+−	+−	✗	✗	+−		
12	−	●	+	+					
13			+	−					L−
14			−	−					
15			+	+					
16	+	(−)	+−	+−	✗	●	+−		
17			−	−					
18			+	+					
19			+	+	R				
20			+	+					
21			−	−					
22	+	(−)	+	+−	✗	●	+−		
23			−	−					
24			+	+					
25			−	−					
26			+	+					
27			−	−					
28			+	+					
29			−	−					
30			− (+)	− (+)					
31			−	−	R				
32			+	+	R				
33			−	−					
34			+	+					
35			+	+					
36	+	(−)	+	+	✗	+	+−		
37	+	(−)	+	+	✗	+	+−		
38			−	−					

May 16, 1951

H267 is V_1 R Gal –

from 843 a Mal –

	LAC	MAL	XYL	MTL	Sm		Ems
~~1~~		~~– (+)~~	~~– (+)~~	~~– (+)~~			
2		–	–	–			
~~3~~		~~– (+)~~	~~– (+)~~	~~– (+)~~			
4		–	–	–			
5		–	–	–	R	R	
6		–	–	–			
7		–	–	–			
8		–	–	–			
9		–	–	–	R	R	
10		–	–	–	R	R	
11		–	–	–	+	R	
12		–	–	–		R	
13		–	–	–			
14		–	–	–	R	R	
15		–	–	–			
16		–	–	–	R	R	
17		–	–	–			
18		–	–	–			
~~19~~					~~R~~	~~R~~	
20		–	–	–			
21		–	–	–	R	R	
22		–	–	–	R	R	
23		–	–	–	R	R	
24		–	–	–	R	R	
25		–	–	–			
26		–	–	–			
27		–	–	–			

LAC	MAL	XYL	MTL	Sm		Ems
–	–	–	–	R	11	–
				S	13	

LAC	MAL	XYL	MTL	Sm	
–	+	+	+	S	14
–	+	+	+	R	2
–	+	–	–	S	16
–	+	–	–	R	1
–	+	+	–	S	1

Respreading microdomains
UV - diploids

d50

May 19, 1950?

H267 $.3 \times 10^{-6}$; .01 ml / plate

10^{10} AM Streak ① all plates.

A = control
B = uv 20

1. 10^{20} AM. 2. 11^{05} 3. 11^{30} 4 1^{15}

5 200 6 320

1	0	(8,0) (18,2) (8,2) (6,1)		B 15,10 6,7 ① pink.
2	45m	(16,2)		12
3	1:20	14		7,9
4	2:55	20		3,12
5	3:40	~~10~~ 40 pinks mostly 2m		4
6	5:00	46,24 ca 6		32 -, v, ① 2-13 / film

June 26, 1951.

Program: Streak out W-1 on EMB agar. Velvet Print Transfer to plain and to selective medium. When V_1^R clones are detected, take plain incubation for second streaking. Repeat until V_1^R colonies are obtained by indirect selection without exposure to specific selective agent.

P26. Streak out W-1.

A27. Restreak single colonies. Make test ~~transfer~~ Prints

A. Preliminary test (whole culture not recently reisolated: more likely to produce clones from V_1^R in vitro culture). A2:

Transfer somewhat smeared.

Tr. 1. 21 V_1^R } 6 congruences, presumably clones
Tr. 2. 13 V_1^R }

1. Pool pickings from homologous sites on plain transfer plate. Restreak. A28

B } Single colony platings. ~~Transfer~~ Print to P_1, T_1 1, 2
C }
D }

B. 6 clones. 19 V_1^R; 14 V_1^R

1 C 7 clones 30, 29.

D 1? clone. 12, 12

A29. Pool pickings ~~from~~ homologous sites and restreak on EMB Lac. save original cultures Transfer to plain, T1 agar.

2 A 15? clones; ca 25 mutants Restreak from 5 sites

B 13? " ca 40 " " " " (without pooling)

C 12 " all v thick streaks 18 muts.

D 1 21

A3-4. 14 clones; 37 mutants. Pool from 5 sites into ca. 1 ml.
Plate serially to further dilutions.
Suspend B, C experiment.
D transfer to plain; 2 T1. 7/3/51. Hold in refrig.

7/4/51. ~~Transfer D3~~
D3: 3-4 clones (?) ca 20 mutants.
Plate as #4:

A4. Transfer to series of T1 plates at 1, 3, 6" spreading

****

This procedure is obviously not working properly, presumably because of confusion between mutants within an inoculum to a plate, and those which occur during the growth of colonies on the plate. The samples from homologous sites are probably too small to have a reasonable probability of carrying an identified clone to the next inoculum.

The procedure should be modified as follows: A broth culture should be spread or streaked on plain agar, and permitted to grow for just a few generations. The thinly grown plate should then be printed to selective and plain agar, and the clones identified. The homologous site should then be inoculated to a small volume of broth, which is then restreaked in the same manner.

Lee Adelberg
Fed. Proc. 1952

Aberrant Linkage

June 26, 1951

A W1177 × 1632 EMS Lac.

B W1635 × 1632

Count from plates: (Lac)

A.	+	−		B	+	−
	17	30			187	18
	2	27				
	13	33				
	14	26				
	46	116	162			

"B" B Lac+ } selections
"C" B Lac− }

Picks A "at random" to EMS Lac. B 100+ 48−

Paint ~~Transfer~~ to EMS Lac, Mal, MTL, sm; T1 for sensitivity

	Lac	Mal	S	MTL	T1	Gal	Xyl
A. 1	−	−	R	−	S	−	−
2	+	−	R	−	S	−	−
3	−	−	R	−	R	−	−
4	−	−	R	−	S	−	−
5	+	−	R	−	S	−	−
6	−	−	R	−	S	−	−
7	−	−	R	−	R	−	−
8	−	−	R	−	R	−	−
9	−	−	R	−	S	−	−
10	−	−	R	−	R	−	−
11	−	−	R	−	S	−	−
12	−	−	R	−	S	−	−
13	−	−	R	−	R	−	−
14	−	−	R	−	S	−	−
15	−	−	R	−	R	−	−
16	−	−	R	−	R	−	−
17	−	−	R	−	R	−	−
18	+	+	S	+	S	+	+
19	−	−	R	−	S	−	−
20	−	−	R	−	S	−	−
21	+	+	R	+	S	+	+
22	−	−	R	−	S	−	−
23	+	−	R	−	S	−	−
24	+	−	R	−	S	−	−
25	+	+	S	+	S	+	+
26	−	−	R	−	S	−	−
27	+	+	S	+	S	+	+
28	−	−	R	−	S	+	−
29	+	−	R	−	S	−	−
30	+	−	R	−	S	−	−

A

	Lac	Mal	S	Mtl	T1	Gal	Xyl
31	+	−	R	−	S	−	−
32	+	+	S	+	S	+	+
3	−	+	R	−	R	−	−
4	−	−	R	−	S	−	−
5	+	−	S	−	S	−	−
6	+	−	R	−	S	−	+
7	−	−	R	−	R	−	+
8	+	−	R	−	S	−	−
9	−	−	R	−	S	−	−
40	+	−	R	−	S	−	−
41	+	+	R	−	S	−	−
2	−	+	R	−	S	−	−
3	−	+	R	−	S	−	−
4	−	−	R	+	R	−	+
5	−	−	R	−	R	−	−
6	−	−	R	−	R	−	−
7	−	−	R	−	S	−	−
8	−	−	R	−	S	−	−
9	+	−	R	−	S	−	−
50	+	+	S	+	S	+	+
51	+	−	R	+	S	−	−
2	−	−	R	−	S	−	−
3	+	+	S	+	S	+	+
4	+	−	R	−	S	−	−
5	+	+	S	+	S	+	+
6	−	−	R	−	S	−	−
7	−	+	S	+	S	−	+
8	−	−	R	−	S	−	−
9	−	−	R	−	S	−	−
60	+	−	R	−	R	−	−
61	−	−	R	−	R	−	−
2	−	−	R	−	S	−	−
3	+	−	R	+	R	−	+
4	+	+	R	+	S	−	+
5	+	+	S	+	S	+	+
6	−	−	R	−	S	−	−
7	−	−	R	−	S	−	−
8	−	−	S	−	R	+	−
9	+	+	S	+	S	+	+
70	−	−	R	−	S	−	−
71	+						
2	+						
3	+						
4	+						
5	+						
6	+						

	Lac	Mal	S	MR	TI	Gal	Xyl
71	+	−	R	−	S	−	−
72	+	+	S	+	R	+	+
3	+	−	R	−	S	+	−
4	+	−	S	+	S	+	+
5	+	−	R	−	R	−	−
6	+	+	S	−	R	−	−
7	+	+	S	+	S	+	+
8	−	−	R	−	R	−	−
9	+	+	S	+	S	+	+
80	+	−	R	−	S	−	−
81	+	−	R	−	S	−	−
2	−	−	R	−	S	−	−
3	+	+	S	+	S	+	+
4	+	+	S	+	S	+	+
5	−	−	R	+	R	−	+
6	−	−	S	−	S	−	−
7	−	−	R	−	R	−	−
8	−+	−+	R	−+		−	−
9	−	−	R	−		−	−
90	+	+	R	−		+	+
11	−	+		−	R	−	−
2	−	−		−	S	−	−
3	+	+	S	+		+	+
4	+	+	S	−		+	−
5	+	+	S	+		+	+
6	+	−	R	−		+	−
7	+	−	R	−		−	−
8	−	−	R	−	R	−	−
94	+	+	S	−		+	−
100	+	+	S	+		+	+

=Lac+ selectants

852B

B2

	lac	Mal	Mtl	S	TI	Xyl	Gal
1	+	(−)	+	R		+	+
2		(−)	+	R		+	
3		(	+			+	
4		+	+	R		(−)	
5		+	+	R		+	
6		+	+	R		—	
7		+	—			—	
8		(−)	+	R		+	
9		+	+			+	
10		(−)	—	R		—	
11		—	—	R		—	
12		(+)	+	R	R	+	
13		+	+		R	+	
14		+				+	
15		+	—			+	
16		+				+	
17		+	—			—	
18		+	—			+	
19		+				+	
20		—	—	R		+	
21		+	+			+	
22		+	+				
23		+	+				
24		—	—				
25		(+)	+	R			
26		+	+				
27		+	+			=	
28		+	+			+	
29		+	+		R		
30		+	+			+	
31		+	+			—	
32		+	+	R		+	
33		+	+			—	
34		—	+	R		+	
35		+	+			+	
36		+	+	R		+	
37		+	+			+	
38		+	+			+	
39		+	+			+	
40		+	(+)	R		—	
41		+	+			—	
42		+	+			+	
43		+	+			+	
44		+	—			+	
45		+	+			+	
46		+				+	
47		+				+	
48		+			R	+	
49		+			R	+	
50		+	+	R		+	

B1

	lac	Mal	Mtl	S	TI	X	Gal
51	+	+	+		R	+	+
52		+	+			+	
53		+	+			+	
54		+	+			+	
55		—	+	R		+	
56		+	+	R		+	
57		+	+			+	
58		+	+			+	
59		+	+			+	
60		+	+			+	
61		+	+			+	
62		+	+	R		+	
63		+	+	R		+	
64		+	+			—	
65		+	+	R		—	
66		+	+			+	
67		+	+			+	
68		+	+			+	
69		+	+			+	
70		+	+	R		+	
71		—	+			+	
72		+	+			+	
73		+	+			+	
74		+	+			+	
75		+	—			—	
76		+	+	R		+	
77		+	+			+	
78		+	—			+	
79		+	+			+	
80		—	+	R	R	+	
81		+	+	S		+	
82		+	—	S		—	
83		+	+	S		+	
84		+	+	R	R	+	
85		—	—	S		—	
86		+	+	S		+	
87		+	+	R		+	
88		+	—	S		—	
89		+	+	R		+	
90		+	+	S		+	
91		+	+			+	
92		+	+			+	
93		—	+			+	
94		+	+	R		+	
95		+	+	R		+	
96		+	—			+	
97		+	—			—	
98		+	+			+	
99		+	+			+	
100		+	(+)			+	

Repeat M- segregation
W1111 x W1632.

July 17, 1951.

Cross on D: MBB, sm) gar.
Test "protoplasts" by plate transfer to D: BB, sm)
Tests very clear.

Plate	M-	Total
1	8	101
2	1	27
3	4	84
4	2	85
5	5	87
	20	384

Also pick 20 at random for M+. Purify on EMB Lac.
Pick to EMB.

M-	7 Lac+	13 Lac -	
M+	3 +	11 -	

not significantly different.
M- grew very well on plating media.

Isolates: M- all grew on BMS, not BB, ✓.
M+ " " " ✓

on EMB Maltose	M-	3 Mal+ (absolute)	others -
	M+		all Mal -

Selection, possible lac −

852 C

	Lac	Mal	S	Mtl	TI	Gal	Xyl
1	−	+		−		+	
2	+	+		+	R	+	
3	−	+		+	R	+	
4	+	+	R	+		+	
5	−	+	R	+	R	+	
6	−	+		+		+	
7	−	+	R	+	R	+	
8	−	+		+		+	
9	−	+		+		+	
10	−	+		+		+	
11	−	+		+		+	
2	+	+		+		+	
3	−	+		−		+	
4	+	+	R	+		+	
5	−	+		+	R	+	
6	+	−	R	−	R	+	
7	−	−	R	+		+	
8	−	+		+		−	
9	−	+		+		+	
20	−	+		+		+	
21	−	+		+	R	+	
2	+	+	R	+	R	+	
3	−	+		−	R	+	
4	+	+	R	+		+	
5	+	+		+		+	
6	−	−	R	−	R	+	
7	−	+	R	+		+	
8	−	+		+		+	
9	−	+		+		+	
30	+	+	R	+		+	
31	−	−		−		+	
2	+	+		+		+	
3	−	+		+		+	
4	+	+		+		+	
5	−	+	R	−		+	
6	−	+		+		+	
7	+	+	R	+		+	
8	−	+		+		+	
9	+	+	R	+		+	
40	−	+		+	R	+	
41	+	+	R	+		+	
2	+	+		+	R	+	
3	−	+		+	R	+	
4	+	+		+	R	+	
5	−	+		+		+	
6	+	+	R	+		+	
7	−	+	R	+	R	+	
48	+	+		+	R	+	

Counted 852C Lac-mly.

852C'

Mal	S	MR	TI
+	S	−	S
+	S	+	R
+	R	+	R
+	S	+	S
+	S	+	S
+	S	+	S
+	S	+	S
+	S	−	S
+	S	+	R
−	R	+	S
−	R	−	R
−	S	−	S
+	S	+	S
+	R	−	S
+	S	+	S
+	S	+	S
+	S	+	S
+	S	+	R
+	S	+	R

Selected isolations

852
D

June 29, 1951.

Cross B EM5 Lac. Print 3 plates to (173 Lac-, 174, 174) for specific selections. #1, 2 rather smeared (use back support wipes)

#3 OK

Pick all Mal- (Lac-) and Mtl- from # 3 plates (including overlaps).

Comm. Lac: 10 - 163 + / 173

Mal ~~29~~ 30 } 7? overlaps

Mtl 21 }

Lac-Mtl 1? ov.
Lac-Mal 0?

S^R : 27 : 8 Mal+
19 Mal-

From the interaction of the - Lac, Mtl, Mal selections, in cross with unselected tester of B, we should note recombination linkage which is maybe interesting.

Summary

Lac	Mal	S	Mtl	T1		A	B		Adj
—	+	R	+	R			/	1	1
—	+	R	+	S	1	1 /			
—	+	R	—	R	3	2 //			
—	+	R	—	S	4	3 ///	/	1	1
—	+	S	+	R			////	4	3
—	+	S	+	S			卌 ///	7	6
—	+	S	—	R					
—	+	S	—	S			//	2	2
—	—	R	+	R	3	2 //			
—	—	R	+	S			/	1	1
—	—	R	—	R	22	12 卌 卌 //// /	/	1	1
—	—	R	—	S	36	26 卌 卌 卌 卌 //// /		/	15
—	—	S	+	R					
—	—	S	+	S					
—	—	S	—	R	1	1 /			
—	—	S	—	S	2	1 /	/		
+	+	R	+	R			//	2	2
+	+	R	+	S	1	2 //	卌 卌 卌 /	16 13	13
+	+	R	—	R					
+	+	R	—	S	1	2 //			
+	+	S	+	R	1	1 /	卌	5	4 5
+	+	S	+	S	9	16 卌 卌 卌 /	卌 卌 //// 卌 卌 卌		5
+	+	S	—	R	1	1 /			
+	+	S	—	S	1	2 //	卌 ///	8	7
+	—	R	+	R	1	1 /	/	1	
+	—	R	+	S	1	1 /	卌 /	6	5
+	—	R	—	R	1	1 /			
+	—	R	—	S	11	19 卌 卌 卌 ////	///	3	
+	—	S	+	R					
+	—	S	+	S	X	1 /	//	2	
+	—	S	—	R					
+	—	S	—	S	1	1 /	///	3	

A

卌 卌 卌 卌 ////

852

Adjusted (to 100)

Summary used in CSH 1951

	Lac	Mal	S	MH	T1		
	−	+	R	+	R		1
	−	+	R	+	S	1	
	−	+	R	−	R	3	
	−	+	R	−	S	4	1
	−	+	S	+	R		3
	−	+	S	+	S		6
	−	+	S	−	R		
	−	+	S	−	S		2
	−	−	R	+	R	3	
	−	−	R	+	S		1
*	−	−	R	−	R	22	1
	−	−	R	−	S	36	
	−	−	S	+	R		
	−	−	S	+	S		
	−	−	S	−	R	1	
	−	−	S	−	S	2	
	+	+	R	+	R		2
	+	+	R	+	S	1	13
	+	+	R	−	R		
	+	+	R	−	S	1	
	+	+	S	+	R	1	4
*	+	+	S	+	S	9	45
	+	+	S	−	R	1	
	+	+	S	−	S		7
	+	−	R	+	R	1	1
	+	−	R	+	S	1	5
	+	−	R	−	R	1	
	+	−	R	−	S	11	3
	+	−	S	+	R		
	+	−	S	+	S		2
	+	−	S	−	R		
	+	−	S	−	S	1	3
A	%+S	28	21	15	17	67	
B	"	85	84	72	73	88	

Segregation of M−

July 3, 1951.

A W1177 × 1632
B W1635 × 1632

Cross on 1. D(0) + BMB$_1$ sm for segregation of M+/−

July 7. B' B, grown together 1.5 hours 2. EMS Lac for linkage data. on D(0)

July 3: A: numerous colonies on BxB, sm.
B 1 " 6 plates!

Repeat B, B'. July 6.

A. Test, by velvet transfer, M segregation from A.
Not all scoreable.

3. 83/83 M+
2. 7/48 M− } 12 M− ✓
1. 5/62 M− }

also recover 12 M+ ✓) 14 ± —.

B' 9 colonies. Test on +, − M.

all are M+. ∴ M is + [illegible] Th
TL
−
S −.

Suggestion: In "B", is Lac linked to TL?
Test by crossing BM S^R × TL s^s
on TL sm agar
and studying segregation
of TL, Lac.

(see over)

Lac character of M+, M- selections

B'	M+	6 Lac+	2 Lac-
A	M+	12: 3 Lac+	9 Lac-
	M-	11: 2 Lac+	9 Lac-

Linkage of Lac to M? Not supported by these data.

Lac- mutants in W1634

July 6, 1951.

W1634 (Cellobiose-fermenter). Irradiated 8 secs on EMB Lac
~~20~~ x 50 = ~~1000~~ colonies.
Some sectorials undoubtedly ignored. Many morphological sectorials.

Spot Lac- on EMB Glu for lac- tester pairing.
By ~~Lanston~~ Hotz:
13 lac- all are sucrose cellobiose xylose galactose maltose and glucose positive

Save 1. lac- 854 1

July 12, 1951.

20 x 50 = 1000 on EMB glucose No definite mutants
15 x ~~40~~ = 600 " " " " " "

7/16. Check Vaughn's cultures 776-835, 837, 839, 840, 841 1-5 s.o., EMB glu, cell, suc, lac

7/17. all are ++ or +. #1, 3 probably most generally suitable
= 835 (Vaughn 129) = W 1647
= 839 (Vaughn 168) = W 1648

7/17. a 1634
b 1647 — uv resistant
c 1648

854-(2-4)

a. ~~3 possible lac-~~ from 12 x 400 = ca 5000 ✓
c 1 " " " 8 x 100 = 800. ✓ (+ spread very well!)
= 854-5

All 4 mutants grow rather poorly. Are negative on galactose, glucose, xylose, mannitol, maltose, lactose, sucrose and cellobiose

see over

(1648??)

W1647. UV

1 Glu- mut on 5 plates, variably recorded

? Lac- or ±

(1). Glu- Cb- Suc + Mal-Lac-
Xyl+ Gal+

Designation of W1647 almost certainly correct, but conceivably was substituted for W1648. Designate 1647a for immediate source of Glu- mutant (W1677)

Penicillin effects on K12

July 8, 1951.

24 hr. K12 culture .5/10 ml fresh Penassay + penicillin as indicated.

1. No penicillin
2. 1 unit/ml
3. 5 units/ml

* P9. Fifth 1, then 2; 3 then W1177 (4) as control.

	1	2	3	4
A. Plate .1 ml on EMB lac	↓st	~~↓st~~ 1+	1 col. lac+ 2 minute lac−	
B. " .1 ml with W1177 on ~~E~~ EMS lac				
C. Broc. 10 ml Penassay	↓st	↓st	Turbid	Turbid
D. " " + W1177.	st	1 minute lac−, ?	stuck out	

AA etc. same but N10., (~~W677~~)

probable lakes

	1	2	3
AA	++	++	
BB (W671)	++	++	
CC	2 lac+		st.
DD	7 lac+	7 lac+	st

Possible "pobbyglis" ? in 3B, 2D
Residuals in D: (0)

This experiment u.v.:
Sealing gasket on filter
used for 1 and 2

With 1–5 units, no morphological effects were noted.

Penicillin effects.

July 11, 1951.

P12. P12.

K12. 1 ml + A Penicillin in Penassay tubes ~~++++~~
B

1	A	0	++	
2	B	50	++	Normal size + motility.
3	C	50	++	many filaments. some minute granules
4.	D	120 u/ml	±	inhibited growth.

Filter 1, 2, 3 through separate 8-9 lb Mandler filters. Pass turbid (ca 10^8) W1177 broth culture each bulk sterility. Plate this filtrate on EMB Lac (= E) and inoculate 1 ml into Penassay (= F).

		1	2	3
A.	Inoculate filtrates 1 ml, Penassay	—	—	K12 only ++
B	" " " " + W1177. Plate after 24.	0	0	~~++~~ K12
C	Plate " .1 " EMB Lac	0	0	0
D	" " EMB Lac + W1177	0	0	0
E		0	0	0
F		—	K12 only ++	K12 only ++

A few particles per ml ~~survive~~ survive filtration, if penicillin has been added. No unique genetic activity is noted. However, higher titres of FA, and heat-inactivation are needed to parallel Salmonella findings. W1177 control satisfactory.

Also plate filtrates into serum agar (+ penicillin ?)

F: streak out on EMB lac + or − see to find W1177.

75u Penicillin K12 1% inoculum. 4½. 37°
" R.T.

A. Sediment and filter supernatant. 14 under 81b filter
B. Follow with untreated W1177 >1% (.01/ml) control.

---

1. Plate A on serum agar; 0.1ml
2 " " nutrient " "
3 " " EMBlac 0.1 "
4 Incr 1ml samples into Penassay.
5 " " " + .5ml W1177
6 Plate B on EMB Lac sen
7 Incr 1ml Penassay.

Conclusion: Filter not satisfactory. 6 showed ca 50 lac-
1,2,3 each showed ca 400 500 (lac+). 4 all turbid.

Refilter unirradiated suspension, 141b Mandler hypothesis all sterile. No protoplasts from 5, 7

Conclusion: Penicillin may result in slight augmentation of lethality of K-12, but no consistent effect noted. No genetic effects whatever noted.

5. showed minute colonies on EMS lac after 3-5 days

Pick and streak out on EMB ~~S~~ lac, D/O). – 3 days: no growth on both

[Probably T-L+ revertants growing on carryover]. not + on former.

8 hour W1649 + 855b Inc'd in Primary.

9 " W1632 " " " "

Wash + plate heavily EMS lac

No colonies

Conclusion: No evidence of genetic activity in penicillin treatments of K12.

TL- +-1 in linkage orientation

- F2 Crosses

July 6, 1951.

Cross D TLB, sm W677 x

A W1367 No yield ~~Sm 88 T~~
B W1302 No yield.
C W1490 2 colonies / 2 plates — 1 grew out: B_1 —

D W1368 x W677

D' (do., grown together 6-8 hours).

7/17 D: Transfer plates to D (sm TLB$_1$);; EMS Mal sm TB$_1$.
Only 1 out of about 60 grown on EMB B$_1$.

D' ~~Most grew on EMB B$_1$, few on TLB$_1$ (results mix up of plates)~~

↓ Streak out auxotrophs on EMB Lac to purify for further characterization. 8 saved.

#1 ~ T-L-B$_1$ - S^R Lac- W1649.

~ all 8 are T-L-(B$_1$-) S^R Lac- Mal- Xyl- Mtl- Sal- (S^R Mutant ??)

7/19/51. E. W1649 x ~~W1368~~ W1632 on EMS Lac, Mal, Mtl.

EMS Lac 95%+
Mal " +
Mtl 90%+.

∴ Haploid strict recombinants also give aberrant linkage results.

Alternate page

Material from 853 W1635 x W1632.
852

EMSMR 41- 359 ~~total~~

Strokes from D10) to EMS Lac.
Also collect Lac- from EMS Lac plates

Repurify all Lac-.

Plate count
EMS Lac. 18 - : 187+
MR 9 - ~~55~~ 64 ~~total~~ 160
From D10) strokes to EMS Lac 13/4 - : 245 total

Read prints from Lac+ and from Lac - separately.

a. Transfer all Lac+ to MR, Mal.

b. Transfer 50 Lac+ also to Xyl, T6, T1

c. Transfer 28 Lac- to MR Mal T6 T1 Xyl

3/16/52 Conclusions?: Lac, Mal are independent

LAC +	Lac	Mal	MH	EMB Xyl	S	T1	T6
1			−	−		R	R
2			−	−			R
3							R
4							R
5		−		±	R −		R
6			−	−	R −	R	R
7							R
8							R
9		−	−	±	R −		R
10			−	−			R
11							R
12							R
13					R +		R
14							R
15			−				R
16			−	−			R
17					R −		R
18							R
19							R
20							R
21						R	R
22	−						
23	−	−	−(+)	±	R −		
24				−			R
25		−		−			R
26		−	−				
27							
28			−				
29							
30			−				
31			−	−			
32							
33		−	−	−	R −		
34							
35							
36		−	−	−	R −		
37							
38							
39							
40				±	R −		
41			±		R −		
42							
43		±	−	−(+)?	R −		
44							
45				−			
46			−				
47							
48							
49							
50							

(1)

✓Malate EMB

only five Lac−V6 crossovers.

ALL EXCEPT #13 MAL−

	LAC	MAL	MTL	EMB XYL	EMS LAC S	T_1	T_6
51							
52			-				
53		-	-	±	R -	R :	R
54					R (s)-	R :	s ✓
55	-	±				~~R~~	
56							
57							
58							
59							
60							
61					R ✓+		
62	+	-	?	-			
63		-			R -		
64							
65							
66		-				R :	R
67		-	-	-	R -		
68							
69			-	-			
70					R ✓+		
71			-	-			
72							
73							
74				-		R mvc	
75	-		+ MUCOID			R ~~lac -~~	
76		-	-	-	R -		
77			-	-			
78			-				
79						~~R~~	
80	-						
81	?					R :	R
82							
83		-	-	-	R -		
84	+						
85			-	-+			
86						R :	R
87							
88							
89							
90			-	-	R -		
91			-				
92							
93							
94		-	-	-			
95	-		-	-			
96							
97							
98							
99							
100			-		R -		

3

	LAC	MAL	MTL	XyL	EMB S	EMS LAC T1	T6
101							
102					R +		
103	−					(R)	
104							
105							
106							
107							
108							
109				+			
110							
111							
112							
113							
114			−			(Mucoid R)	
115							
116							
117							
118			−	−			
119							
120							
121							
122							
123					R +		
124							
125							
126							
127			−+	−+			
128							
129							
130							
131		−					
132							
133			−				
134							
135		−	−	−	R −		
136		−	−	−	R −		
137							
138							
139							
140							
141							
142						R .. R	
143							
144							
145							
146	−						
147		−					
148				±			
149							
150					R +	R . R	

4

	LAC	MAL	MTL	XYL	CMS LAC S	T_1	T_6
101							
152		−	∓	±			
153							
154			∓	−			
155						R	R
156			∓				
157							
158		−+			R^+		
159							
160		−					
161			−	−	R^+		
162							
163						R^{Lac-}	
164							
165			−	−+			
166							
167							
168	−+						
169							
170	−	−+				R^{Lac-}	
171			−	−		R	R
172			−	−			
173							
174							
175			−+	−+			
176							
177	●						
178							
179							
180			−	−+			
181							
182							
183							
184							
185		+	−	∓			
186							
187	∓			∓	R^{+Lac-}		
188			−	−			
189							
190						R	R
191							
192							
193		−	−	−	R^-		
194						R	S
195							
196							
197							
198	∓						
199							
200			−	−			

	LAC	MAL	MTL	XYL	EMS LAC S	T_1	T_6
201							
202		∓			R⁻		
203			−	∓			
204							
205	− ✓					R	
206					R⁻		
207							
208							
209			−	∓			
210							
211			−	−			
212		−	−	−			
213							
214							
215						R Lac−	
216			−	−			
217	∓ ✓						
218							
219					R⁻		
220							
221							
222					R⁻	R	R
223		∓			R⁻		
224							
225							
226							
227						R Lac+	
228							
229		−	∓		R⁻	R	R
230					R⁺		
231					R⁺		
232							
233		−			R⁻		
234							
235							
236							
237			−				
238					R⁺		
239		−	−	− (+)	R⁺		
240	∓ ✓				R⁻	R Lac−	
241							
242							
243					R⁻	R Lac−	
244							
245							
246							
247							
248							
249							
250							

	LAC	MAL	MTL	XYL	S	T_1	T_6	
1						R	S	37
2			–	–	R MAL+		S	
3						R	S	
4						R	S	
5							S	
6							R	42
7						R	S	
8							S	
9							S	
10							S	
11						R R	S	4
12						R	S	
13							S	
14		–	–	–			S	
15		–					S	
16		–	–	–	R –		S	52
17							S	
18							R	
19						R	S	
20			–	–			S S	6
21					R MAL+		R	
22						R	S	
23						R	R	
24						R	S	
25							S	
26		–	–	–	R –		S	
27			–	–			S	
28							R	

Lac+

Lac –

July 10, 1951

P10

1. Inoc. Isolate U-1 to Permassay., NSA slant

~~Spread~~ Spread .1 (a) and .01 (b) ml on E4B lac. Assay .1 ml i T1.
9:30 A12

~~4P~~ 4P Print to I T1 agar.

A 13: Read:

a. 46 clones; 31 singles; (1 plate); 2 1/2 singles (second plate).

b. 10 clones; 6 singles (on one plate only)

Pick from b sites to rush broth.
Spread .001 ml; streak. Incub 4 P11 - 10:30 AM
Print A 10:00 AM. 13.

~~AM~~ .001 ml: 15 clones 10 singles (1)
5 " (2)

(3) Pick 5 sites.

- Assays of successive isolates. 1×10^{-6} .1 ml 91,5 mic.
2.5×10^{-6} .1 ml ca 500.
3×10^{-6} .01 ml ca 1000.
4×10^{-6} 38/582

N15 Plate at various dilutions
Shows incubation:
Print to T1.

A streak: 1 clone is nearly confluent portion. Restreak quickly, also testing and compare cross-streak with T1, as against random isolated clones. Pick 1 clone = 1

B 10^{-5} Semi confluent. 9 clones; no singles.
Pick 3 clones = 2-4 streak out
pool to Permassay.

See over:

1. Random tests on streaked colonies. / T1.

1.	10 S.	0 R
2.	"	"
3.	25 S	5 R.
4.	8 S	2 R

∴ successful isolation of V_1^R by indirect selection

2. Dilute 858-4 10^{-6} plate. 1 ml. 3 plates.

#2 smeared - too wet

Permit to T1 after 9 hours (pinpoint colonies)

1.	12 V_1^R	301	total
3.	16	261	"
	38 /	562	

Pick best isolated homologous colonies. Test against T1 and streak to purify.

✓ each of 4 V_1^R

2 random V_1^S.

Store single colony as 858-5 ... grow + transfer broth to test stability

Note In final plating a few plaques of contaminant phage (identified as T1) were found. However, no evidence of T1 was found in the broth series, or in selection line. It seems unlikely that this could interfere with achieved result. However, see 859 for repetition of general expt.

Indirect Selection: W-112 / T1.

July 17, 1951.

Initiate single colony broth cultures 11 AM. = 859-1

P17 Plate EMB lac, Incubate ca 5 hours. Print to T1 plates .01 ml

5 clones; Total: 16; 12

N18. Picks clone sites to Penassay.

3-8 PM. $^{A}10^{-2}$, 10^{-3}, 10^{-4} ml plated on EMB lac

8 PM Print to T1 plates

A19. A. 23 clones, 1-3 singles.

B 1 clone 2 singles

C 1 clone 1 single.

Pool clones from B and C to Penassay. 859-3

N19. 2 hour tube probably ca 3×10^8) plated out on EMB lac

12 N19 — 10^{-3} A, 10^{-4} B, 10^{-5} C

q^{30} A20. C: (ca 600 cells) No V_1^R

B: 2 clones; 1, 0 singles

A: 16 clones; 2, 0 singles.

Pool 2 sites from B and 2 from A to Penassay 859-4.

10 A21. 10^{-6} dilution: 3 plates. After 4 hours, make replica transfer.

A

B 12 mutants; 793 Total

C

Streak out 4 of the V_1^R.

Test against T1. V_1^R ✓

Place associated V_1^R on slant.

Initiate serial transfers in broth, 5A, B, ...

858 and 859. Each transfused serially through 10 5ml tubes Penassay with loopful inocula.

8/3/51. Plate out on EMB glu. Replica to EMBLac. T1. all colonies are V_1^R. W1485 control lysed on T1 plates.

Total counts:

858	859.
290	131
277	150
269	166
836	447

Replica Efficiency

July 24, 1951.

A. Compare fresh and 4 day broth for clones.

1. Dilute 58-161 in EMB (4 ml) 9 AM.
2. Spread " " " " 315 PM.

325. Replica to T1 agar, pairs.

B. Dilute 58-161 10^{-5} Spread .1 ml, let dry.

① Replica to series of plain agar.

② Assay 10^{-7} ml for count.

③ As ①, but 10^{-5} ml originally

			(Replica removed) ↓				
(B) 2:	10^{-7}	0	1	2	3	[illegible]	
		99	91	7	1		
1	10^{-6}	(1000)	± 10^3	42	19	12	10	6
3	10^{-5}	(10,000)	± ca 10^4	331	171	108	109	87

∴ About 10-25% of initial cells are removed.

5-10% deposited on first replica, with indicated "decay" thereafter.

8/30/61 — see experiments with E. Klein on efficiency of colony transfer,,

Singlets and clones
Replica plating

July 25, 1951.

2¹⁵ P.M. W112 (859-1)

1a. 1 ml + T1 assay 190
b. 02 ml + T1 assay. 90 ~~78~~

2a ~~.02 ml~~ .1 ml Replica to 2 T1 plates immediately
b .02 " " " "

3a .1 " " after incubation 2¹⁵ -
b .02 " "

2a	15	4	0	) no sign of clones.
2b	2	0	6	
3a	73	65	53	many clones.
3b	14	10	5	
2bb	11	8	4,	2nd Replica suis from 2b after incubation.

Use 2bb and 3b for published figures of replicated clones

7/27. Nonclonal replicas.
c1 .1 ml W-1 culture transfer (EMB) as initial
c2 1 ml (sed + conc to .1 ml ⇒ 10:1)

Primary segregation ratio: H226.

July 28, 1951.

Use technique of (respreading microcolony)

2-wk old culture H226. 10^{-6} dil.

Control: .1 ml spread over plate; .01 ml in line

Expt'l. .01 ml in line 11:30 AM. Respread after 7½ hours

mostly segregated for N.S. 7 PM.

EMB Lac.

Repeat, new H226.

7/29. Mostly segregated. By replica, almost all Lac- are prototrophs. 1 aux. Lac- noted = 862-1 see 868

7/30. New H226. → (Lyophil) from nigle colony. 10 AM. – 3 PM = 5 hours. 10^{-6} .01 ml.

Control plating .1 ml: 166 23

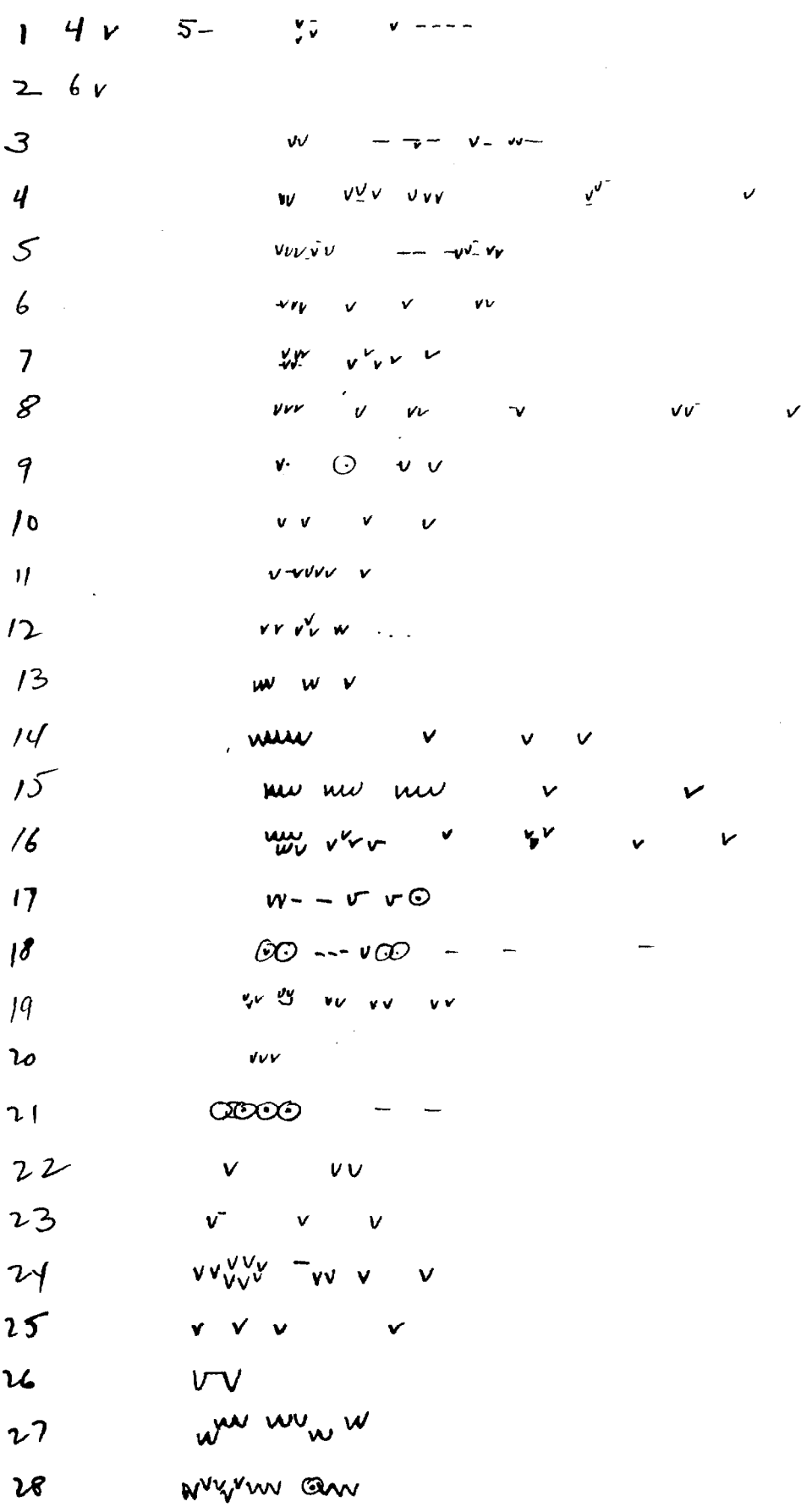
.01 ml brush. 29 4

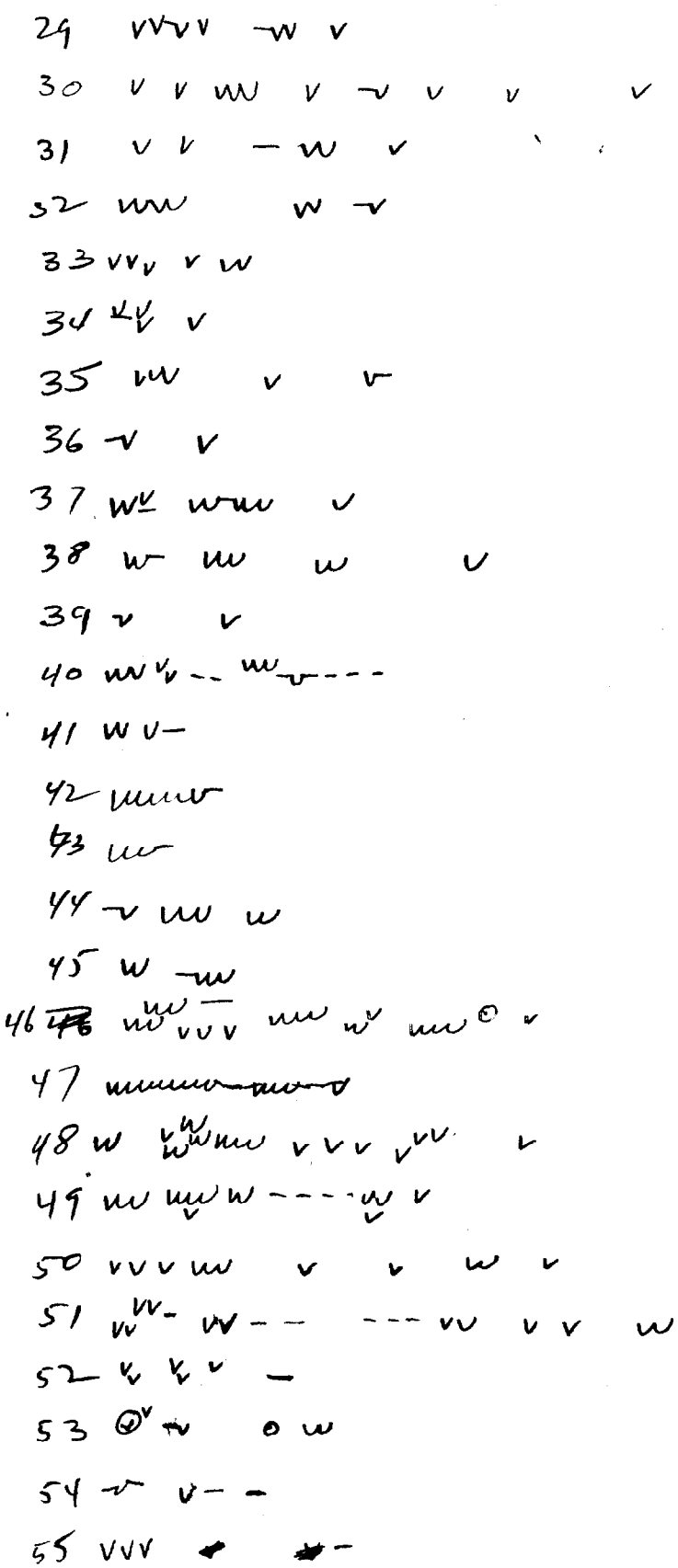
Probably 2× too many cells per brush, but many likely

From replica plates, most Lac- are auxotrophic;

Replica Lac- "segregant" solutions to EMS Lac

Clones





56 V V V V V

57 V V V V V V

58 V V ⊙ V

59 ⊙ V

— clones

15, 7, 24, 10, 17, 16, 15, 21, 19, 21, 14

many unique clones. Pick possible new — from lacV clones as best possible. Pick to EMB Mal

---

all picked were Mal+. #52 MalV, prototrophic all others auxotrophic.

all Xgl+ except 29, 57.

Most likely most of the Lac− were previous segregants

see 867

W. K. Maas cultures
Inter-sterility.

July 30, 1951

A. KIGT-h- } pts.
B. KI h2-arg }

Add put to all plates, EMS Lac
1 plate per x.

1. A x W-1
2. B x W-1
3. A x H245
4. B x H245
5. A x H290
6. A x W1606 (es BM-)

KI GT-h-
= put-multicam-hist-

KI h2-arg
= put hist arg.

8/2/51. all plates barren. Other crosses with W1606, H245, H290 were fully fertile.

Maas says put+ mutations are futile.

July 29, 1951.

a. Irradiate on EMB agar 6-20 secs.

b. Irradiate washed suspension 2 ... 20 secs. Drop ½ ml into Penassay. Use 4 sec treatment

7/30 Plate on EMB Lac

Ca 300 tested for auxotrophy by replica: all X+.

No Lac, or Mal variants on ca 20 plates. Many colonies suggesting morphological characteristics.

ca 300 more tests for auxotroph. ~~I doubtful: Retests~~

10 x 350 = 3500. No auxotrophs detected!

Total, ca. 4000 tests!

Plate on EMB Mal sm. [E. Woodworth records show W1647.]

Variable types, some Mal ±. All Lac+.

1
2
3

9/20/51. Problem adopted by E. Cohn.

W1606 × H293 ; W1675 × H290
S^D × S^R

865

Mostly lac+. Replica to EMSlac, EMSlac sm. for S^S.
ca 200 colonies — grew exquisitely on
EMS Lac, ± Sm.

Aug. 4, 1950 ~~W1606 ×~~
B W1675 × H290.

C. W1675 × H294 on EMSlac, ± sm
TL lac-Mal-S^D M-lac+Mal+S^S
8/5/51

"1675" proved to be BM- lac+Mal+S^D
(probably W1606)

Losses n.g.

July 30, 1951.

M290 × M293

a. EMS Lac b. EMS Mal

M- Lac v Mal- S^S TTL- lac v Malt S^R

a. 20 picked. 11 Lac v. 9 thus lac- (Lac v.)

b " " 8 Mal-
~~8~~ 7 "+"
3 Mal v
2 ?

Test ~~[redacted]~~ single colonies on EMS Lac; EMB Mal / sucr.

~~[redacted]~~:

	Mal	S	#
b.	—	S	8
	+	R	5
	~~4~~ v	V	2?
	+	S	4
a.	—	S	9
	+	R	1

Mal v relatively infrequent in this cross.

Grow 866-1 on D(Lac) for mucoid patches on EMS Mal.

Diploid microcolony segregations

August 2, 1951.

See 862 .01 ml; $.3 \times 10^{-6}$ per plate $11\frac{30}{}$ -

assay .1 ml EMB Lac

V	-
14	6
9	3

Mal

+	-	V
1	0	23
9	1	26

11^{30} - 5PM.

ca 200/ml. About 2 clones / plate.
Agues with 2-3 ~~pla~~ strains not required.
On EMB Lac 6 plates. EMB Mal 3 plates

Clones:

—		EMB Mal
	1^1 V	1 V
1	1^4	1 V
		1 +
	1^{10}	1 V (+, - also!)
	no segregants	1 mixed V, +.

No useful data but method is substantiated.

8/5/51. H226 A) .01 ml 10^{-6} B) .01 ml 3×10^{-7}
D . Lac count; many —. (assay: 14 V, 17 —)

5 usable Lac V clones, ca 10/. 2 possible segregants, but crowded.
Streaks on EMB Mal. (Both Mal +) ✓

8/6/51. As above. Total 25 plates, mostly too crowded Mostly Lac V
E 47 possible useful clones (5-20) ~~a~~ altogether. (5½ h. mic.)
Pick Lac - to EMB Mal · 12 : all Mal +. (2 Mal V)

8/7/51 4½ h. mic. ~~Loop~~ standard loop method — Few clones
F should use 2 x. Many Lac - !
(over)

G, H. UV 20 secs. ca 50% surv.

Results inconclusive. Increase in Mal+ in both intact and resp'ed.

Method probably is unsound.

auxotroph diploids. 868

July 30, 1957 FA

A. H226, grown in D(Lac), no treatment. auxotrophy noted in replica platings. Nutrition, Mal, S tested in such platings.

H267, grown D(Lac). UV 30 secs. Grew heavily in D(Lac) +

B. BM

C. TLB$_1$.

Plate on EMB Lac, Replica tests for auxotrophies. B gave numerous auxotroph Lac$^-$; C very few.

	Type		Nutr	Lac	Mal	S	Mal fermd.	S
A.		1	M	V	V [redacted]	[redacted] S		H294
	6	2	TL	V	+ V	S	H245 type	
	6	3	TL	V	+ V	S		

			Nutr						
B	3	1	M	TL	−	−		−	R
	4	2	TL	M	−	−	V	−	R
	1	3	M	TL	+	−		[redacted] V	[redacted]
	5	4	M	TL	V		S	V	S
	1	5	M	TL	+	+ V		V V	[redacted]
	6	6	TL	M	V		S	V	S
	3	7	M	TL	−	−		−	R
	−	8	+	+	+	−		V	S
	4	9	TL	TL	−	−		−	R
	3	10	M	M	−	−		−	R
	5	11	M	M	−V		S	(V)	S
	5	12	M	M	+V		S	(V)(+)	S
	−	13	MTL	MTL ?	+	V		V	V
	1	14	M	TL	+V	−		V	V
	3	15	M	TL	−	−		−	R
	4	16	TL	M	−	−		−	R
	3	17	M	TL	−	−		−	R
	1	18	M	TL	V	−V		V	V
	−	19	+	+	−	−		−	R
	3	20	M	TL	−	−		−	R
	−	21	MTL	MTL ?	+		S	(+)	S
	3	22	M	TL	+	−		−	R
	3	23	M	TL	+	−		−	R
C	2	1	TL		V	−		V	V
	2	2	TL		+	−		V	V
	2	3	TL		+	−		V	V

		Mal	S	
A1	M	V?	S	= H294
A2	TL	V	S	
B1	M	—	R	H291
B2	TL	—	V	
B3	M	V	V	—
B4	M	V	S	(H294type)
C1	TL	V	V	

Cross Attempts with E coli B
Phage T3

August 3, 1951.

Mix 1 drop culture or phage T3 (10^{-3} lysate) to 5 ml Penassay
Incubate overnight.

A 8/3

1	Y44		
2	Y51		
3	Y44 + Y51		
4	Y44 + T3	clear	
5	Y51 "	"	
6	Y44 + Y51 + "	"	

→ #1, 2, 3, 6 plated. all grew without plaques on EMB.
No protoplasts after 7 hours.

Sediment turbid cultures. Plate on D(o)

Repeat A, with heavier bacterial inoculum (ca. 4/ml)

1-3 turbid. 4-6 clear. Streak out 4-6. Also W1664/3 passages

B 8/4

4-6 remain clear!
No true 1664/3 formed as survivors (phage tests)

W1663/3 obtained from platings on EMB. = W1679.
Purify by 3 streakings on EMB and retube —.

C 8/12.

1	1679		Protoplasts? 1
2	" + T3	±	1
3	1679 + 1665		1
4	1665		2
5	1665 + T3	Clear.	
6	1679 + 1665 + T3.	± turbidity.	3

Sediment. Also inoculate 2 and 6 into Penassay.

a) The parents are not completely stable
b) No detectable effect. The crosses had numerous ● (L?) forms.

See Notes by E. Cohn

August 29 (ff.) 1957.

a) Diauxotrophes from PF9 (meth-) and PF12 (leuc SR).
UV 40 sec., resuspended cultures in saline from exh. Asolectin broth
Wash proc 1:100 in D(C) + meth + leuc + 1000 u/ml penicillin,
↑ aerate overnight. Plate on EMB bac after 10-12 h.,
replica to D(ML).
PF 9 ca 15% auxotrophes.
PF 12 ca 3% . to pack to EMB for Rechecks.
On Rechecks:
PF9. 7/8 and 31/40 = 44/48 OK as auxotroph
PF12 14/20 OK.

see 870a. for Random series.

b) Plate mixed suspensions from b) at ↑. PF 9, PF12 and PF9+12 (uv. grown together). The last showed high counts (prototrophs) (ca 5000/ml) but considerable counts 5/5 1000?/ml) these were in the separate cultures also.

Rhynia Plate Tests

8/04

		Gr.	HC	Vits	A1	A2	A3	A4	A5	YNA.	EMB
PF13	①	1	1			+					
	②	2	3				+				
	③	3									
	④	4	4					++			
		5	2			+					
		6	3				+				
		7	2			+					
		8	3				+				
		9	2			+					
		10	2			+					
		11	2			+					
		12	3				+				
		13	2			+					
PF9	①	1	2			+					
	②	2	3				+				
		3	2			+					
		4	2			+					
		5	2			+					
		6	2	X.		+					
		7	2			+					
		8	3				+				
		9	3	all but S3		± ?	+				
		10	2			+					
		11	3				+				
		12	3	None x			+				
		13	2			+					
		21	3				+				
		22	2			+					
		23	3				+				
		24	2			+					
		25	3				+				
	③	26	4			+		++			
		27	2			+					
		28	2			+					
	④	29	1		++						
		30	2			+					
		31	2			+					
		32	2			+					
		33	2			+					
		41	[redacted]	[redacted]	[redacted]	+		[redacted]	±	++	[redacted] ++
		42	2			+					
		43	2			+					
	⑤	44	4		++	+					+
		45	2			+					
		46	2			[redacted] +	+ −				
		47	3			[redacted] −	+				
		48	2			+					
		49	2			+					
		50	2			+					
	⑥	53	YNA								
		54	[redacted]	AH ERRATIC.				See over		±	

all A3 – Rechecked by replica:
all were Trypt. –

Discard these, and all A2 – (presumably related.
Isol. nVal or IV)

and PF 26.

Keep other PF members and preserve mixby tubes.

September 4, 1951.

	PF									
PF12:	19	[redacted]	L+: A2	Iso [redacted]	Val [redacted]	I.V. [redacted]	[redacted]		o	Iso-Val
Add arc	20	A3	A3	Val	tyr	tryp.	Val+tyr		o	Tryp
	21		HCV	Yeast	RNA	N.Z.		o		??
	22	A4	A4	hist	thre	glut	pro	trp.	o	hist
PF 9:	23	A2	M+: A2	L.	IL	Val	I+V.	I+V+L	o	Iso Val
Add meth.	24	A3	A3	Val	tyr	tryp	Val+tyr		o	Tryp
	25	A4	A4	hist	thre	glut	pro	trp	o	Hist
	26	[redacted]	A1	Lys	Arg	Cyst	o			Cyst
	27	[redacted]	A1	Lys	Arg	Cyst	o			Cyst
	28	YNA.	M+ YNA.	Pur	Pyr	Guan	Aden	Xanth	Hypx	RNA o

(Guanine

For crossing, try Meth Cyst [redacted] × Leu Iso. PF 26 × PF 19.

These cultures pumped by dipping by Henry's method. Also try non-dendritic experiments. Add suspension directly to plasma or powdered SiO_2 gel, previously sterilized in tubes.* Seal off in air unless thin is added in tubes.

9/4-5. Attempt crosses of PF 26 × 19. a) Plate separately on minimal b) Grow together in P-C assay; c) UV o's. on washed cells, inoculate P-C assay sep. and together.

(Record of date by registered letter.)

Crossing Attempts with Pseudomonas fluorescens 870c

Sept 4, 1951. ff.

see 870b.

PF 26 × 19
Inc plates 3-4 days.

a. Grown separately.

4/4	1	19	0 0
	2	26	0
	3	26 + 19	5 v. small (cont ??) PF ??

b. Grown together

9/5	1	19	0
	2	26	10 !
	3	(26 + 19)	2 .
	4	(26) + (19)	1

4 PF
PF.

c. UV 60s; grown together

	1	19	0
	2	26	0
V. heavy	3	(26 + 19)	1, 2? (pinpoints, microcolonies)
	4	(26) + (19).	2' Not PF. Papillate clones! Not PF

Hold in refrigerator for later study.

1/16/51. Situation at D(0):

1 } "a 3"
2 }
3 } b 2
4 }
5 }
6 }
7 } b 3
8 }

all S's

Pseudomonas ??? doubtful.

9 } b 1
10 }

D. 1. PF 9 = M- 6 5 petite strains.

2. PF 19 UV L-IV-S^R 0

3. PF 19 UV + PF 9. 3 large + many small → >100 petite strains.

4 = 1+2 4 + (1-2) 9 "

Replica to EMS tube sent: None SR

This result is rather indecisive. ① Repeat with tests for SR
② Survey other mutants for stability. Test PF 2, 3, 4, 7, 8

see 769

Use PF 12 = L-S^R × PF 28 = M-Su-

P. fluorescens

9/21/51. Stability tests on auxotrophs.

Grow on NA. 48 hrs. Harvest, wash and suspend in 3± ml. Plate 1 ml samples on minimal agar. (probably ca 10^9 cells)

	Prototrophs
PF 2	9
3	0 , same background (Marin ?)
4	0 " " "
6	> 100
7	> 500
8	10
12	21
28	ca 30 (for Guan + Meth)

Monoauxotrophs of choice are [redacted], PF3 , (4)

Try × PF 28

10/2. PF28 (uv) × PF12. SM

PF12	9	T^R 3^S
PF28uv	0	—
from together	3	3R
" separately.	2	3R

No evidence of recombination.

Replica to EMS ± Sm.

Is PF12 homogeneous?

10/6: Yes!!

Experiment should be repeated

10/3 Stock culture streaked out on EMB Lac. Colonies replica plated to EMB Lac Sm. All of ca 50-100 were SR.

The PF12 S^S may have been contaminants

10/F. Nitrate Purnassay cultures. (PF-12 from colony on Sm.)

Expt. completed ca 10/20. Just as above.

PF12	ca 50	all SR	By replicate
" 28uv	0		
" 12+28uv	ca 50.	all SR	"

No evidence of recombination!

Pseudomonas fluorescens

870s.

Summary of Crossing Expts.

10/22/51.

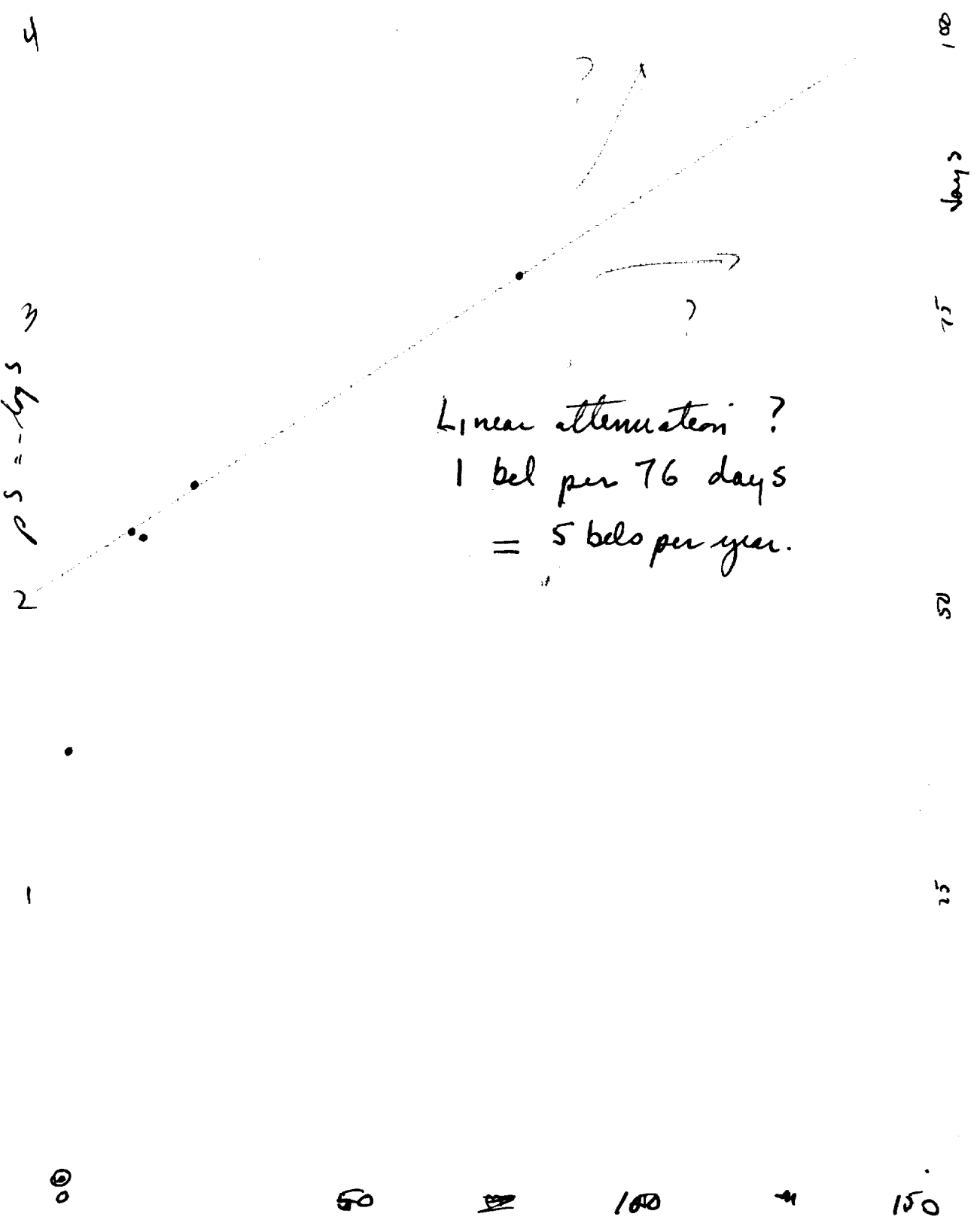
Diauxotroph combinations: L-IV- M-Cys PF 19 x 26

PF 26 gave a few prototrophs on dense platings.

Mono-diauxotroph combination (SR mutant):

PF 19

Try PF 19 x 28.


4
3
2
1
0
50
100
150
100
75
50
25
days
Linear attenuation ?
1 bel per 76 days
= 5 bels per year.

Solid desorbent for pressing cultures

511

September 4, 1951.

a) See typewritten memorandum

9/5. b) Dry K-12 by various methods to compare viability. (From heavy suspensions washed to saline 2% from NA slants.)

			9/18/51 contents/10ml broth
1.	$CaCl_2$ dr. (Hershey, Brown)		2.5×10^7
2.	In contact with granular silica,	air	2.5×10^5
3	"	"	vac.
4	"	powdered	air
5	"	"	vac.

9/4 c) Lo. PF 21

1. gran SiO_2 air
2. " SiO_2 vac.

9/5. Open 1, 2, suspend in 10ml Penassay and titrate. (10^{-7})

c1: 2 c2: 17

872
B

September 30, 1951.

Resume: Survival.

		Days		pS	Count: 1.1/10 .1/10 .1/10 } .1 ml = 1x 10^{-6} ml from 10 ml
9/6/51.	Initial	0	3.4×10^9/ml [10 ml]	0 (9.53)	340
9/7		1	11×10^7	1.49	11 1; 187/100
9/14		12	1.9×10^7	2.25	
9/15		14	$.2 \times 10^7$	2.23	20/10
9/29		23	1.3×10^7	2.42	13/10; 1/1; 80/100.
11/24	~~[illegible]~~	79	2.4×10^5	~~[illegible]~~ 3.14	1/10 17/100 245/1000

September 6, 1951.

A. Broth, duct. 1K12, 10 ml Permassay, aerated overnight. 15 11 A1 1

B. ", sedimented, resuspend in 1 ml (1:10)

.05 ml per tube.

1. Gran silica, Air ||||||
2. " " Vac ||
3. Pdr Alumina Air
9/6/51. 4. " " Vac
5. Hi-dry $CaCl_2$ - Vac |

Assay B. (.05/10; etc.). Count 299, 376, 675

1.1/10
.1/10
.1/10 .1

(= 10^{-6} ml sample from 10 ml.) Original titer 6.7×10^9 cells/ml.

Each tube received 3.4×10^9 conc. 10:1 duct broth.
3.4×10^8

Hold in refrigerator

9/1/51. Assay sample tubes. Empty tubes into 10 ml Permassay, rinsing washings from walls. Dilutions schedule as above:

872 B1 (.05/10); 1.1/10; .1/10, .1/10. Plate .1

		Count	10x	100x	Assay, ml (in 10 ml)	Survival (pS)
A	1		2	15	2×10^5	1.3
	2		15	121	1.5×10^6	.3 !
B	1	11	161		1.4×10^7	1.3
	2	10			10^7	1.3
	3	0			0	—
	4	0			0	—
	5	14	188		1.4×10^7	1.3
		340.				

Ca 95% "Process Loss", and attenuation in 24 hours.

9/14/51. ~~B2~~ B1 1:2) 11 187 1.9×10^7 2.2

9/15/51 B1 B5 20 0 0 ! ~~7~~ ~~2.1~~

September 15, 1951.

Harvest from Permassay tubes. Concentrate in enzyme 20 ml
ca. 30/1.5 = 20:1 Use .05ml per sample ≐ 1/ml

a) Titrate initial samples (1.1/10; .1/10; .1/10; .1/plate) ~~rigor~~

Silica tubes ~~ferrously~~ balanced; store in vac. over Drierite.

		Count	Assay/tube
Initial assay. A.	58-161	34	$.34 \times 10^9$
	W-1177	148	1.5×10^9

Until 10/11/51, Silica used was Davison, 05-08-09-216.

New lot received 10/11/51 (Grade); 40-08-09.226

10/11/51.

B. ~~H295~~. Harvest, 10:1, .05 ml/tube. Assay $\frac{100 \times 139 \times 10^{-8}/2}{\text{ml}} = 3.5 \times 10^9$ per tube

	Count.	Surv.
1. Old Silica	33	
3. New Silica	<10	
2. Silica Grade 923 Mesh 100-200.	<10.	
4. Activated Alumina	1-2	

10/12/51 ~~Plate~~ add tube to 10ml. Plate 10^{-5} ml

Very low survivals. Should be repeated.

Department of Genetics
University of Wisconsin
Madison 6, Wisconsin

January 1, 1952

Preservation of Bacterial Cultures on Silica Gel

This circular was written in response to a number of inquiries. Judging from these, present methods for preservation of bacterial cultures are not entirely satisfactory, and it would be a real contribution to laboratory technique to work out a better one. Unfortunately, I can only suggest a principle that seems very plausible, but that has not yet been empirically justified.

The working principles are (1) that suitably dried bacteria should survive just as well in a sealed tube under air as in vacuo, and (2) that if this is correct, chemically inert desiccants such as anhydrous silica gel could greatly facilitate the practice. The following arrangement has been tried: small vials or tubes are filled nearly full with silica gel (Davison Company, Baltimore; Grade 40, 6-16 mesh). About 1 to 1.5 gms. of silica fits well into the tubes used. The tubes are plugged, then baked in a sterilizing oven at 160-180 C., 2 hours, to dry and sterilize the tubes. These are stored in a desiccator. The bacteria to be preserved are suspended in 2% peptone. About .05 ml. is pipetted directly to the silica, and the end of the tube sealed off. The tubes were then stored in a refrigerator. The water disappears very quickly. To regenerate the cultures, the tubes were broken, and the silica poured into broth. Considerable gas is liberated. After about an hour to allow redispersion, viable counts were made on the broth. The 24-hour survival, in apparently dried condition, was quite high (about 10%), but this was more encouraging than long-term experiments. After four - five months, the survival has been low, of the order of 10^{-5}, and some tubes are inviable. In its present form, the method is not a success, and cannot be recommended for long-term preservation and storage. I think that it could be greatly improved, without complication, by experiments leading to a better suspending fluid, and possibly by drying the cells on a layer of glass bead over the silica. What is most needed is an improved theoretical understanding of the biology of suspended animation in successfully dried cultures.

Despite its shortcomings, the silica gel tubes provide an ideal method for mailing cultures. They are not affected by undue cold in the way agar slants are, and probably ought to be more resistant to high temperatures as well. The mechanical strength of small sealed tubes allows them to be sent with simple padding in an ordinary envelope, and the absence of any liquid minimizes possible hazards from breakage and leakage.

I hope that other workers with suitable facilities to study preservation problems, or with a potential interest in the biophysics of suspended animation may be able to make some use of this suggestion.

Joshua Lederberg

September 25, 1951.

A. K-12 overnight, aerated. Dilute 1:10 (est. 2×10^8/ml)

A 2 .02 ml Assay original K-12: 5.7×10^9

A 5 .05 ml

B2 1:1000 (est 2×10^6/ml). .02 ml

Assay. B2. ① Ca 2 gm. Silica ② Ca .5 gm Silica

4/29/51. A2 ③ " ④ "

2 gm samples may have been exposed to heat glove to cell during sealing.

		Survival	pS
1	6	6/114	
2	16	16/114	
3	8.75	8.75/114	1.115
4	> #3 ca 10%		1 ±

A2 init 1.14×10^6/tube. 11,400 per plate = pS 0.

B2 init. 114.

October 7, 1951.

A. For S^D/S^S Cross on EMS lac, EMS Malsm.

B. 10/8/51. H292 x W1709

No yield!!!

Check fertility of W1709. Grow on Penassay + 1000 u sm/ml (Hi) 50 u (Lo)

(10/11/51)

A	1709 Lo	x W1490	High yield	
B	"	x H292	n.g. - overgrown	H292 ? Probably used 295 by error
C	1709 Hi	x W1490	V. High yield	
D	Hi	x H292	n.g.	
E	1709 Hi	x 58-161	3-4	
F	1709 Lo	x 58-161	3	

Note reduced ~~yield~~ yields of $S^D \times S^S$ cross. (Residual sm in S^D cells?). Should compare $S^S \times S^D$; S^R grown on comparable sm medium, and $S^S \times S^R$ on plain nar-sm broth.

---

su876A A W1734 x H290 low yield on EMS lac (Ca 5/); Ca 200/EMS Malsm.

su877 B " x H291 . Very low yields Ca 4/ EMS lac ± sm.

876 A

W1734 × H290
Mal+ S^D Mal- S^S ||

A. EMS Lac.
B EMS Mal sm.

Streaked out EMS lac, EMB Mal, EMB Mal sm
High yield, bit n.g. 24h.

A.

	Mal	Mal sm.
1	v?	
2	v	
3	v	
4	?	
5	v	
6	v?	
7	v	
8	v	

growth much sparser. colonies indeterminate. May be ~~Ss~~ S^D/S^S with substituted S^S segregants.

Presumably S^S dom S^D

Repeat 10/19/51. see 877.

10/22: ca 5-10 purplate Picks 1-4.

10/22 Picks 5-8. Brush 1 or 2 v. small Lac+?

	Lac	Mal	EMS lac sm	
1	+ nov	+ nov	+	S
2	+ "	+ "	+	S
3	+ "	+ "	+	S
4	+ "	— "	+	S

not S v

No colonies on replica to EMB sm.

see 87B.

Picks from EMB Mal.

	Lac	Mal	lac sm	Mal sm
5	++		- few thy	+ (few) only
6	++		"	"
7	++		"	"
8	++		"	"

Pick single colonies from EMS. Brush /sm EMB lac, Mal.

No Mal v

11/2/51

	S^D		S^R Mal	
C	W1734	x	H 301 v	No yield.
D	"		H 302 -	Yields very few, improved mar sm.

Plate on EMS lac Streak sm on some plates to establish gradient of sm concentrations.

D. Experiments continued in desultory fashion.

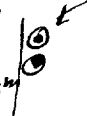
Many prototrophs fail to grow further. In numerous (20-40?) tests, lac- were mostly Mal-, some Mal+, 2-3 Mal- but no S^D were noticed.

Investigate further S^D prototrophs. 10 restreaked

	EMB Mal	
1	+D +R	-
2	+D	-
3	+D	-
4	-	
5	-	maybe S^D or S^D/S^R
6	-	
7	-	
8	-	
9	+D	-
10	+D	-

3, 4, 7, 9, 10 did not produce good colonies on EMS lac sm (1004/ml)

4 (single colony?) shows most definite signs of being a heterozygote. Pick single "Mal v" colonies



0 Mal- 4D 1-3 s.c.

4D4 grows on EMS lac sm

5 shows strong Mal+ S^D reaction and a single ~~to~~ Mal- S^R.

Purity of initial inocula in doubt

		EMB Mal //sm	EMB lac //sm	EMB lac sm	EMB Mal sm
D4:	1	+D	-D	-	+
	2	+D, -R v?	v, -	(D?) v -	- v? +
	3	"	-	(D?) -	+, -, v?
?	4 - mix	- R	v, -	v -	-

Pick clear single colonies, Mal v or lac v and streak on EMB, EMS ± sm

W1734 × H291
Mal+ S^D Mal- S^R

8 77

A EMS Lac
B " Mal sm

A.

	EMB Mal	do. + sm.
1	V	V
2	V	V
3	V	V
4	V	V
5	V	V
6	V	V
7	V	?
8	V	V

On EMB Mal, a more or less "normal" segregation is observed, Mal+ predominant (not necessarily not V.) With sm, Mal- is much more prominent, suggesting that S^D/S^R in presence of sm does not compete well with ~~Mal-~~ S^R.

If S^D is present here, it is presumably recessive to S^R.

B. Mal V very doubtful. Same appearance ± sm.

Lost by overheated incubator.

Repeat 10/19/51. 10/21/51. Ca 5-10 colonies per plate EMS Lac. Pick (1-4.)
10/22 Pick (5-8) including numerous small Lac?

	Lac	Mal.	EMS Lac	sm
1				
2	+	nov.	+	S
3	+	"	+	S
4	+	" ?	+	S
	+	"	+	S

Test colonies as EMS, EMB ± sm. from EMB by replica plate.

#39, occ. colonies from thick streaks gave Mal- colonies on EMB Mal sm. Mostly Mal+ S^S.

Picks doubtful colony from 1, 2, 3. 1a, 2a are Mal+
3a is Mal V. Mal- on EMB Mal sm. ~~Photographed S on EMB~~
~~Maybe either~~ S^R/S^S or S^D/S^S, ~~probably the former~~
Not photographic: probably H291 parent!

	Lac sm	Mal sm
5	- only	- only
6	"	"
7	"	"
8	"	"

all are S^R/S^S

Note H291 found to be Mal V.

Preserve H298

878

4PM 10/16/51

A. Dilute 1:100 in

1. Water abc = 1-3
2. Saline abc = 4-6
3. Peptone 2% abc = 7-9

Initial assay: Lost
Probably ca 2×10^9

B. Inoc .05 ml in
a Old Silica
b New "
c Sran "

C. New Silica, Peptone dil.

.01	10	0
.02	11	0
.05	12	0
.1	13	0
.2	14	5
.5	15	4
1.0	16	5×10^4

ml.

ca. 1.5g silica per tube.

Remained wet

11/18/51. Initiate assays. Predicted initial would be ca. $2 \times 10^7 \times$ [illegible] $\times .05$ per 10 ml tube, $= 10^5$/ml. Plate .1 ml and .001 ml samples.

#1,2,3,5,8 showed no survival in .1 ml samples. However, every tube grew out after 48 hours (Cells bound to silica??)

∴ Water dil., ~~on pep calcium gel~~ on any, or saline or pep on "new" silica not very good.

#15. 4 colonies at 10^{-1}

#16 5 colonies at 10^{-5} ! (but this not dried)

Many apparently "Lac+" colonies.

11/20/51 Plates of remaining tubes: 4,6,7,9,10,11,12,13 Sterile #14 shows 5 colonies at 10^{-1}

But all tubes except 4,6,11 are turbid.

(over)

These results are very discouraging. However, they may be the result of early detection or a poor medium for the initial assay was lost in an overheated incubator. Lf 872 B1.

Glu- × Gal- = Lac-?

10/20/51.

W990 = Y10 Glu- Lac+
×
W618 = 58-161 Gal- Lac+

Cross on EMS/Gal. Replica to Gal, Lac, Mtl.
Picks the 4 combinations: all are Lac+!

	Gal	Glu	W
	+	+	1741
	−	−	1742
par.	−	+	1743
par.	+	−	1744

In the cross, Gal+ >> Gal−
Mtl− >> Mtl+

suggesting that the Gal and Glu− are both linked to Brn.

[W619 was also tested, but this is Lac ÷. The genetics of the loci here is not known. Should try W618 × W251, 252]

On fermentation tubes, rather slow ambiguous reactions were seen.

W1742 was grown on Y+Lac plates, harvested to water and suspensions tested for glycolysis in 1/100 buffer, 1% sugar BCP.

(15 minute test). Lactose ++
Glucose +++
Galactose ±.

This suggests differential adaptation of glucozymase to lactose! Compare glucose, lactose grown cells.

W251 (Lac+ Glu− Gal±) × W618. Good yield
Isolate several Glu− Lac+ Gal−
W1752 1753.

W251 in fermentation tubes is Gal++ Glu+ Lac±! in contrast to appearance on EMB plates.

BB 545

Streptomyces griseus.

11/8/51.

Culture WAc 1 Received from E.S. McCoy. Transfer to Nutrient Agar slants, streak out mother media. Limited ~~vegetative~~ growth on EMS bac, Glu, Gal. No growth on EMS. Good growth, limited sporulation, on D(a) agar. Culture inhibited by streaked loopful of ~~sus~~ 10^5/ml.

11/14 (1) Harvest spores from n.a. slant in 10 ml H_2O with vibrator, ca 10 minutes. Count ca 2×10^8 in counting chamber. Adjust to ca 10^8/ml in H_2O. Many clumps; ca ~~80~~ 80% single spores. Dilute + plate out on nutrient agar.

A) control B) uv 10 sec. C) uv+120 sec.

Plate 10^{-6}, 10^{-5}, 10^{-4} A., B, C.

For irradiation, dilute ~~to~~ to 10^5/ml predicted count.

A 6 111 → 4 poss. tested, all +

B 6 125 ← Replica to minimal agar transferred 8 poss. retested 2 auxotrophs: WAc-2, -3

No appr. kill at 60 sec. Try 90 sec. 120 sec. 150

11/16 Dilute to nominal 10^4/ml

A 1 .05 ml	204	No auxotrophs
A 2 .02 ml	233	
B 90 sec .1 ml	48	
C 120 " " 0		
D 150 " " 0		survival.
E 180 " " 0		

6, 14, 4, 8, = 32 ~~auxt~~ no auxt

p.8 F 90 sec, Dilute [redacted] to nominal 10^5/ml .05 ml/plate

5 plates, ca 80 scorable/plate (+ bacterial contaminants!)

2? aux. No.

(See over)

G 60 sec ca 500 colonies 1?? WAc 4

Grows slowly, compatibly on minimal agar.

Probably more slowly than WAc 2.

1 colony noted as producing pale yellow pigment on minimal agar.

I	60 sec	8 plates	ca 90/	720
J	90 sec.	12 "	ca 45/	540
				1260 colonies.

10 possible mutants:

2 from J

8 " I (5 on 1 plate!)

Eventually 13 possible mutants.

4 show v. low residual growth on minimal agar (1, 2, 6, 10) = WAc 5-8

Others are mixtures or slow type. Recheck replicas from streak plates

S. griseus mutants and crosses.

WAc 3 x 4

11/20/51.

WAc 2	A1	Slow growth on minimal, vits?	Arginineless
3	A2		Leucineless.
4	A4		

WAc-2 grows more slowly than + but eventually (3-5 days) gives considerable growth. Admixtures with WAc 1 show no improvement, either by cross-brushing on minimal agar (D(0)) or by restreaking from X on nutrient agar.

879

WAc 5	6	A1	
6	10	A2? Syph. WAc 3	
7	1	A4	
8	2	A4?	
9		Slow growth.	

1. When 1st streaked on minimal agar scattered colonies with poor growth in periphery of streak.

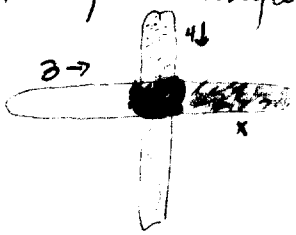
12/5 Cross brush suspensions of WAc 2, 3, 4 on minimal agar.

12/2/51. Controls: WAc 3 has a barely visible residual growth; 2, 4 show definite residue, (2>4).

2 x 3 Definite improvement (aerial mycelium) at cross-brush.

2 x 4 Marked " " " " "

3 x 4 Heavy aerial mycelium at cross brush and at canyons regions:



Proper demonstration of interaction may depend on using suitably "negative" mutants.

12/6. Prototale from x, cf. parent

---

4 A4 ✓ A1 ?
5 ✱ A1 ✓
6 V1+, A4, A1, A3, A5 esp. A2.
7 A4 A1 ?
8 A4 (weak ?)

on others, reaction is graded with ++ toward old spot of WAc 3

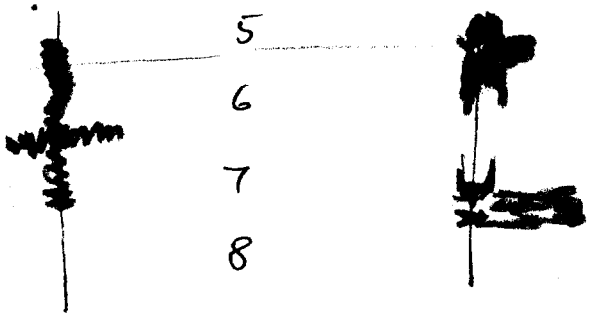
[illegible] synthesis reactions on these plates [illegible] WAc 3

WAc 8 shows strong synthesis with 880 - 9 (WAc 9)

Cross brush var. comb WAc : drops spread., but apparent interaction is

5 x 3 (maybe 5 x 6, actually)
6 x 8 " ?

3 x 7 very clear.



WAc 8 WAc 3

Repeat on separate plates 12/8/51.

	12/10	12/12
3 x 5	-	++ (no spont reversions in controls)
6 x 8	++	studies are indefinite owing to syntrophisms. All +++
5 x 6	++	

11/20/51.

See 765

Original slants W1327–1330 were restreaked for purposes of recovery and storage. All are predominantly stable +, –, or slow, no unstable forms seen at first sight. Mal– forms are apparently stable now! This is contrary to former result of high instability. Attempt repeated replatings!

W1729 was noted as a similar Mal v type. Lyophil culture predominantly Mal v. Following experiments are designed to ① establish patterns of instability and ② (experimentally reproduce the peculiarities of W1327 which is probably now lost.

Reisolate of W1729 Mal– → Mal v+, thus resembling W1327 original behavior.

11/21/51. Restreak ① and slant W1729 ca = Pure Mal+ / Mal v, Mal–

1. Plate Purnassay susp. EMB Mal. ① Hold in incubator at 37.
② store 1:100 dilution in. Hank's.

A Streak plate from slant 28 +v 36 –P No stable.
B Plate from ①. All streaks either +v or –P

C Plate 11/21 : 1, 2. All +v or –P 1 pure + ✓
D 11/22 all +v or –P
E 11/24 " "
F " "

all 884.

3. Bros. Both with a –, + colony.

W1327 x W1394. 882

November 23, 1951.

EMB Lac, Mal

Mal++ > -

No Mal V noted in platings of pooled probings

November 22, 1951.

See correspondence ca. 11/51 with Umbreit & Ogrisley

Stanier recalled the report (J. Bact 1949) that S^R mutants of the Hussey + Gratia strains of E. coli were not improved in growth by aeration. RYS suggested that this might be a directive effect of sm on spontaneous S^R survivors (similar to Ephrussi's petite forms). Several S^R mutants of E. coli K-12 were tested, and did not differ from S^S in improved growth with aeration on Penassay, Minimal or Tryptone broth. (incl. W1177, & other K-12 S^R).

Ogrisley sent two cultures labelled S and R respectively (Hussey strain). These did not show NAI. effect, but Ogrisley later wrote that the early experiments were not readily reproducible.

The cultures when streaked out are highly heterogeneous, and show a majority of minute colonies that do not grow for ± 48 hours.

11/20 Test S1, R1 (sm. colo.). Both show AI on Penassay.

P21 Restreak S1 and R1 (as received). on EMB Lac, Nutr. Ag.

A22: large colony forms apparent on EMB Lac Practically no growth visible TSA. Incubate

12/16/51.

WAc 3 grown on nutrient agar bottle.

Susp. ca 2.5×10^8/ml. Dilute to 2×10^3/ml. UV 90 sec
plate on nutrient agar. n.g.: survival ca 500/plate!

12/28/51. Same suspension. Dilute from nominal 2.5×10^8
to 10^3/ml. 90 sec uv. Plate ,2 ,1 ,05 ml
ca 10×35 colonies. 4?? None mutants. Recheck.

Plate WAc 3 on TSA+sm100. (ca 10^8 plated; 10^2 colonies appeared)
Restreak on TSA+sm. Pick clean colony to Dtc slant as presumed sR
mutant

W1729 : Selection in Series on non-Maltose medium.

November 24, 1951.

Streaked from EMB Mal to nutrient agar A: Mal+v
B: Mal−

Note: A forms somewhat larger, rougher colonies

11/24. Picks 4 colonies from A, B. Restreak on EMB Mal and NA

11/25 A: Mal v (ca 94%) and Mal− in all 4 streaks
B: Mal − P " " " "

∴ Maltose is not immediately necessary for instability.
Inoculate from nutrient agar to Pomassay, EMB Mal and NA
Difference between Mal v and Mal− noted again in these streakings.
(Looks for mutational equilibrium)

	A	B
1	20+ 8− some+?	ca 1%+
2	84+ 34− some+ slower	100+: 17−
3	37+: 40−	[illegible]
	70+ 71−	[illegible] stable ??
	[illegible]	ca =

A3 shows no + stable
B3 + stable ?

A state approaching pseudoequilibrium is reached from either side.
Stable +, (− ? also) occur in B series.

Repeat with colony streaks. Streak Mal+v colony (A3) on EMB Mal; Nutrient Agar
= C1

6/24/52. Restreak slant of A1, B1. A1 → mostly Mal ±, and − v
B1 → all Mal − P
∴ no change in stability.

Stable + seems to accumulate in successive transfers, but so gradually that skeleton mutation process can't be excluded.

WAc 3 x 4. 885

December 7, 1951 et seq

see 880

12/2 Cross Bunch 3 x 4 on minimal agar. Prototrophic interaction seen
12/6 Restreak from X, also parents D(o) agar.
12/9. WAc 1 Heavy spor. growth
WAc 3 no "
WAc 4 faint colonial background.
WAc 3x4 Mostly like WAc 4. about 20 heavily spor. colonies on thick streak.

mycelium Ditto., especially around mycelial fragments.

12/9. Restreak (spores) from spores A., mycelium B. to minimal agar.

12/11 +++ growth, apparently pure in A, a few residual - colonies in B. Restreak spores on minimal medium.

12/14. A. 12 colonies on thick part of streak
B. Blanks.

Restreak from 12/11 plate., A, B and from 12/14 A. to minimal and complete. Also replica 12/9 to " " all prototrophs.

12/16. All streaks +++

Compare 886.

1/2/52.

Repeat cross brush. Heavy residual growth of WAc 4, but sporulation only of interests. Restreak 4 sporulating ~~sg~~ sations 1/5/52

1/10/52 Occasional sporulated colonies finally developed. (5 days!) Restreak

1/16/52 Residual growth crumbling WAc 4. WAc 4 shows too much residuum to be a satisfactory mutant New growth probably at coincidence of WAc 3 - WAc 4

WAc 3 x 5

886

December 8, 1951.

A 12/7/51. Crossed on minimal, but WAc5 app contam ⇒ shows spots of +.
B See 880 Reverse. Cross-Bush WAc3 x WAc5 on minimal agar. 12/8/51

A ∘ B after 3-4 days show prototrophic interaction, no signs of syntrophism.

A. 12/11 Restreak spores on minimal. 12/14. ~~Very few colonies A, none B. (good inoculum ?)~~

~~12/14 Restreak from 12/9 plate to minimal, complete; also replicate~~

12/20/51 Table photographed

No Table photographed

or

(12/12-12/14. at room temp.)).

B. 12/12/Restreak spores on minimal agar (separate areas). Isolated
12/16. prototroph areas; background mostly auxotrophic. Repeils to minimal medium.

12/20/51. 1: Scant large colonies, background smaller (new heterokaryons?); residue of background heavy — (auxotroph parent) Restreak from 12/16 plating
2: no prototrophs.

12/25/51. Over two prototrophs, mainly satellited. By late growth, diffuse prototrophy (= formation of heterokaryons?) Restreak 1, 2 (centers of prototroph growth).

→ complete. Replica to minimal +, - Incubate ca 37° 1 each.

gave sporadic prototrophs on minimal. (over)

Heterokaryosis only?

886

1/1/51. plate 12/16/51 [illegible]

Strain largest prototrophs in minimal.

1/6/51 1 sector largely prototrophic, about 50%

"prototrophic" colonies, may be partly sectored? Restreak some of these —

886A. 1-4. 1/10 flat growth not sporulated.

WAc 11 : Mutation mm.

WAc 19 x x.

12/29/51.

Harvest from 4 day bottle nutrient agar.
Yield: 3×10^8 (ca 15 ml.) cell count

Dilute to 1.5×10^3/ml. UV 90 sec. Plate on D(Ac)

1/1/52 Considerable variation in pigmentation. Moderate # sectored colonies (spore texture; pigment ?) on ur plates. 1 yellowish slow, wetted mass on plate: streak out + compare with col. from control plating (might be S. griseus). Methylest dark slight but measureable difference presented.

ca 14 x 100 colonies replicated. 11 poss mutants recovered by brushing spores on D(o); D(Ac). 3 are clearcut mutants with little or no residuum. Pick from D(Ac) to eg. spore suspensions:

WAc 18, 19, 20. Slant. Cross brush these with each other & with WAc 3 on minimal agar.

	WAc 18	WAc 19	WAc 20.
WAc 3	0	0	0
WAc 18		++ past intersection	
WAc 19	++ past intersection		
WAc 20	± " "		
	no residue	1 reversion ?, sl. residual	no residue

1/10/51. Restreak 1/16/52

A WAc 19 x 18 dark gum growth at intersections of smaller colonies.

B WAc 19 x 3 Numerous, moderate sized white colonies

C ~~WAc 18 x 20~~ occasional large dark colonies against mucoc. background.

1/16/52. (room temperature:) WAc 3 x WAc 19 very heavy dark gum growth at intersection. Background very light.

WAc 18 x 20 similar, no background.

WAc 19 on this plate shows considerable background (from intrusion ?)

Streptomyces from NRRL; mutation runs. 888

12/31/51.

Cultures received

a WAc 13 S. venezuelae
b 14 S. lavendulae
c 16 S. coelicolor

15 and 17 sporulate poorly on D(O), DAc yellow color

Scrape spores directly from slants to 1/2 ml H_2O. Estimate density by cytometer. (ca 10^8). Dilute to calculated 10^3/ml; Treat as 883.887.

1/3/52 c OK.

b, a. v.g. in uv set (too much killing?)
b sporulated poorly on D(Ac), OK on D(O). right uv killing
a. count on D(Ac) low, on D(O) o. low uv survival.

Retransfer single colonies to DAc bottles for spores
WAc 16 showed two (more?) types of colonies: dark red (on DAc) and UV light orange red. Repick + test mixture of/difference. Red colony was used for stock. Light colonies were less acid fast by asparagine. No mutants in production runs.

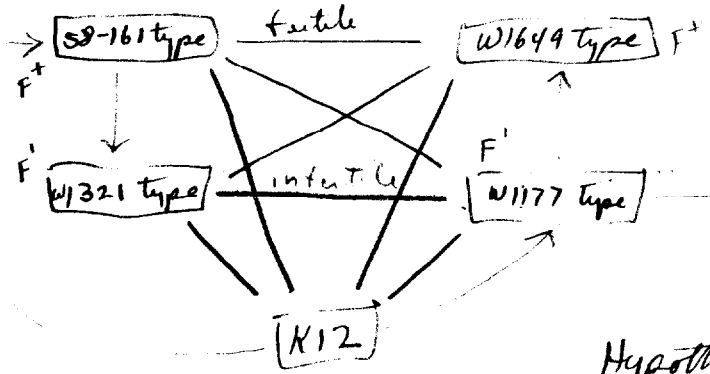
1/10/52. Re-start DAc) agar bottle for spores.

Rec 894

Incompatibility.

1/4/51.

EM+ noticed that W1321 (and subline W1578 from W518) was highly infertile with W1177. Her tests and those of this experiment give following relationships



Hypotheses: W1321, W1177 both carry the same mutant incompatibility allele F', all others are F+ and are freely self- and inter-fertile.

1/3/51. The following crosses were carried out on D10.

F' W1321 x

		Yield
F'	W1177	0
F+	W1267	+++
F+	W1649	+++
F+	W1678 (K12; mal; str-)	+++

(58-161 x W1177 controls should have been included)

λ is segregating

1/5/51. Initiate following crosses: 58-161 x W677

		Yield	SM	Lac
1.	Y10 x (W1607 + 58-161)	++		
2	Y10 x W1607 + 58 161	++	Mostly Lac+ S^S / Some Lac- S^R, Lac- S^S, Lac+ S^R / all lac+ S^S	
3	Y10 x W1607	—		
4	Y10 x 58-161	++	all lac+ S^S	
5	W1607 x (Y10 + W1649)	+++	Mostly Lac- S^R, some Lac+. Some S^S?	
6	" x Y10 + W1649	+++	all lac-	
7	" x Y10	-	+S^S	
8	" x W1649	+++	all Lac-	

(Note Sbac was mistakenly tried!)

Mating train D10 to EMS Lac, no plate-cal Sbac, D10

This is evidence that F' x F' crosses in presence of F+. F' is thus deficient in "donor" and "fertile" [illegible]

Conjugat	W1607 x	1304	++	1022	+++	W1014 x W1015	+
tester		674-680	—	1015	+++	x W1649	+
F' fertile		471	+++	K19TH	—		
Lac+		-11	—				

889: 1 8 hybrid [illegible] on LMS lac

1	+, −	wkyane
2	0	controls 2-4
3	0	n.j. ?
4	0	58-161 suspension ?
5	+, −	
6	−	
7	0	
8	−	

Fertility situation experiment [illegible] Stuehrer except pistol-pkt to test for ♂ to use for self-incompatibility expts.

Recombinations, additions of F's

1/7/52 W1810 × K12 (SRF) — (Mal+ and −)
" × 1177 } D(0) —
" × 1649 } —

1/9/52 Add Hairin to part of plate
1/11/52 Norfleet

1/10/52. Do there a connection between linkage modification and F+?

~~1 W1178 × W1607 : segregation of Mal, S.~~
2 W588 × W1607 " " " lac
~~3 W1178 × 58-161 Control, Seg. Mal, lac, S.~~ (cut. plate)
4 W677 × 58-161 " " " "
lac− → lac+ Mal− → Mal+

W1178 seems apparently contains segregation of F+

A.

do λ inherent to F+/F− ?

B.
1 W1321 × W1027 (F−) } no D(0). } infertile
2 F− × W1267 (F+) } very fertile

1 ~~58~~ × 1607 lac− → lac+
2 ~~677~~ × 58-161 lac− → lac+
3 W1800 × W1178. Mal+ >> Mal− (∴ BMF− × TLB, F+ f1 shows same ~~showed linkage as BMF+ × ...~~)
RU 896.
4 1178 × 58-161 Mal+ > Mal− lac+ > lac−
5 1178 × W1607 Mal+ > Mal−

Conclusions:
1. Status of W1810 Unsure, clouds situation
2. B shows that λ is not directly related to F
3. F+/F− not directly related to abnormal linkage of F. TLB1.
at least F+ × F− = F+ × F+

1/11/52 W1367 } × 679 SRP } Both fertile, segregating lac.
W1607 }
∴ 679 is F+

(see over)

W1813 x Y10 +++

W1303 x Y10 +++

679-183 x W1607 ++

Confirms that 679 = F^+

679-680 = F^-

W1033 xx
Hfr.

570

1/6/52

Harvest 10ml Fermasay tubes to bulk. Conc xx are 1/ml each parent pair. D(a) plate after 3rd generation, called Dil xx. Use 1% "this".

No counts before plating.

		Conc	Dil
A.	1 58-161 x W1177	40	3 (+1?)
	2 1033 x "	ca 70 (½ are small col)	12
	3 58-161 x W1649	50	0
	4 1033 x "	17	0
	5 58-161 x W1678	~~3~~ 12	~~3~~ 0
	6 1033 x "	~~3~~ 31	0
	7 ~~Mass [illegible]~~ W1811 x 1033	0	

These yields on the whole are very low. Repeat.

A 1/15/1. "Conc" as above. "Dil" = dilute only W1033, do not alter ~~W~~1177

	Conc	Dil
1 1033 x 1177	+++	
2 " 1649	+++	

No effect of Hfr noted in conc. 58-161 x W1033 x 677

Re-cover Hfr!)

B. Aerated cultures. Resuspend in 10ml for conc. 1:1/10 for dil. Inoculate D(a)

	Conc	Dil
11 W1033 x ~~58-161~~ W1177		
12 58-161 x W1177		
~~13~~		

No yield. Except for rather low yields sporadically in A, this confirms the sterility of aerated cultures. Try 58-161 x W1177 x 1649 aerated non aer — in various combinations — to see if it may not act via F+.

1/9/52

Following cultures grown in Penassay.

1. 58-161 + W1607 + Y10 (100ml) Sedimented cells and filtered supernatant.
2. 58-161 10 ml
3. Y10 10 ml.

Nofiltrate.
Add "normal" 1:1 Penassay. Incr. W1607 11:20 AM – 4:20PM
(Also incr Y10 in separate culture).

Plate 0 1 2 3 & Y10 on D(0) .2ml 1607; .1ml Y10

1/18/51 No prototrophs in any of the series 0, 1, 2, 3 or when filtrate of 58-161 was used. F+ substance may not be present in filtrates of grown cultures.

1/10/52 Sinterglass U tube, 15 ml Penassay each side.
58-161 + W1607 + Y10 (A) vs W1607 + Y10 (B). 11 AM - 5:30 PM

Plate 6PM on D(0), EMS bac

1/11/52. A ++ B – . 1/12/52. A +++ B — mostly bact, few –

stimulus is not filtrable!

Do stimulus inhibited?
Grow $F^- S^R + F^+ S^S$ together, then inoculate into one broth & select out S^S. Test $F^- S^R$ residue from time to time.

UV Activation of 58-161 for recombination (Hayes) 892

1/9/52

Harvest 58-161 from 10 mL Penassay. Irradiate (in 2 ml saline) for 100 secs. UV. Incor. Penassay YCx Broth 20 minutes. & dilute and incorp x W1177. (.1 ml each) prototrophs

Control: 6

UV : ca 200!

11:35 - 12:05.

This confirms Hayes' claim. Repeat with F⁻ x F⁻ and

1/11/52. Repeat similar experiment to "activate" λ⁻ 58-161, W1607, W1248 and W677.

58-161 F⁺ A, A'

W1607 F⁻ B etc.

W1248 λ⁻ C

W677 F⁻ D.

		EW1649
1	AD	+++
2	A'D	+
3	AD'	±
4	A'D'	+
5	BD	++
6	B'D'	+
7	B'D	+
8	CD	+++
9	C'D	++
~~10~~	~~A E~~	
11	A' E	
12	B E	
13	B' E	

A' + D should be all hr- } experiment ruined to a great extent by W1607 is activated by big growth in ppt. presence of UV < controls. not also UV

892a. 1/14/52. Cluster BD: effect of yeast extract incubation (as is control for UV) on 1607 F-.

1. W1607 x Y10
2. W1607 (Y.Ex. Broth 20 mins ....) x Y10

D(o) ca: A = 58-161 B = W1248 C = Y10

b. 11	AC	60
12	A'C	0
13	BC	300
14	B'C	10
15	A"C	30

No activation! Dose too high?

Controls are YEx.
A' UV
A" ~~dead~~ saline (glycerin) control

(see over)

892 C

1/12/52 ± ① W67 ② W67 uv } × W1649

② was sterile

① gave high yield,

numerous lac+ heterozygotes. [Is W1649 het ?]

① 4 lac+ → all lac v 3 Mal+ S^S
1 Mal− S^R

33 addnl lac + or ? colonies picked from EMS.
16 definitely lac v. Others may be + or v. 6 −.
Replate single colonies. Spot on EMS lac. Brush / sm EMB / Mal

17 verified lac v. 15 Mal+ } all S^S.
2 Mal +− }

3/27/52. Recheck: W67 × W1177 highly infertile
W67 × W1876 " fertile.

∴ W67 is a very weak F+.

1/10/52

Grow 58-161 A, A' in 10 ml Penassay I aeration (+ antifoam)
W1607 B, B' Harvest aerated 5 hr & unaer to 2".
Y10 C C'
W1649 D D'

Harvest and cross in following combinations; on D(0).

A · C	+++
A · D	++
A · C'	++
A · D'	+
A' · C	0
A' · D	+++
A' · C'	0
A' · D'	++
~~B · C~~	~~0~~
B · C	0
B · D	++++
B · D'	+++
B · C'	0
B' · C	0
B' · D	+++
B' · D'	+++
B' C'	0

(1) $B^a \cdot C^a$ infutile as before

(2) A · C or A · C' futile

(3) ~~A' C or A' C'~~ all crosses with D or D' were futile

(4) A' infutile with C or C'. ∴ A' behaves like F^- whereas D' retains F^+ character.

Resistance of DF^+ to F^- behavior on aeration should be verified.

1/13/52 Repeat. A = 58-161 B = ~~W~~ Y10 C = W1649 1 = standing 2 = aerated [3 = oxygenated but A+C from slant inocula did not grow]

A1 B1	+++
A1 B2	++
A2 B1	0
A2 B2	0
A1 C1	+
A1 C2	+
A2C1 ~~A2 B2~~	~~+~~ ++
A2C2	++

This confirms previous study. 58-161 aerated behaves like F^-. Can W1649 be inhibited by oxygen ?

1/11/52

1/11 Grow W1607 with 58-161 (and separately) in Permassay.

1/12 (A) Streak out to recover W1607 as lac- 10 or/plate to agar (B) Inoculate mixed culture, 1:50 into broth to remove bulk of 58-161 11:30 AM – 4 PM.

(C) 1st cultures (before sm selection)

			D(o)	EMS lac	EMB for 58-161
1	58-161	× Y10	~~+++~~		
2	1607	× Y10	0		
3	58-161 + 1607	× Y10	+++	+ –	
#2		× Y10	+++	all +.	

2nd series (4 PM, sm selection)

4	(58-161 + 1607)	× Y10	+++	+ , –
5	"	W677.	0	all –
6	1607		6	

3d series (9±0 2d sm selection) 4 P 12 – 4 30 PM.

7	58-161 + 1607	Y10	+++	+ –
8	"	W677		all –
9	1607	Y10	0	

~~#0~~ 11 AM 1/13/52. 3d sm selection

10	58-161 + 1607	Y10	+++	+ , –
~~11~~	~~1607~~	~~Y10~~		

effect of 58-161 on W1607 ~~seems~~ to persist

All 896 also.

A. About 7 lac– : 1 lac+. Collect about 40-60 lac– colonies for reisolated W1607. Inoculate Permassay 1:45 PM. → 1/14 No lac+ in streak. No lac+ detected in streakings of 4, 7. (except 1-5 papillae $< 10^{-3}$).

11	W1607 (58-161 + W1607)	× Y10	+++	+ –
12	W1607	Y10.	0	

C. 1/14 Single colonies from restreak of A from Permassay. O = mass culture

PM 1/14.

	D(o)
1	0
2	+++ = W
3	+++
4	+++
5	+++
6	+++
7	+++
0	+++
W1607	0

× Y10

∴ 6/7 cells were transduced to F+ by growth with F+.

Streak out on Gal EMB. to test heritability of fertility from single colonies

F⁻ prototrophs

1/11/52.

1 } F⁻ × F⁻ (S^S) presumably of F⁺ { 889-1 ♂ 18 Lac⁻ 15 were S^S
2 } { 889-5 ♂ 13 Lac+ 4 were S^S → 3 were Lac⁻ after purn. 1 Lac+ (2A)

3 Prototrophs from W1800 × [redacted] W1178

4 " " #58-161 × [redacted] W677

	SRP × W177	W1649
1A	+++	+
B	+++	+
C	+++	+
D	+++	+
2A	+++	+
B	+++	+
C	+++	+
D	+++	+
K-12	+++	+

No F⁻ from presumably F⁻ × F⁻ prototrophs. But see 895 for probable explanation.

Aeration effect: post-aeration

January 14, 1952

Ca. 75 ml 58-161 harvested from Pennassay to 30 ml saline.

4 ml aliquots in each tube 2PM – 4 30 PM 37°.

			A × Y10	B × W1649
1	Refrigerate in saline		3 4	10 11
2	Aerate	" "	1 ✓	20 40
3	"	" D(o)	1 ✓	20 40
4	Oxygenate	" saline	0 –	60 –
5	"	" D(o)	0 ✓	8 ✓
6	"	" Pennassay (1:3)	100	40
7	Initial assay		3 5	2 4
8	Incubate	in saline	0 ✓	1 3

A

Antifoam added to each tube.

Results ambiguous owing to low controls.

Repeat Ca 50 ml → ca 2 ml. [× Y10]

1/16/52	1	Initial assay .1 ml		11 50 –
	2	.7 ml + 5 ml D(o)	aerate 1hr.	+++
B	3	" "	incubate.	++

∴ Aeration of washed suspensions is ineffectual.

Repeat aeration effect expt again.

1/18

			1/20	
	1	58-161 × W1177	++	This proves that the aeration effect is related to F (cf 3, 4 which are XX W1177, W1177F+ resp!
C	2	" × 899-5 —	++	
	3	58-161A × W1177	– ✓ 1/22	
	4	" × 899-5 —	+++	
	5			

58-161 str. grown in aerated D(o) + BM or TLB,.

1/19.

			A21	
	1	58-161 × W1177	+	++ (>100)
D	2	58-161A × W1177 A	– ± ?	5 cols
	3	58-161A × W1177	– ± ?	6 cols.
	4	58-161A × W1817p A	++	++
	5	58-161 × W1817p A	++	+
	6	58-161 × W1817p	++	++
	7	W1607 × ~~W1177A~~ W1817p	++++	+++
	8	" × W1817p A	++	++
	9	58-161 × W1177A	++	++

Again, the aeration effect was not absolute but correlation with F+ is quite clear.

58-161A: aerated in Pennassay from 12N20 – 10A21

C: CO_2 bubbled from ca 6P20 – "

E.	1	58-161	W1177	+++	+++	8. 58-161 W1304	++ 18 cols
	2	" A	W1177	±	2 cols.	9. 58-161A W1304	++ 45 "
	3	" A	W1817	+++	+++		
	4	" C	W1177	±	28 cols.	No effect of ageing	
	5	" C	W1817	+++	+++		
	6	" 4da.	W1177	++	++		
	7	"	W1817	+++	++		

Aeration : air vs N_2 8976

1/24/52

Penassay : 58161 4PM – 10AM.

1 –
2 aerate heavily
0 N_2 bubbled heavily. Growth very poor.
Start agars with 1:20 inocula from ①. 11AM – 4 30 PM.

1 –
2 aerate
3 N_2
4 CO_2 ($\frac{1}{4}$ M/10 $NaHCO_3$)
N_2 still inhibits. 4 ÷ 1. 2 >> 1.

Harvest and cross
A W1177
B W1817

1/26/52 all plates sterile!
No apparent source of error

Plate 1/28

1 –
2 aerate (old) strongly
3 " weakly (ca 10 bubbles/minute. little growth stimulation.
4 N_2
5 CO_2

A × W1177
B × W1817

	A 1/24	1/30 A	B 1/30	B 1/24
1	+	+	++	++
2	–	–	+++	+++
3	++	++	++	++
4	+ delayed	+	++	++
5	++ delayed	++	+++	+++

∴ this air not inert gas that causes F+ → F–. Air doesn't seem to influence washed cells, however, according to 8

Oxygenation ; W1649 ; aeration

1/15/52

Grow overnight in Permassay.

A 58-161
B " + Air
C " + O_2
D W1649
E " + O_2
F W1607
G Y10.

+ = 5-20
In general, ++ = 20-100
+++ = 100-600
++++ = >600

		20h.	44h.
1	A D	+	++
2	A E	+	++
3	A G	+	++
4	B D	++	+++
5	B E	++	+++
6	B G	++	+++
7	C D	++	+++
8	C E	++	+++
9	C G	++	+++
10	F D	+	+++
11	F E	-+	+++
12	F G	—	0

No experiment

cells removed ??

Cross delayed.

1/16/52. Repeat test of aeration effect

	A x Y10	B x W1649
1 58-161	++	++
2 58-161 aerated 3		+++

Note divergence of W1649 x with aeration!

1/17/52. A = 58-161 B = 58-161 A C = W1816 D = 1816 A. E = 58-161 48hr F = Y10 "

				48hrs.
1	A	Y10	+++	++
2	B	"	±	5
3	C	"	+++	+++
4	D	"	—	+
5	B	679-680 F+	±	+
6	B	679-183	+++	+++
7	B	WH77	—	8
8	B	W588	+++	+++
9	B	W1304	+	+
10	B	W1605	+++	+++
11	B	W1649	+++	++
12	C	"	+	+
13	D	"	-	++
14	E	Y10	++	+
15	E	F	+++	++
16	E	W1649	+	+
17	A	F	+++	++
18	B	F	++ small	++

In these experiments, inhibition by aeration was not absolute. There may be recovery on the plate themselves. The effect parallels the modification of F+ to F-, possibly excepting reaction with W1304 which should be checked in a controlled experiment. Again note possible greater futility of F+ x F- vs. F+ x F+

aging 36h. no effect
aeration 58-161 parallels W1816 +++ effect

1/15/52

1. Washed cells. Mix 58-161 and W-1607 in saline. Incubate 3 - 9 PM. a) Streak out on EMB Lac; b) Inoc. Penassay + sm 10ug/ml. c: λ^+ All XX x Y-10 unless indicated

2. Grow 58-161 + W1321 in Penassay. a&b as above. (a showed background of Lac+ and lambda plaques. 2c λ^S (Therefore λ has not been transmitted whereas F+ has). lysogenic

3. Transmission from lambda-sensitive to [redacted]. W-1655 + W-1607 in Pennassay. Then a&b c: λ^+

4. to sensitive. W-1655 + W-1321.... c: λ^S

5. F+ to W-1177. W-588 + W-1177 a & b. (b showed rare + pap. b culture was pure.)

In all cases, Lac- colonies from a) were pooled to make fresh inoculum. Cultures were restreaked to control success of resiolation of F- component. Growth from sm-Penassay in b) was used directly, in each case with only barely detectable Lac+ residuum.

6. Transfer via lambda? 58-161 streaked out on W-1321 on EMB Lac sm. Plaques restreaked. Individual colonies picked and tested for lysogenicity. #1 (out of ca 25) was lysogenic and restreaked, rechecked. Single verified lysogenic colony retained for test of F: (X y-10)

7. Single colonies of W-1816 from stock culture (itself reisolated) streaked on EMB Gal(-). o = stock.

	Tests (x Y-10 exc. 5, x W-1607)		
	a	b	c(= retest pooled Lac- colonies from test streaks of b)
1	−	7 cols. + ?	w1607cont: −
2	+++	++	+++
3	+++	+++	+++
4	++	+++	+++
5		+++	C: 1 +++ 2 +++ 3 +++ 4 +++ 5 +++ 6 +++ 7 +++ 8 +++ w1177cont − 4 = W1817 (W1177F+)
6	−		∴ λ does not transmit F+. Cf. 2.
7	1:+++ 2+++ 3+++ 4+++ 0+++ W-1607 −.		F+ of W-1816 is therefore heritable at least 50 cell generations.

8. Crude supernatant of 58-161. Add sm 10ug/ml. Inoc. W-1607, incubate 1-7 PM. Limited overall growth! Reinoculate loopful to Penassay sm for cross inoculum, and streak out on EMB Lac. (ca 1% Lac+)

9. Whole culture 58-161 inoc. ca 1:20 sm Penassay. Add W-1607 as above.... < .1% Lac+

10. Dense cell suspension 58-161 aerated, as above.... < .1% Lac+

Crosses of sm-selected W-1607 treated component.

8	++
9	+++
10	+++

Is this due to the residual cells? Should be plated out without 2d growth cycle.

Washed cell mixtures are inefficient in transfering F+. λ and F+ are destruct.

Persistence of ~~aeration~~ effects on F+ 900

January 17, 1952.

From various expts. streak out aerated cultures that have shown partial or complete inhibition of F+. Pick single colonies to 1 ml Penassay and test for F (x y10, W1177 or W1607) by "unwashed" crosses.

A. 898C - B (58-161) 5 cols } all F+
B. 898C - D (W1816) 5 cols. }

C 897 1/18 58-161 5 cols. F+ ~~F+~~ F+

D 897 D 58-161 synth. 5 cols. F+

E 897D 58-161 A 1/20 → 1/21 11AM → 4: 1F+ 2F- 1F± ? E3F+ E1F-✓

F " " C. 4: F+

G Remov. E for iterated aeration 1/21, 2:30 PM. → 2F+ 2F- : G1F-✓ G2F-✓

H " 1/22 11AM 3F+ 1-? (contam. in test plate)

I " 1/22 5PM 2F+ 2- ? "

J " 1/22 830 PM ? cont? F- "

K " 1/23 900 AM F+ (K suspension x W1177 was +)

L " = ~~W+830~~

M " Save E1, G1, G2 for further tests. Colon. tested TLB, -

N " a) ~~Re-~~Transduction to F+ ∴ 4:4.

b) comp. xx W1177, W1817 : for just test, Strikly in all combinations!!

LA Fast growth for F-? Aer. 58-161 to aer. Penassay ca 1:100 12¹⁵
B Streak out 12:15 3¹⁵ Rem. } Hold in refrigerator
C 5¹⁵

Contaminant or cultures transposed?

No persistent F- found in this series!

Transmission of F+.

Jan 21, 1952

Clear Supernatant (2 centrif.) of 58-161 = A ~~[illegible]~~ Assay: ca: 3×10^6

A. 1. A + W1177 (ca 10^7/ml) + sm 12° – 2:30 Inc.

~~2. A + W1177~~ Refrigerate } v. little growth

3. 58-161 cells ca 10^7/ml + W1177 10^9 + sm in Pennassay Inc } considerable

4. " " 10^8/ml + W1177 + sm " Inc } growth.

Streak out EMB lac after 2½ hours incubation.

1 : Ca 1% +
~~3~~ : No +
4 : Ca .1% +

Pool 20, lac – colonies as inoculum for crossing test.
For ' series, pick as many colonies as possible.
Check all inocula by streaks on EMB lac

A1	–	✓
A1'	++	✓
A3	++	✓
A3'	+++	✓
A4	+++	✓
A4'	+++	✓

Note A1' alone: – ✓✓

Cells are evidently very efficient in transfer of F+.

B 1/24/52. W1177 ca 10^8 + 58-161 in Pennassay 4PM – (washed cells)

		A	B	C
1.	58-161 ca 10^7			
2	58-161 ca 10^8	} Incubate 2 colonies	++	++
3	58 161 supernatant (1:10) (ca 10^8 cells)		–	–
4	58-161 ca 10^8	Refrigerate in Pennassay.	–	–

A. 2 cols } W1177 Reisolated and tested.
B 10
C ∞ cols

1/26. all plates bare except 2B, 2C.
∴ 10^8 cells transfer ca 10% F+ in 1 hour. But retest 2A.
F+ may not be all or none at first growth!

Cross each × W1607 (unwashed)

Assortment: 2B: +++ 2A, 1C, 3C, 4C: –
2 colonies: – probably contaminants

Sources of F+ ?

Jan. 21, 1952.

F+ may be produced by other bacteria, fertile and infertile. Test by growing progenitors overnight in broth with W1177, followed by streaking out on EMB to separate W1177 component. Cross extracted W1177 with W1607 for F+ test.

		W1177 found	F status of W1177 (×W1607)
1	wg 1 K12	✓	+++
2	2	2-3 cols.	—
(3)	3	✓ Lac+. Reisol+ ✓	+
4	4	ca ½	—
5	5	✓	—
6	6	✓	~~~
7	7		
8	8		
9	9	Lac−	
10	10	— (Mal) ✓ ✓ ✓	—
11	31	✓	—
12	B	✓	—
13	ML	— Mal · many big cols ✓	—
14	W1811	✓ ~~Lac+ Reisol−~~ − also SR.	ATT) W/Lac− ingrowth Reisols (screen)
K0	1 16	—	
	2 17	90%	—
(W-1551-5) 155	3 18	— ✓ ✓	+ ? (Reisols)
	4 19	✓	1 col. Reisols
	5 20	½	—
15	W1305	✓ Mal ✓	+++
21	S. gallinarum	✓	—
22	pullorum	✓	—
23	LT2	½	—
24	LT22	<10	—
25	LT7	<10	—
26	coli 1	— few cols. ~~Reisol−~~ ✓	+(+) —
27	coli 2	⅓	—
28	Ponna	½	—
29	coli 5	✓	—
30	coli 4	✓	—
31	coli 3	— v. few Reisols ✓	—

M14 broth inoculated in 10 ml Penassay 10:45 AM 1/22.
Add sm 10 μg/ml 4:30 PM. Streak out (A) 9 PM. Replica lac or Mal- next day.

Transmissible F+ seems to be confined to K-12. Infertile strains are evidently not restricted by this mechanism. Except 3 ?

#14 again scored Lac+ on check although care was taken to pick only Lac- colonies!

Repick from same plate. Streak out Lac+ check test.

Sources of F+ ?

902a

			suspensions of ex-W1177	× W1607	
32	wg 6			++ ✓	Restreak suspension for A2 test.
33	wg 7	lac⁻ - "peculiar later"	++	T	
34	wg 8			—	
35	11			+++ ✓	
36	12		+	+++ ✓	
37	13		—	T why? ✓	Check - 37 is lac⁻ prototroph; wg 13 is lac⁺ prot. of possible exct.
38	14			+++ ✓	
39	15			—	
40	16			+++ ✓	Prototrophs not found on request
41	17		+	3? ✓?	
42	18			—	
43	19			—	
44	20	all Lac+	—		
45	21		++	T	
46	22		++	✱ - sec!	46 is lac⁺ auxotroph but wg 22 is similar
47	23			—	
48	24			1? ✱	
49	25			1? ✱	
50	26		—	T	
51	27		—	T	
52	28	lac⁻ also -	—	T	
53	29			— ✱	
54	30			1? ✱	

Reticulas: all +++ (×1607) above EMS lac⁻ cultures

3B
14B*
18B EMBlac: all + Pick + streak from EMBlac sec.
18A2: Repick from same plate → ✱ → + → ∴ W1811 = F+
14A2 (Repick W1177 colonies from same plate as 14A1) → +
14A1 (see above): Mixture of lac⁺, lac⁻: evidently not clearly picked.

+ When streaked out on EMBsm, the mixed culture of W1811 + W1177 showed plaquing in the thick streak. Origin of phage?

WG-16 also found to be lysogenic on same basis.

Confirmation of F+ transfer from this strain

9036

Jan. 28, 1952.

A) The following wg x are fairly clearly F+ :

WG: 1, 6, 11, 12, 14, 16, 21

B) The following are uncertain F+

~~WG~~ 3, ~~17~~ W1553

C) The following were inadequately tested

wg ~~7~~ + ~~13~~, ~~21~~, ~~(22)~~, ~~26~~, ~~27~~, ~~28~~, ~~20~~

Note: wg 22 is auxotrophic. A Mal- Lac- prototroph was obtained from wg 13 + W1177 selection

---

B: Repeat or verify with W1177

C: Repeat transfer of F+ to W1607 for comparison of F+ against number/flows wg.

Repeat 36, 41, 44

B

36	smeared.	+ ?	+
37		~~+~~ — def.	—
41		±	+
44		±	—
46	all −.		—
50			—
51			—
52			—

C. F(wg x) to W1607: 6, 11, 12, 14, 16 Label W1607 (F wg 6 ...)
Note: ~~D 12~~, ~~D 40~~ OK; D6, 11, ~~12~~ not F+ on first test See 908 0

D Transfer F+ to W1177: wg 6, 11, 12, 14, 16 ~~36~~ 21 ~~44~~ 17 ~~D44 not F+~~

E Test Kauffmann O Types (W1551 −) for F+ to W1177. Label per O-Type
example: E3 = W1553
Rebuilt 3, 6-12. all F−

February 19, 1952.

(Dennis) all recent attempts to repeat F+ transfer from W3x have failed. Technique consisted of brushing on sm loop full of W3x. Are these F+ perhaps reverted to F-?

Repeat 902-C-D by two methods: (a by direct streaking from mixed culture to E/MB lac (b by interrupted streaking on EMB surface.

lac-: D12, D14

a: C16 D16 C11 D11 — masscultures prove F+ proceed to isolation from single col.

C12, C14 turbid or impure. Reisolate

C-D 3, 6, — no signs of F+.

→ C3: lysed? colony motile (lac-). No peculiarity in matrials

b. C-D 3, 6 — no F+

C11 F+ (weak)
C12 F+
C14 F+ (weak)
C16 OK. F+.

D11 F+ strong
D16 no F+

Test from washed cultures

Isolate F+ from a, b as follows

C11	a	D11 a
C12	b	
C14	b	
C16	a	D16 a

Try 3 and 6 again

C, D11, 16 single colonies F- by pencil repl. plate test.

Redtest 2 single colonies via broth tubes.

	A	B		pool	single col.
D16	−	−			
D11	+, ±	+	✓		
C14	±	+	✓		
C12	±	+	✓		
C11	++	+	✓		
				C3	−
				C6	−
				D6	−
				D3	± 2nd
				C16	−
				C21	+
				D21	++
				C17	±

F+ transfers from mg×. 902cc

March 16, 1952.

A) The following are F+ transduced, but single colonies not yet recovered as F+ :

		S. col.	
D 3	•	none in pool.	
C 21	•	2 cols in #1, [redacted]?	
D 21	no F+ even in mass pool		
C 17	" • "	" " #4 pool?	
~~D 35~~	—	✓	single colonies: 8/8 F-?

tests passing: old [redacted] some F+ unstable?

B) Not yet transduced even in mass culture:

c 3	✓ ×	#1, 2, 4, 6/8	
c 16	✓ ×		
D 16	✓ ×	#/8	1 pol.×
c 6	×		
D 6	×		
c 17	×		

C) Not yet attempted:

C 35 ✓ - 1 colony in F+ bit of pool!
× ×

D) accomplished Rec 923

C - D 11
C - D 12
C - D 14
D 17
D 35
C 21 ?

Can wg × acquire F+.

1/26/52

1. Y19
2. WG-3
3. WG4
4. WG31
5. WG4 (W1713 Lac+)

6 903-F1
7 " G1
8 " G2

Grow propositus in 1ml Penassay overnight. Mix with W1817 12NZ6. — A27. Streak out on EMB lac, (A) reisolate Lac+. Grow these with W1177. (B) Reisolate W1177 streak 1/29 and test ×× W1607.

Notes: #2 +overgrow - in first step

#8 ~~W1177~~ W1817 carried along with lac+ in second step.

Save 903 A 6-8 for fertility test

Results × W1607

B.

1	+++		
3	+++	✓ Lac-	If reliable, this would indicate that
4	++	✓ Lac-	wg 3-4-31 could become F+ if not so
5	turbid		already.
6	+++		
7	+++		

A. 1.(×W1607) — ! Recheck (cf. B1) Br nucleus, ×W1607, F+

B

1	+++	
3	+++	WG4 } can become F+ on contact if not already.
4	+++	WG31 }
5	—	∴ WG24 is F-, remains so

Repeat: Compare original WG4, WG31 as donors of ~~F+~~ (=C) vs. F+ deriv. (=D) to W1177

✓ WG4,31 originals do not donate F+ to W1177 (✓ × 1607)

WG4, WG31 after exposure to W1817 become F+ donors.

		×W1607
W1177 (WG4)	C	—
	D	+++
(WG31)	C	—
	D	+++

Interactions of wg x F+.

February 10, 1952

Cultures from 902. C are W1607 F+ D are W1177 F+. (All 902)

1	C6	x	D6	− −
2	C6	x	1177	− −
3	C11	x	D11	− −
4	C11	x	1177	− −
5	C12	x	D12	++ ++
6	C12	x	1177	− −
7	C14	x	D14	++ ++
8	C14	x	1177	− −
9	C16	x	D16	− −
10	C16	x	1177	− −
11	W1607	x	D6	− −
12	"	x	D11	− −
13	"	x	D12	+ ++
14	"	x	D14	+ ++
15	"	x	D16	− 1 col ?

~~16~~	~~58-161~~	x	W1177	
17	A	x	"	3
18	B	x	"	+++
19	C	x	"	2
20	D	x	"	++

A = aerated overnight B = remoistened 10 min. ea. 3 hours
C " " " 6½
D " " " ~~each~~, 6½ hours

~~All~~ cultures harvested at 11 AM; 1:15 PM; 4:45 PM and refrigerated to 5 PM for plating

Reisolate W1607, W1177 (F+, wg x)

W1808	x	1177	+++	
		1817	+~~+~~	
1809		1177	−	} of 1809 F+.
		1817	+	

W1611	x	1607	±	turbid
		1875	++	
		1177	±	
		1876	+	

behaves like a partial F−

W1611		161	++	
		1607	1 col	
		1177	−	
wg 4		1817	~~+++~~ −	

F states ?

1205	1607	turbid	−
	1875		−
	1611		++

W1145	1607	turbid	−
	1875		+
	~~+++~~ 1611		−

Segregation & role of F+ in outcrosses

904

January 26, 1952

1/27 (+M)

A	W1446 wg4	× W1607	1/21 T		
B	"	W1816	++	T	4 n.g.
C	W1451 wg3	W1607	12	wg3 probably F+	
D	"	W1816	18		
E	1446	× 58-161	28		
~~F~~	"	× ~~W1830~~	~~6~~		
G	1451	× 58-161	40		
~~H~~	"	× W1830	~~1~~		

900E1

~~Status of W1830~~ 4 n.g. ~~is still doubtful~~.

PM

~~Aseptic~~ ~~to E.H.S~~ Cenaya on fuchsin (0).

In view of 903-3, "segregation" would not be meaningful (Unless F+ can not be transmitted extracellularly in minimal.)

W1868 (wg12)	×	1808 . 31	+++
		1607 . 1-	+++
		1875 . 1+	+++
		1451 . 3	+
W1808 (wg31)		1451 . 3	++
		1868 . 21	+++

W1811 Stability: Summary & Expts.

Lysogenicity

1/28/52

In course of 902 - 14A, plaques were noted in W1177 that had been grown with W1811. The lysogenicity of W1811 / 1177 was confirmed. The plaques are readily seen on sm agar (suppressing W1811 bacterial growth.) Check other post-cultures from Maas! all of his strains proved to be lysogenic on W1177. Their history is given as K12 → * KIT (ts) → KI put aboard. Presumably phage entered (mutated?) at *. ↓ ↓ Since KIT series is futile, the phage is not related to W1811 stability.

1/28 Grow W1811 i and s 58-161, W1177.

1	58-161 + (W1177 · 1811)	+	W1811 stability is not absolutely transferred in mixed cultures
2	(58-161 · 1811) + W1177	++	
3	( ) + ( )	++	
4	58-111 + W1177 + W1811.	+	
5	See 897 for control, 902-14B.		
6	(58-161 + 1811) + W1817	+++	

2/1/52 Further work on this phage assumed by E.T.L. W1811 has been verified as F+ (902). The phage acts equally on W1177, W1817.

1811 ×	14/51	wg 3	~~cont?~~	
	1611	4	−	
	1607	1 −	−	
	1868	12		
	1808	31	! ?	: Repeat → 4 colonies
	1205	wg 29	5 colonies	
	1145	wg 2		

W1678 - aeration effects and F character 906

1/29/52.

1/26/52: 1678A × 1607 ++++ F− !
1816 + F+ !

Repeat 1/29-30/52.

				1/31	2/1
1/30	1	W1678 ×	W1607	++++	✓
	2	"	W1816	+++	✓
	3	"	W1177	+++	+++
	4	"	W1817	+	9 cds.
	5	W1678aer	1607	+++	✓
	6	"	1816	±	+
	7	"	1177	++	++
	8	"	1817	−	−

1678	1830	−
1817	1830 good	−
1177	1830	−

: Of aeration has any effect on W1678, it is to decrease its fertility with F+. Do W1678 a "super" F+.

pl 907

Growth conditions and the F- phenotype

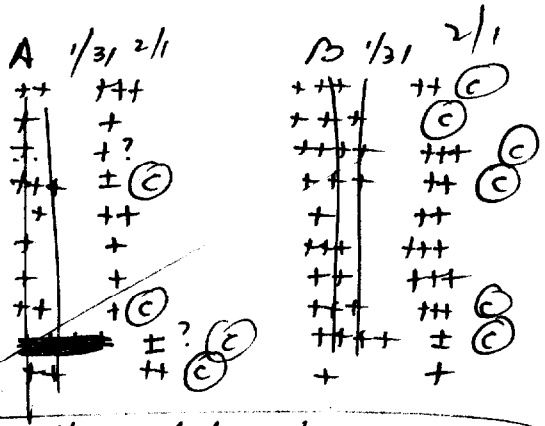
58-161

January 30, 1952

	Temp.	Vessel	Medium		
1	37	10 ml tube	meat	× 1177	A
2	"	Plate	liquid	× 1817	B
3	"	tube	aer		
4	"	Plate	agar		
5	30°	tube	meat		
6	"	"	air		
7	"	plate	liq.		
8	"	"	agar		
9	44°	10 ml tube		v. poor growth	
10	"	plate	liq.		

25-30°

A



As in previous expts., adjust approx. for cell density.

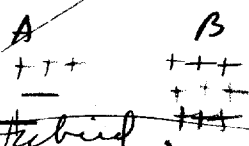
Note very high yields of 44° cells!

Many of these plates show contamination: (c)

Repeat comparison of 30°, 37° (30° thermostated).

58-161

1	37°	–
2		air
3	30°	–
4		air



plates hybrid: ~~contaminated~~

B

These results all n.g. owing to contamination

C

Growth conditions and F-phenotypy of various strains

2/1/52

	58-161 Temp.	Aeration	A.	B.
1	37	+	× W1817 — (2 cols.)	× W1817 +++
2		−	+ ++	++
3	30	+	± (3+ 4 cols.)	++
4		−	++	++
6	1678-37°	+	× 1607	+++ +++
6		+	1816	− 7
7		+	1177	+ +
8		+	1817	− −
9	1678-37°	−	1607	+++ ++++
10		−	1816	+ +±
11		−	1177	++ +++
12		−	1817	+ 4
13	1678	+	58-161	+ 5
14		+	58-161 A	+++ ++
15		−	58-161	40
16		−	58-161 A	++++
17	1816 ·		1177 ·	+++ (contains.)
18	1816 A ·		1177 ·	− 16
19	1816 ·		1817 ·	+19
20	1816 A ·		1817 ·	+70
21	1817 ·		1607 ·	+++++
22	~~1817 A~~ 1177		1607 ·	− −
~~23~~	~~1817 A~~		~~1816 ·~~	
24				

∴ 30° does not ameliorate aeration

1678 and especially 1678A are relatively infertile with F+, highly fertile with F−

Does 1816 respond to aeration?

2/2/52	23	161	~~W1817~~	−	++	(aeration was interrupted)
	24	161	W1817	++	+++	
	25	161 A 37°	1177	+	++	
	26	161 A 37°	1817	++	++	
	27	161 A 26°	1177	+	+	
	28	161 A 26°	1817	++	+++	
	29	161 A plate 37	1177		5	
	30	161 " 37	1817		+	?

W1817 crosses ~~[illegible]~~ show [illegible]

2/1	31	161 44°	1177	++	∴ 44° does not produce F− non-inducible fertility.
	32	"	1817	++	
	33	"	1177 44°	++	

Conditions of aeration for F-phenocopy 907b

February 4, 1952.

		A × W1177A	B × W1817	C = W1177	
1	58-161 37°	++	++	++	} inconclusive 1
2	" aer	+	+++	+	
3	" 26°	—	+++++		
(growth same as 3) 4	" aer.	—	++++		
5	W1816 × W1177A		+++		} inconcl.
6	W1816 A × W1177A		+		
7	W1816A × W1817		++		
8	W1817 × W1607 A		+++		} inconcl.
9	W1817 A × W1607 A		++		
10	W1817 A × W1816		—		

× 1177. all cells washed, stand in saline 4 8PM

11	161 37°	heavily grown	++	
12	161A	"	6	
13	"	"	±	} dilute media
14	"	28°1/10"	—	
15	"	26°	1 colony	26°!
already heavy 16	161A. Reinoc 10AM from 11		145 PM. +	
17	" " "	12	" +	
also heavy. ~~18~~	~~15 continued~~ to		—	
~~18~~	~~16 Reinoc~~ 2PM - 5PM			
19	16 " " "		++	Moderate growth = unsaturated
20	17 "		+	" " saturation

1. low temperature; dilute media do not mitigate aeration effect. Phase of culture cycle? (Cf. 19-20 vs. 11; 16, 17) 12

Suggested experiment: Aerate cells to saturation. Assay. Reinoculate and assay at partial, complete saturation.

See 907c

A = aerated overnight B = reinoculated, aer ~~8~~ hours
C " " "
D = reinoc noaer.

21	58-161	× W1177
22	"	A
23	"	B
24	"	C
25	"	D

cells harvested at 10:30 AM; , refr. to plating at PM.

aeration effect

2/10/52

A are aerated overnight (2/9 - 2/10 5PM - 12M).

1	58-161 × W1177	+	+
2	" A × "	−	−
3	909-1 × W1177	+	+
4	909-1 × W 909-4	++	++
5	909-4 × W 1607	+++	+++
6	W1607 × W1177	−	−
7	909-1A × W1177	−	−
8	909-4A × W1607	−	10 col.
9	909-1A × 909-4A	−	−
	909-1 pur × W1177	+++	
	909-4 pur × W1607	+++	

2/13.

11	1875 × 1177	++	++
12	1875 × 1876	+	++
13	1876 × 1607	++++	larger, more numerous than 12 & 11
14	1607 × 1177	−	−
15	1875A × 1177	±	+
16	1876A × 1607	±	+
17	1875A × 1876A	±	8
18	58-161A × 1177	−	1
19	58-161 × 1177	++	++
20	58-161B × 1177	−	6

} little effect of aeration.

B = 58-161A + Pennessay 4:30 − 6 . See 908-15,16

∴ no recovery in this interval.

2/15

21	58-161	× 1177	+	1
22	58-161A	× 1177	÷	
23	58-161B	× 1177	+	

B = 58-161 alcpriot suspended in supernatant of 58-161A for 2 hours, & then sedimented.

∴ 58-161A supernatant had no effect on 58-161

Transduction F+.

2/1/52

ca. 10^9 each cell type in ~~Penassay~~ 3 ml. $3^{30} - 4^{30}$ PM.

				× W1607
1	58-161 + W1177	Bre.,	Penassay	+++
2	58-161 A + "	"	"	+++
3	58-161 + W1177	Defr.	"	—
4	W1678 + "	Bre	"	+++
5	W1678 A + "	Bre	"	+++
6	58-161 + W1177	Bre	D(o)	—
7	" "	Bre	D(o) + MTLB,	—

For assays pool 2 (A) and 10 diffuent (B) W1177 isolates.
× W1607 . . 2/6 A and B agund in each case.

∴ F is transfund in Penassay but not in synthetic medium under comparable conditions. This agrees with behavior of three-way crosses. Try growth in synthetic for longer periods. Brenlation also seems to be necessary. Aerated cells, presumably phenotypically F−, still transfer F+.

8 58-161 + W1177 in D(TLB, BM) aerated. ξ overnight.
9. " " " " " "

Reisolate W1177 by streaking out and via sm.

The labels on 8, 9 were unfortunately lost. What was probably 8 failed to show transfer; in 9, 10/14 isolates were F+
Repeat experiment 2/11/52 (was 10:20AM) – 5 10PM)

8 1F+/15 tot
9 5F+/12 tot.

also streak after overnight. 8-9A. 1st Replica tests n.j.

Transmission of F+

Febr. 12, 1952

11 —
12 sus 1000/ml. —
13 heat 56° 30m. —
14 boil 5m 1 colony ?

It has been previously established that W1811 washed cells do not evoke fertility in W1607 x W1177 on D(O) agar.

add ca 2×10^9 cells W1811 1 ml to 1 ml Penassay + ca 4×10^9 W1607.
Treat tubes as indicated. Incubate together from 12 30 — 2 PM.
(Pretreat W1811 for heat experiments etc...). Rewash. Plate with W1177
This transfer technique; assay m.g. Phenotypic delay in F+ ??

15 58-161 + W1607 in Penassay
16 58-161A + W1607 " " of 907 20.
test single colonies. Replica tests m.g.

Phenotypic ~~lag~~ + F+ transduction.

February 16, 1952. W1305 ⇒ W1177. Ca 10^9/ml in Penassay. 37° 2 PM — 3 30

		×1607, immed.	
21	W1177 in Penassay	—	Single W1177 exists.
22	(W1177 + W1305) in Penassay	+++	(Plates contain. but ca 10/5 F+)
23	W1177 original	—	
24	W1177 (Pen.) + W1305.	—	

1305 controls all —. Phenotypic delay in F+ is not
borne out by this experiment.

Reactivation of 7/1 ?

90 76

2/19/32.

W1305 from 2 TSA plates to 10 ml. 1 ml to 10 Penassay. Latex old
Ca 10^9/ml each. W1177 = .

x ~~W1177~~ W1607 | 2 and 3 sterile
1 ca 2/1305 : 1 1177

1 No treatment. Incubate together 3PM – 5 30
2 Bath 5 mins
3 Heat 56° 30 mins.

1. showed many protoplasts; 2 and 3 – ?

but plates are contaminated!

Repeat.

4 Control 1305 + W1607. x W1177. +++ Contam ?
5 Heat 56° 30m.

W1305 : .1 ml sterile

W1305 stock ? or plates contam.?

F+ from different sources.

January February 1, 1952.

Mix culture: Grow overnight. Streak out on EMB Lac; EMB lac sm Rectivals from sm to EMB lac → pure cultures. Pick from 1-5 cultures for pool for initial tests. Not s.c. pure!

1 1607 K12
2 " 58161
3 " 1678
4 1177 K12
5 " 58161
6 " 1678

✓ by crossing to W1177 or W1607 resp. all now F+

		x			
1	2	1177	++	+++	
2	2	5	++	+	
3	2	6	++	+	
4	2	1678	+++	++	
5	3	1177	±	++	
6	3	5	±	+	
7	3	6	+	+	
8	3	1678	++	+	
9	5	58-161	+	+	
10	5	1607	++	++++	
11	5	1678	—	4 cols	
12	6	58-161	+	++	
13	6	1607	++	++++	
14	6	1678	—	2 cols	
15	1678	1607	+++	++++	
16	1678	1177	++	++	
17	1678	58-161	++	+	
18	1	4	+	+	
19	1	1678	++	++	
20	4	1678	+	6 cols	

Summary.

	F−	F^{161}	F^{1678}	F^{K12}	(BM test)
W1678 ×	++++	++	+/++	÷ ++ ÷ ++	÷
1607 (F_{1678})		++	+	+	
1607 ($F_{58\text{-}161}$)		+++	+	+	

Note 1 × 4. Compare

1 × W1177
4 × W1607
1 × 4
1177 × 1607

∴ W1678 is relatively infertile with F+ whether derived from 58-161 or W-1678 or K-12.

F− reinfected with F+ from various sources behave in the same way. There is no evidence that W1678 carries a different F, but the infertility of F+ × F+ is emphasized especially in × W1177 F+.

2/5/52.

Grow 08161 30° 3 TSH plates. Harvest, wash x 4 ml, dry overnight

2/5/52 Extract H_2O ca 5 ml. → A) Sup. B) Sediment

Extract B with 1/2% saline overnight refrig.

Add 1 ml supernatant + 1 ml 10^9/ml W1177 to 5 ml Serum.

Inc 5:05 PM - 8 PM Streak out on EMB lac

2/6/52 No lact+ colonies seen. Pick individual and pool colonies

(1) Replica xx test from EMB Streak

(2) Test 4 single colonies, and ca 40 pooled colonies.

x W1607.

all F− Crude extract: no transmission

Aeration effects

Febr. 19, 1952

I. Harvest 58-161 from 2 TS Agplates. Ca 3/4 of this into 10 mC fresh Penassay (~~broth~~ & 10^{10} cells/mC) aerate 12:15 – (3-4 PM ?) = B.

1. ~~58~~-161 ×W1177
2. 58-161 A plates contam.
3. " B + ×1876 +

Repeat: aerate in Penassay organisms of 58-161 A = B.

4 58-161 B
5 58-161 A
6 " + W1305 in Penassay. (Und 58-161 ca 10^9/mC. 12:25 PM – ") ×W1177 — contam
7 " —— " " ++ contam.
8 58-161 +++

Compare transfer (F+)

~~Transfer to~~

2/17/52.

W1177

1. 58-161 + (W1177) in Penassay — Ca. 10^8/inoculum.
2. " " aerate — 12 hr — 2P
3. 58-161A + (W1177) "

Stationary and test results

1	2+/8
2	0/12
3	2+/12

∴ 58-161A donates F+ · Aeration may inhibit transfer

1½ hr. min. Penassay

1	58-161A	x W1177	—	
2	" + W1305		2	
3	" + W1305A		1	
4	" + W1811		6	
5	" + W1811A		4	
6	" + W1607		5	—
7	58-161 + W1607		18	—
8	58-161A - incubated		2	
9	W1607 + W1305		—	
10	" + " A		—	
11	" W1811		—	
12	" " A		—	
13	1305	no X	—	
14	1811		—	

58-161 x comp?

From 4 and 5, strain F+ may have stimulated 58-161A but exchange even to W1607 was limited. Note: W1607 was aerated!

Todo: Compare 1607A ; 1607 as receptors of F+ from W1305. But note also low yields on 7.

W112 x W1435
Structure of diploids

2/19/52.

W112 x W1435
lac 1b- lac 1a- V_6^R Het.

EMS lac.
D/O → EMS lac } ca 15 plates
ca 100-200/plate.

3 lact found. 4+ mixed set 4 in 3d.

	1	lac ++	R	–
	2	lac ++ slow	S	–
	3	lac ++	R	– 1.
H304	4	lac v –	S	RS
	5	lac ++	S	
	6	++	S	
	7	++	S	
	8	++	S	
	9	++	R	
H305	10	lac v	S	
	11	++	R	
		W112	R	
		W1435	S	

~~Ctrl. #2. ca 1 lac-~~

4R 7S

lac- 1st 1b lac- 1st 1b

Test segregants (mass streaking) of H-304-5.

H304 6 lac+ all S 5 lac– all R
305 7 lac+ all S 5 lac– all S sic!

H304 is therefore presumably A; H305 may be B.
(may have arisen by mutation in a $\frac{+-}{-+}$!)

B: or { ——— ——— ——— ——— } A

If so, the lac – of H304 should be stable, i.e. A-B-V_6^R
of H305 should be unstable A-B+ V_6^S.

Picking the mutations of these – (pooled) and compare with parents.

Re-streak single clones, test +/+, 1- from each. I6.

H 304 7 lac- 1S 6R 8 lac+ all S

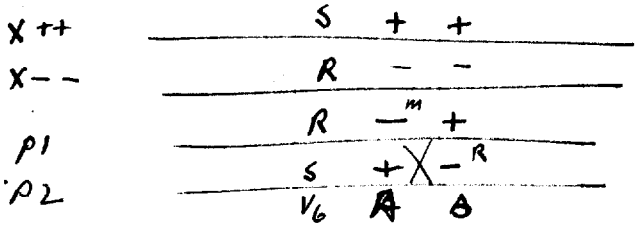
H 305 9 lac- all S 9 lac+ all S

This confirms previous page.

Check pooled lac- for unstability. of parents

	48h. EMB	lac	
H 304	M	V_6^R	
H 305	S	V_6^S	
W1431	M	V_6^R	
W112	S	V_6^S	
Lac+		V_6^S	

∴ These two lac- component appear to be parental, not complementary crossovers.



H 304 = X++ / P1

H 305 = X++ / P2

Repeat cross 3/13/52 8 plates EMB S lac (×400) 1? lac+ ✓ Saved as H308

9/3-12 slow? lac+ 1-

3/17/52. 4 × 600 2 Lac+ 9/3:13-14

13: probably lac v, Sup. 12

14 lac+

3/27/52. 8 × 400 cols. 4 Lac+ ✓ + v? → weak + few-sg? + + 15, 16, 17, 18.

5 × 300 0

February 22, 1952.

Closest comparison would be W1368 $^{+R}$ x W677 $^{-S}_{+S}$ x W677F+. Latter is being prepared.

2/22. Crosses on D(o) ± sm.

		D(o)	D(sm)	
1.	58-161 $^{+S}$ x W1177 $^{-R}$	++	+	=
2.	W1368 $^{+R}$ x W677 $^{-S}$	+++	—	=
3.	W1802 $^{-S}$ x W1876 $^{+R}$	+++	—	
4.	58-161 $^{+S}$ x W1876 $^{+R}$	1	?	—
(5)	W1875 $^{+R}$ x W677 $^{-S}$	+++	—	

] agrees with Hayes

2/25

11	1368 $^{+R}$	677 $^{-S}$	$\frac{4}{2}$		$\frac{3}{4}$ +++	—
12	1368 $^{+R}$	677F+ $^{+S}$	$\frac{1}{2}$ +	SM ±	$\frac{3}{4}$ ++	0 SM ± — 1
13	1607 $^{-R}$	677F+ $^{+S}$	$\frac{1}{2}$ ++ ✓	++ ✓	$\frac{3}{4}$ +++ ✓	—

(1-4): ± (14 cols.)

2/26

14	1802 $^{-S}$	1876 $^{+R}$
15	58-161	1876

F+'s pooled. (1-4)

∴ Hayes' observations are again confirmed. Also 677F+ becomes competent on sm ages, and the sm effect is related to compatibility.

677F1- are F+ / F+ F+ / use F1 - mate = W1876

			o	SM
16	1895	1177	+++	+++
17	"	1896	++	+
18	1811	W1177	—	—
19	1678	1177	+++	++ ±
20	1678	1876	1 col.	—
	~~1895~~	1811	2 ?	

note near sterility F+ x F+

Repeat a). (old 1368 swap.)

	o	SM
11	++	—
12	+	—
13	+++	—
14	+++	—
15	++	— ?

b)

Result not confirmed!

o	SM	
~~0~~ +	1 col?	medium ?
8	"	
~~0~~	3	
++	—	
+	—	!

Try 1678 ^{S} R

		D(o)	D(sm)
3/10/52.	W1368 x W677	++	0, 0
	x W1896	+	2, 1

conclusion upheld (F+ and resistance to sm-ffute, but not statistically!

3/12/52. Repeated:

		D(o)	D(sm)
W1368 x	677	+++	0
	1896	+	3

Again. also note greater compatibility of F+ x F-.

~~The survival factors may be due in part to the low fraction of s^R protoplasts in the 1896 x. Rather than using a comparison of~~

Repeat the test cross for role of F+. F+ x F- combinations maybe more futile

				EMS/Mal	D(o)	D(sm)	
A	W1876 x	58-161	ca 15%	+++	—	—	! of W1177 x 58-161
B		W1802	"	+++	—	—	

Repl. to EMS Lac, Mal

as before, 1802 and 58-161 are similar x W1177 F+

Repeat x 1177 also! see 915.

				D(o)	D(sm)	
1/29 A	~~58-161~~	x	W1177	+++	+++	∴ Hfr behaves as F+
B	"	x	1876	+++	+++	in this context.
C	1802	x	1876	+++	—	
D	58-161	x	W1177	+	3	Yields generally
3/19 E	"	x	W1876	++	—	low. Bears repeating.
C	W1802	x	W1876	+++	—	

		D(o)	→	D(sm)	D(sm)	
3/22 D		++		5^S / 49	20	Note instability of C
E		+++		56R / ca 200	—	on sm as reported.

Proportion of breakthrough / segregants s^R is evidently greater for D than E. F+ x F- S^R differs from F+ x F+ S^R.

This consistent with the concept of "relative potency" of BM and TL whereby BMF+ x TL is actually BMF-!

Equivalent of parents in F+ x F−

A. W1802 (BM F−) × W1876 (F+)

B ~~58~~ 58-161 (F+) × W1177 (F−) ± Sm (A+ ; B−)

C. 58-161 (F+) × W1876 (F+)

also see Smith, 1947.

915a was conducted by Mrs. D. C. Gosting. It appears to show that W1177 F+ behaves like filial W1177! Repeat and of other F+ strains.

3-3-52
DG

A

lac	mal	SM
+	+	S
+	−	S
+	+	S
+	−	S
+	−	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
−	−	R
−	−	R
+	−	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	R
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	R
+	+	S
+	−	R
+	+	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	S
+	+	S

B

lac	mal	SM
−	−	R
−	−	R
−	−	R
−	−	R
−	−	R
−	+	R
−	−	R
+	−	R
−	−	R
−	−	R
−	−	R
+	−	R
−	−	R
−	−	R

B cont.

lac	mal	SM
−	−	R
−	−	R
−	+	S
+	−	R
−	+	S
+	−	R
−	−	R
−	−	R
−	−	R
−	+	S
−	−	R
−	−	R
−	−	R
+	+	S

C

lac	mal	SM
+	+	S
+	−	R
+	+	S
+	+	S
+	−	S
+	+	S
+	−	R
+	+	S
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+	+	S
+	−	S
+	+	S
+	+	S
+	+	S
+	+	S
+	−	S
+	+	S
+	+	S

			mEMS Mal	D(o) Rep	bac	14al
note near equal +/-	A	58-161 ×	W677	− > +	24+ : 20− ;	20− : 5+
	B	"	W1177	− > +	~~28+ 18−~~ ! !	40− 6+
	C	"	W1817	+ > −	50% −	8− 62+
	D	"	W1876	+ > −	5% −	<10% −
	E	"	W1896	+ ≥ −	61 + 6 −	ca 10% −
~~⇄~~	~~≡~~	1368	1896		10+ 4−	
		"1368	677		23+ 29−	

. . . "aberrant ~~⇄~~ behavior of filial TLB, - stocks is entirely explained by their F+ character !! Instability of segregation ratios is fully explained.

Is phenotypic modification of TL F+ parent possible (by acetate ?)

See 915a for quantitative data here quantitatively confirmed.

Glyfed

			Lac	Mal	D/O	Lac in D/O	EMS lac or Mal.
A.	W1678 ×	W1607	−>7+ −>7+	+++	−>+		no effect of F+.+ on lac segregation obscured by linkage? cancer inhibit.
B	"	W1875	3− 2−	++		−>7+ !	
Prof/lac; linkage? C	"	W1177	9−	3+ 12− ++	−>+	ca 20% M+	
D	"	W1876	2− 14	1− 21	all lac−?	6M+ 12−	
Cyst Meth. E	W1687	1607		++			also mostly SR
F	"	1875		++			
G	"	1177		1−			
H	"	1876		++			

J.	58-161 × W1177F+ : 902	D 12	Mal:	13+ : 13− !	Partial effect ?
K		14	Lac:	ca 90%+.	
L		17	Mal:	ca 35− ? 8+	minimal effect !
~~M~~				numerous sectored cols.	
M	1802	D12		ca 90% lac+	
N		D12		" "	
O		D11		1 Mal− 2M+	low fecundity !

Restreak D17 to verify purity.
Repeat D12.

A-B. Lac segregation similar in W1678 × W1607 — 1875 (F — F+)

C-D " " " " " ; Mal+ may? be increased in × F+ ?

C-D should be repeated.

	Lac	Mal	M+
C	ca 10%+	4+ 1−	32+ 12−
D	2+ 10−		2−

		Mal		
1802	1177	ca 5% −		
1895	1177	ca 10%+	many sectored	
"	1876	"	"	"

Thus confirms:
a) 1802 ≅ 58-161 × 1177
b) 1895 × 1177, 1896 ≅ 58-161 × 1177

Repeat J-O ^ & single cols.

		#	%lac	Mal	
58-161	D17	-1	− ≥ +		∴ D17 result above probably due to admixture
		-2	+ > −		
1802	D17	-1	[illegible]		
		-2	+ > −		
161	D12		ca 30%lac−	ca = +, −	D12 should also be repurified and checked
1802	D12			ca 30% Mal−	

915
SUM

Conclusion: in following crosses the lac and Mel markers follow the parent indicated first:

	×		F (key fertility)	
	W1177, W677	58-161	−	+2
	58-161	W-1876, W1896 (also W477, etc.)	+2	+3
	W1802	W1876	−	+3
	W1607	W1678	−	+5
	W1875	W1678	+2	+5
	W1177	W1688	−	+5
	W1876	W1678	+3	+5
	W1687	W1875	+1	+2
	W1687	W1876	+1	+3
	W1177	W1687	−	+1 nearly infert.
	W1607	W1895	−	+10
?	W1177	"	−	"
	W1875	"	+2	"
	W1876	"	+3	"
	W1607	W1649	±	+..
	W1177	W67	−	±

~~Sa921~~ 921, 915d. 916a.

Hfr. 916

2/27/52

"Hfr" received again from Cavalli ca. 2/24/52, after retests on W1033 showed no Hfr activity. Store as W1895.

a) Platings of W1895 at 10^8 and 10^6 per plate, in comparison with 58-161 showed 100-1000 x as many prototrophs

b) Effect was same x W1177, x W1876. ∴ does not depend on F- Opposition;

c) In one experiment, aerated Hfr was still F+ (same yield vs. 1177, 1876) but not highly fertile as Hfr. Control showed macerated Hfr still +++ fertile.

d) 1895 dil. x W1611, 1678 ⇒ few prototrophs! Repeat

e) 1895 of. A. showed Hfr x W1177 ± aeration
also Hfr x 1876 does not accord with c).

3/10/52 f). 1895 - 1895A. x1678. Showed Hfr from dilute plating, but A +++ / ++
x 1177 A ++ - ++++

This may reflect a partial F- phenocopy effect of aeration.

g. Grow W1895 in broth with W1607, W1177, W1876. Streak out, recover, and test by replica plating

A. 1895/1607. 1895 12 cols. Hfr x W1177
1607 2 " F- or Hfr? = 916G-2

C. 1895/1177 → 11 cols
1876. → 11 " } Hfr x W1177.
∴ No back dissimulation

B. 1177/1876/1895. 1177: 8 cols. + Hfr? x 58-161
1876: 11 cols. No cols x 58-161
Both: No cols x W1607.

Is this W1177 F- Hfr? Recheck after isolation. = 916G-1

(These mixtures should be repeated)

W1607/1895 = 916G-2

3/12/52 M. Recetion: subcult.

	conc.	1177	1876	dil.	1177	1876
1895		++++	++++		all ++ - 2/3 Lac -	all ++ - ; 2/3 Lac -
1895A		+	+++		1	4

Repl. to EMS lac.

student out on NSA: By replica 20/20 all Hfr!

∴ again, there seems to be ① an incomplete effect of recetion on the F+ character and ② a depression of Hfr in compatible crosses.

Note absence of F+ effect of W1876 x 1895 cf. 58-161

3/12 i. Repeat, using "moderate dilution"; ca 3×10^8 cells 1895 or 1895A per plate

	1177		1876	
1895	1	+++	2	+++
1895A	3	++	4	+++

No marked effect.

Repl. to EMS lac.

lac - : lac + Estem.

1	3:1
2	2:1
3	2-3:1
4	3:1

∴ again, W1895 x F+ shows no modification of segr. ratio!

916 "gA" 1-2. 1607/1895 Both gave seemingly very high yields with W1876, — with W1177. Same questions arose with W1177/1895. See above. ?Hfr. gA1 = 916 & 2.

W1875 tester was day-old. Today's broke!

916 & 1	x 1607	—	
	x 58-161	++	
	x 1875	+++	
dil.	x 58-161	29	
1177"	x 58-161	10.	

Closer comparison required!

as half-plates:

916 & 1 } x W1875 { — !
W 1177 } { ++

916 & 2 } x W1876 { +++
W 1607 } { +++

Is close comparison they were very similar 3/21/52

Is 1895 x W1876 less fertile than 1607 x 1876?

M Repeat transfer of F+ from W1895: Grow with 1607, W1177 in hemizygy. Resistant as EMB sm agar. Pool 40+ colonies:

C W1607 / 1895 / x 1177 —
D W1177 / 1895 / x 1607 —

∴ again Hfr does not transfer F+!

① Is F+ found in Hfr? ② Is it absent? Test pooled prototrophs from 1895 x [redacted] 410

Summary

3/11/52. h. aeration of W1895 → ~~no~~ a partial effect on F+, Hfr.
Histidine plates, 1895A on nutrient agar gave 20/20 all Hfr!
1895 gave normal bac, Phal- ratios × 1177, or 1876

3/12. i Similar result.

j. W1607/1895 Neither were modified, either re F+ or Hfr.
W1177/1895

k. Similar result. → l. do. But 916b1 on half plate test was −. (presumably mor cf. k.)

m Retrial of j, via Sm agar, for F+ from W1895.
Both W1607, W1177 (pools of colonies) remained F−.

3/16 n Retrial of m. 1607, 1177 → Remain F−, with +++, ++ × F+ testers.

o 1876/1895. ✓ O/K. 1876 remains F+.

p. Retrial m:

		1607	1895
A	1177/1895	+++	++
B	1177 / 1033	++	+
C	1177 / 58-161.	++	+

Results: (1607) ! − + A presumably reisolated 1895 (fact) proved to be impure (fact) Repeat! — Do this correct?

q. Is 1895 more futile than 1607 × 1876? Genetic study may be required.
Hfr does seem more highly futile Test 2.
about =. Test 2

R. Repeat p

		× 1607	× 1895
A	1177/58-161	+++	++
B	1033	+++	++
C	1895	−	+++

∴, as before, Hfr does not reproduce F+!, but W1033 does.

Methyl-naphthaquinone effects.

3/8/52.

Cf. Colwell 1946 J. Bact. 52: 417.

D/O) + MNQ (from 1% solution in acetone). Strain K12.

		A 24h.	B 48h.
1	0		++
2	.0002 %		+
3	.0005		+
4	.001		+
5	.002		−

A. Streak out on nutrient agar. No "petites" observed.

B. Re-streak. No petites " . #5 was sterile.

Critical concentration may be between 4 and 5.

C.

		A) 24h	B) 48h	A	B
1.	0	+++	++++	A No petites	
2.	.001 %	+	++	" "	no petites.
3.	.0015	−	−	Few saw. pet?	Streak
4.	.002	−	−	"	"

3/19/52 Colwell's observations are not confirmed with K-12! Write him for details

3/27/52 Repeat with Colwell's #II strain = 776-1763 = W1939

		24h	colonies	48h.	colonies
1	0	++	++	+++	−
2	.0005	+	++	++	++
3	.001	−	++	−	mostly +, some dw? Restreak.
4	.002	−	some dw?	−	+ / dw? (practically sterile)

"dw" grew out to full size.

Repeat 4/21/52

		24	48h	colonies:
1	0	++	+++	
2	.001	−	++	
3	.0015	−	−	one small but gave +++ on re-streak.
4	.002	−	−	

Colwell specifies previous transfer on minimal. Try this with W1939 A (single col. isol.) No luck.

September 16, 1952.

9/13. Colwell sent strains again: #1 = original coli transferred on minimal medium. = W1939A
#2, 3, 4 = "SCV" selected with guinine.

9/14-15. Characterization of SCV verified. #1 grew promptly to give large colonies on NSA. #2-4 gave barely visible colonies in 24 hours. By 48 hours, ca .2 mm colonies.

9/15. Inoculate from plates to D(o); Penassay:

9/16: All cultures grew very well, #1 perhaps slightly denser
By D(o) #1 grew more poorly than others! for further work, use 1 and 2 only.

9/16. Inoculate 917-1 and -2 from Penassay to:

	1	2	2days
D(o) liq.	++	+	
Penassay	+++	++	+++
NSB	++	+	
NSB+glucose	+++ acid agglut	++	
D(o) Agar	++ 2days	±	
NS Agar	+++	±	
EMB Glu	+++	+	
EMB Lac	+++	+	

Also, mix 1 and 2 ca 1:5 and streak out no satellite effect observed.

Growth on D(o) comparable to NB, NA. ∴ not likely single tryptophane requirement. In her paper, Colwell refers to MacLeod's "synthetic medium" but does not specify whether HC was added.
In experiments with W1571 (HLB yeast) MNG .002% was lytic in standing tubes. Guinine was lytic only in aeration. Usefulness in place of penicillin still to be verified.

Waksman strains
= W1906

March 10, 1952.

A. W1177 + W1906 in Penassay; streaked on EMB Waksm. Waksman plaque not again
Pick single colonies for F+ test. x W1607. 5 singles — | 6 singles
3 +
3 −
Isolate single colonies pool x+ ++ | pool++
= 902 D35 +3

B. Crossing tests: auxotrophs.

W1907 = 1908 — 1909 control platings —.

1907 x 1909 —

1907 x 1607 —

1875 —

1909 x 1177 ~~+++~~ contaminant coli? Replic to EMS lac: poor growth. prob. contam.

1876 — Repeat

C. W1846 — —
" x 1177 —
A " x 1876 4? → all lac− prototrophs. Fertility supported.
B " x 1607 1 Mal+
" x 1875 —

D. Waksman. SRP x

1177, 1876, 1607, 1875 } rare Mal+ no − each! This would have been classified as doubtful futile to be retested.

E - F (W1852; W1909)

		E	F	
1	1607	—	1?	
2	~~1875~~ SD 161	—	++	ng
3	1177	— (1)	—, —	
4	1876	—		
5	1808	—	4	
6	1678	—	—	ng

E3 grew others did not

∴ part TS strains may be detectably futile, others not (yet)

1177 + 1909 : grown up and together : —, —

1875 x ... ?

WG-35 x

918a

March 28, 1952

1.	W1852 ×	W1177	37°	=	
2	"	"	+ pant.	=	
3	"	"	+ pant. 30°		ca 20 cols. eventually
4	" ×	W1895			
5	W1846 ×	"	+ phage-ridden		
6	W1907 ×	"	1 " "		heavy background lysis
7	W1908 ×	"	ca 1000		

∴ Hfr does allow crossing of wg35 × wg 1., but W1908, not W1907! (φ^R tester should be used)
EML is working on the extraction of Wollmans phage and transfer to wg-1.

---

ca. 4/10/52. EML noted Wallas. to be sucr + - ± on EMB sucr.
Comparison of W1906; W1811; W1852; K12 shows the first 3 to be alike, strengthening conclusion of origin from wg 35. Cf. Shaw for cross-reaction (H?) of wg-1 × wg-35.

4/15/52. Strains received from Maas: K1t-p A) K-1t h2 B).
Grow together in Penassay 3h. Plate ca. 3 ml per plate

W1177 · A	No prototrophs	
W1177 · B	"	
A · B	Minute colonies; background growth (cytotoxicin?)	
A ·	1-5 " "	.
B.	" "	.

?? Was K1t-p or -h2 ever properly crossed with # wg 1? as claimed by Maas? WG-35 behaves in my hands throughout as a nearly sterile wg×, but there is some likelihood of crossing with other wg's.

W1678 deriv. Het crosses.

March 10, 1952.

W1903 (= W1688 s^R) x EMS lac Picks

A W1325 40-50/plate ca 2-:1+ ca 30
B W1178. 5-10/plate. ca 5-:1+ ca 10

Picks lac+ and streak on EMB lac to look for Lac v.

A. 1 lac v → lac v Mal-

B. 5 lac v → lac v 4 Mal- 1 Mal+. No Mal v !

Presumably Mal- are hemizygous and Mal segmental diminuetans also occurs in these "outcrosses".

c. W478 x W1876 (for formal statement). EMS lac, Mal. + → -.
EMB poor - differ. inuetans 40 tests - 8? mixeds.

Recheck from EMS lac.

4 more? / 40 tests.

		Lac v	Mal	
From EMS	1	+	+	
	2	+	-	
	3	+	-	
	4	+	-	
	5	+	-	
	6	v	+	-
	7	+	+	
From EMB	8	v	+	-
	9	+	-	
	10	+	-	
	11	? v	+	.
	12	+	-	

v V, P prototroph for W1927 Wald

Rare of these are useful for reversion analysis.
Cf. Mal status of W478 x W1177

Should try W1178 x Y10F+

F+ transfer: single cell analysis 920

March 14, 1952.

58+161 + W1177. Contacted and separated. ERL micromanipul.

3/12/52 Crowing cells contacted on Nutr. agar. Pools assayed x W1607

			count		F
1.	on needle	5 min	59		−
2.	colonies mixed	2 hours.	65−	303+	−
3	control.		50		−
4			50		
6	↘ → ≪	30"	42		−
5	control		88		−
9	⟩	10"	57		−
11	Near	30 min	79		−

3/26/52.

1	−
2	−
3	−
4	++
5	−
6	−
7	−
8	++
9	−
10	−
1876	++

3/21/52

#	no cells positioned	no. after 3 hrs.	nos. on plate (5-6 hrs) Lac −	Lac +	hrs. in contact
1	3	8, 20	13	30	½ - 2 *
2	2	32	16	crowded	1½ - 2
3	2 (1 dead?)	17	14	0	4
4	3	16, 16, 16	27	crowded	0 - ¾ *
5	2	16, 16	27	65	½ - 1 **
6	2	38	14	crowded	1½ - 2
7	3	27, 13	7	"	0 - 2
8	2	60 (4 hrs.)	22	53	1½ - 2
9	2	14, 12	17	crowded	¼ - 1
10	4	15, 13	35	50	1 - 2

Dividing cells from 58-161 and W1177, placed near each other, grown at room Temp. 2-4 hrs before the microcolonies coalesced. Hrs. in contact is the Time from the first observance of coalescence until the mixed col. was picked up and plated on enr. ag.

* When >1 cell was present originally, it was not definite which coalescence brought + and − Together.

** #5 2 microcolonies were mixed with each other after 4 hrs. when each was about 30 cells.

Inheritance of Hfr.

3/17/52

A W1895 × W477
B " × W677. Very crowded. Pooled controls.
C " × W1896

A. 12 picked. 2 probable LacV. Controls.
(2 Lac-). → 1 LacV = 921A

		crossover?
1	Lac- M—	↓
2	Lac+ TLB$_1$	
3	Lac- M—	
4	Lac+ TLB$_1$	

B. Pool tested for transduction to W 1607. All F- by transduction test! See over

C.

	Lac	Mal	streaked on EMB sm	Pedigree transduced F
1	+	+	Lac	+
2	+	+	Lac	+
3	+	—	Lac	+
4	+	+		
5	+	+		
6	+	—		
7	+	+		
8	+	—	Lac	+
9	+	—		
10	—	—	Mal	+
11	—	—		
12	—	+	Mal	+

Test for transmission of F+ to W1177. Test exposed W1177 / W1607

921B should be tested for fertility × W1177, W1817

A)

1	A1 × A2	3	
2	A1 × 1177	2	
3	A1 × 1876	16	R → 14 S^s
4	A2 × 1607	++	R → Lac sm all - R
5	A2 × 1875	+	(40) " 1+R.

∴ A1 behaves like a weak F+

A2 ~~but~~ like a moderate F+. A1 × A2 not accted for (unless F ÷)

Replica A1 - 1876 ξ EMS lac
A2 × 5 sm

4 unfore
$F_{A2} > 1875$
$F_{A1} < 1876$

In these experiments, Hfr behaves like an F-, with F+ Hfr not hfr.

921B #1 = lac+
2-8 lac-

Need to keep for
SRP x 1876
1177

Prot. (W1895 x W677) F states by SRP test

921B

~~3/30/52~~ 4/3/52

#1 Lac+ 2-8 are Lac-. Use 1 ml Penassay to prepare SRP x 1177, 1876

	1177		1876
1	- 0	+	46
2	- 0	+	22
3	- 0	+	47
4	- 0	+	101
5	- 7	+	42
6	- 0	+	38
7	- 4	+	109
8	- 0	+	77

Second test

1177	1817	control	
0	370	0	
0	108	4	* prep. ~~SRP~~ contents

Can these be re-F+'d ?

	1177	1817	control
B1-1 W1895 11-1	236	27	54
11-2	128	7	21
1	ca 150	5	0
2	21	2	0

Hfr x F- → F- prototrophs which can be transduced F+.

✓ on EMB Mal.

F+ (wg x) Crosses

W1177 x		1876	1678
c11	−	++	+++
c12	−	+++	+++
c14	−	+++	+++
1875	+	+	++
1678	+	/	
1607		++	+++

d 11		d12		d14	
c11	3	c12	+	c14	+
1607	2	1607	+		+
1875	++	1875	+++		+++
1678	++	1678	++		++

c11-12-14 have evidently became again F−

d " " " have retained F+ character, but are much weaker F+ than W1876. D12 (see above ≠ 915 a) may have became mixed F+/F−

March 26, 1952.

R = rutin Q = quercitrin.

Cells from 24 hr Pennassay. 58-161 and W1895 1 ml/10 per sup
1:15 PM — 9 PM.
x W1177

1.*	58-161	Sup. —	+
2.	"	Illuminated (Hanovia/glass) 60 secs.	++(±)? Increase over ?
3.	"	Rutin 1/2 x 10^{-4} = .05 mg/ml	++
4.	"	Quercitrin "	++
5.	1895	—	++++
6	"	Rutin 1/4	+++
7	"	Rutin 1/2	++++

B = plated with .25 mg rutin per plate : 1 +, 5 ++++

* tube probably misentry! Use residual cells from inoculum tube.

Rutin had no effect!

March 27, 1952

					2 Ml/10	11AM – 2 30 PM	
1	58-161				+ (19)	1B (Rectini) +I 65	1C + (15)
2	"	Light 60s.			+		
3	"	Rectini	1/4		+		
4	"	Green	1/4		+		
5	W1895	—			+++	5B +++	(5C: illum Hanovia-glass)
6	"	Rectini	1/4		++++		

Recheck i control for strain effect of rectini on 58-161 x W1177!; light on 1895x.

58-161, W1177 grown in dark (red glass).

b.

1.
2. Fluorescent lamp 10 min.
3. Hanovia (through glass) 4

4 58-161 x W1177 grown without dark precautions

1:	56, 57
2:	64, 61
3:	54, 85
4:	98, 74

no significant effect of light.

F+ Hormone from W 1895

March 26, 1952..

A Cross W1895 with W1607. Plate on D(o), D(sm), EMS lac. x Y10

B. Control septum, grown separately

C. " components. 1 1895 x ~~W~~ Y10

2 ~~W~~ 1607 x Y10

D streak out 1607/1895. ca ½ % lac+ 1 SR

A. D(o) +++ $\xrightarrow{R}$ EMS lac sm 4 SRP. later ca 20 addtl. SRP, some lac− !

D(sm) No SRP. 3 days. ca 100 !

EMS lac ① ca 1000 lac+ No lac−

B. D(o) +++ $\xrightarrow{R}$ EMS lac sm ② 500 + 1 ? 925A1 streak out + check

D(sm) ① 6 SRP, lac+, −

EMS lac all +

C1 D(o) +++ $\xrightarrow{R}$ EMS lac sm 0.

C2

In view of possibility of intercalary crossing of W1607 x W1895, these data provide no support for a hormonal control of F+ grade in a situation where F+ transduction does not occur. See 928

Delayed crossing probably occurs on EMS sm plates.

Streptomycin and streptothricin

March 27, 1952.

Test (cross-bunch) Sm^R and Sm^S strains vs. old streptothricin 10^4/ml. W1922, W1607, W1177, 58-161, W677 all Sth^S
Lack showed a more ~~greater~~ abrupt cutoff in streaks. Cf. previous observations of SM-STH cross-resistance ! ⇒ These concerned cross-resistance at low levels only! (5 u sm = 10 u sth).

3/29. Selections for Sth^R:
W1678, W1177 ca. 10^9 ea + 10,000 u STH. No survivors

4/20 ff. Add 10 ml grown culture 58-161 to 100 ml Penassay + 50 u/ml STH. After 4 days streak and test survivors.
all tested were STH^R SM^S. Picks 1 as W1969. When cross-bunched against loopful of 1000 u/ml, show slight inhibition, whereas 58-161 is completely inhibited. Sth^R may differ from S^R in step-resistance.

Trial run: λ adsorption assay with multiple filter

8/13 ± 152

λ (1439 / woffate) diluted to nominal 2×10^6 /ml.
W1655 young aer culture in NSB, nominally 2×10^9/ml.

Mix in equal volumes 5+5 $4^{50} - 5^{15}$ 37° / incub. (no aer.)

(A) Assay initial phage ~~(also dilute 1:100 and filter)~~ (uncorrected for 1:1 dilution) with W1655. (132, 181) $= 1.6 \times 10^6$

(B) Assay filtrate :1 543 :01 42 .53 ~~.543~~ $\times 10^4$

(C) Assay residue λ and bacteria (173 .1) (25 .01) Bacteria: $.143 \times 10^7 = \frac{14}{96}$: % λ : $.173 \times 10^4$

(D) Assay diluted mixture. .1 >1000

λ 165, 162 1.64×10^4 Expt completed 5:38

Bacteria $.96 \times 10^7$

Initial λ ≐ $1.6 \times 10^6 \times \frac{1}{2} \times \frac{1}{100} = .8 \times 10^4$

λ in mixture = 1.6×10^4

Residue λ = $.17 \times 10^4 \times \frac{96}{14} = 1.16 \times 10^4$

Filtrate λ $.54 \times 10^4$

Low e.o.p for λ, measured by incubating cells?

March 28, 1952.

~~Verification~~ Test of Mal- elimination in Hfr crosses.

Streak out cross-mixture on EMS lac. Scoring difficult — ? ca 10% Mal+ 30% Lac+

Pick small Lac+, Mal for Lac V

3/31/52. EMB Medium rather poor: characterization doubtful. 5? / 24. Restreak

A. Recheck from EMS Lac. #1-5

Repeat 40 addtl. test from cross plate 1? #6

		Lac	Mal
1		V	—
2		V	—
3		V	—
4		V?	—
5	X	—	—
6	X	+	+

~~Save + 4~~ T.O. 6/53.

Note 6/52. Unless these are homozygous, which is unlikely, elimination likewise occurs in Hfr x Het. The important point is not readily tested here, namely whether markers such as Xyl or Mtl = in W1177 would be heterozygous. Selection for prototrophy misleadingly search for TL, M heterozygosity which would be really as useful! Similar cross should be conducted on EMB Lac Sen.

Hfr crosses i limited selection

March 30, 1952

Lac+ S^R had been noted repeatedly in mixtures of W1895 and W1607, etc. There might represent recombinants.

Grow W1895, W1177, W1607 overnight in broth. Mix 1 ml each + 10 ml Penassay. Incubate 11 30 AM – 4 PM. Streak out on EMB lac sm.

are sm-inactivated Hfr cells participating?

1895	no colonies
1607	all –
1177	

1895 + W1177 Ca 1–2% lac+ S^R some apparently sectored, but lac+ very weak (Gal – ?)
1895 + W1607 " " R " lac+ Mal Xyl – S^R Aux.

3/31. 1:5 each 12 N.

A. 2 PM EMB lac: clumping not obvious. EMB lac sm 2+ 37–

B 5 PM EMB Mal sm. 1 Mal+ ca 500 – (streak) EMB lac (clumping ?) streak → R → EMB lac sm Mal sm

~~same rather small lac+ or lac +/– (ca 2-3%)~~ See below

~~4/1~~

4/3/52 Restreak as 928A

1 pure +
1 lac – (+ r ?)

(delete in saline)

4/1 C. As above. 2 45 PM – 4 PM
D. Grow separately, then plate together

1895 tends to self agglutinate

D: 4 × 200 EMB lac sm → No lac+ S^R EMB lac: ca = mixture numerous overlapping colonies

C.1 EMB lac colonies. ~~=~~ about like D, but ca 1% small sectored

2 EMB Lac+ sm:

lac+	lac +/–	lac –	
1	5	78	
2	3	92	
2	3	63	
3	6	116	
1	5	82	ca 2% lac+ 6% lac +/–
2?	8	113	
11	30	546	/587

E. Washed cells — to Penassay +

F. Mixed in saline (dense ca 10^9/ml each) 37° —

G " " " 4° —

H as C. 2 30 PM – 4 15 ++

I " aerated (to stir!) +.+

J 1177 cells + Hfr supernatant. —

studies not EMB lac sen.

K. See 929. 4/4.

Pool data of 929-1 and K:

929-1.	lac+, +/-	—	K:	+	+/-	—
#1-11	7	84	EMBlac sen	0	1	51
"	"	"		1	3	63
			#12-25.	1	1?+1	64
see over.				2	5	48
			EMBlac T1	0	0	60
				0	3	45
3: lac+, lac- RR. ←			#26-28.			
			EMB lac.	67		56

KK. EMBlac see also 931B. 15 Total.

1-12 studied 4/plate. 13-15 1/plate. a) pick colonies 1-4. b) Replica all original plates. Segregation is V, S

	lac+	lac-
1	SR	RR
2	RR	RR
3	SR	RR
4	SR	RR
5	SR	RR
6	RR	RR
7	RR	RR
8	RR	RR

	lac+	lac-	
9	SS ; RR	RR	no SS seen
10	RR	RR	SR → a few
11	SS ; RR	RR	
12	~~pure lac+ SS.~~		No recomb.
14	SR	RR	
13	RR	RR	
12	SS	RR	Recomb?

See 928a

Exp. Tried segregations by deleting in both, right way of segregation of lac+ to lac+.

K: Lac+ and − ∴ all are Mal−, Xyl−.
(3 are V,R selectants)

Among Lac+, $10 V_1^S / 17 V_1^R$

Lac −, $1 V_1^S / 19 V_1^R$ (coupled with Lac+ V_1^S).

a) Keep this pair for test (#32)

b) Test all for pidotegraphy (B, agar).

\# 3/-8 Lac+ } grew on B, agar. Both are B_1 −.
\# 2-10 Lac+ }

∴ 2 B_1 − +++ of 47 recombinants (ca ½ Lac+ S^R).

3.2 Lac+ $V_1^S S^R$ Lac− $V_1^S S^R$ sci

both are T− B_1 − L+

In most tests, T− and L− are not distinguished (on replica plates), but only on reisolates in tubes. L+ V_1^S maybe associated.

4/2/52

C The pure lac+ S^R are especially significant as they exclude the possibility that residual Hfr cells fruiting W1177 microcolonies on the agars (after agglutination and clumping).

C2 - pure lac+ Restreak: 6 ✓ lac+ pure. Pick for Mal, V, test.
C3 - sectored lac+/-

C1. 5: Repl to EMB lac, lac+ for identif. lac/- mean.

4/4/52 C2-3. (lac+) all Mal-. C2: $3V_1^R$ $3V_1^S$

C3: $7V_1^R$ 5 S
+5 +5
12R 10S

C1 in each are, isolated lac+ S^R identified, few or no lac+ S^S! ∴ the small sectored colonies presumably are related to Hfr recombination.

928 A) Repeat 8 lac V? colonies:

1	−,	V,	+
2	+,	−,	V
3	+,	−	(V?)
4	−		
5	+	−	V
6	+	−	V
7	+	−	V
8	+	−	V

Repeat lac V. Restreak EMB Lac, Mal, lac sm; EMS Xyl- Gal V Auxotrophic

C3 Nutritional tests: agar. all these are Mal Xyl Gal- Tubes:

	V_1	BB, TL	B B, M	O		
11	R	+	−			
12	S	+	−			
13	S	+	−			
14	S	−	−	MTL-?		✓ M-T-
15	R	+	−			
16	R	+	−			
17	R	+	−			
18	S	−	−	MTL-?		✓ M-T-L-
19	S	+	+	±[MTL+	I. Probably B- or B_1-	✓ B_1-
20	R	+	−			
21	R	+	−			
22	R	+	−			

Test associated lac- or V_1!

Summary sheets. 928cc

Re-organize C3 tests, lac+ and lac− components

C3 EMB lactose

	Lac+	V_1	lac−	M	Nut. Req.	Pi	all Mal + and −
1		S	R		+	−	
2		S	R		+	−	
3		R	R		+	−	
4		R	R		+	−	
5		R	R		+	−	
6		R	R		+	−	
7		R	R		+	−	
8		R	R		+	−	
9		S	R		+	−	
10		S	R		+	−	
11		R	S	+	+	−	−
12		S	X	+	X	−	
13		S	X	+	X	−	
14		S	R	−	+	−	MTL −
15		R	R	+	+	−	
16		R	R	+	+	−	
17		R	R	+	+	−	
18		S	R	−	•	−	MTL − ; MTL −
19		S	R	+	+	+	− Lac+ B_1 −
20		R	S	+	+	−	−
21		R	R	+	+	−	−
22		R	R	+	+	−	−

KK lac− ETH lac

	Lac		V_1		S		TLB, growth		BM		B_1 no +
1	+	−	S	R	R	R	+	+	−	−	
2	+	−	R				+	+	−	−	
3	+	−	S				+	+	−	−	
4	+	−	S				−	+	+	−	
5	+	−	S				−	+	+	−	
6	+	−	R				+	+	−	−	
7	+	−	R				+	+	−	−	
8	+	−	R				+	+	−	−	
9	+	−	S		S		−	+	+	−	
10	+	−	R		R		+	+	−	−	
11	+	−	S		S		−	+	+	−	
12	+	−	S		S		−	+	+	−	Cross?
13	+	−	S		R		+	+	−	−	
14	+	−	R		R		+	+	−	−	
9a	+		R		R		+		−		
10a		−	S		S		R	+		−	
11a	+		R		R		+		−		

Components tend to be all TL− and concordant MTL− Reminiscent of prototroph tunica. But B_1 not concordant.

Mal, S concordant

See also 931E

#4, S are exc. BM− lac+ V_1^S S^R (basing error)

#9, 11, 12 were Hfr type, + usual pair 12 Cross?

#10 had ① usual pair V_1^R; ② TL− lac− V_1^S S^R ∴ W1177 + 2 Recombinants = 2 zygotes?

∴ 3 TL− types: lac+ V_1^R, lac+ V_1^S, lac− V_1^R Hfr missing

KK: ~~Most sectors are~~ lac+ V_1^R S^R lac- .

of 14 seg. colonies, 3 showed lac+ S^S / lac- S^R, and may not have been recombinants.

The remaining 11, the lac- component (1 part. exception) was V_1^R S^R. The lac + was also S^R, 5 V_1^S, 6 V_1^R.

Sample type colonies from each sector ~~for~~ for further test.

4/3/52. 1ml each / 5 Penassay: 2[10] PM - 4[05]

			lac/S recomb.	
1.	W1895 -	W1177 -	+	> 2% No pictographs with 100x !
2.	W1895 -	W1607 -	+ or ± (weak lac+)	
3.	W1895 -	W1876 -	±	(≤ 1%)
4.	W1678 -	W1876	—	
~~5~~	~~W1802 -~~	~~1876 -~~	—	
6.	W1922 -	W677	—	~~2+ lac - S[R]~~ - Rebuke No.
~~7~~	W1678 -	W1177 -	—	
~~8.~~				

8. W1895+ W1177. Conc. ca 10[10]/ml each fresh Penassay 3[00] PM - 5[20] PM.
0/80 lac -. (Numerous + on EMB lac)

4/4 : W1177 + W1895. overnight.

~~1.5 lac + 4ml~~ 1ml ea + 5ml Penassay 3PM. A
W1177 control. 4:50 B
W1895 " C

Microscopic clumping in C and A.

SM Crosses and F+ gradient.

March 30, 1952.

W1922 = W1895 S^R. Hfr status?
1903 = ~~1895~~ S^R
1678

A)

		D(o)	D(sm)
1	W1895 × W1903	+++	++
2	W1922 × W1678	ca 50	12
3	W1922 × W1876	+++ Hfr? $\xrightarrow{R}$ ~~[illegible]~~. ~~Lac- → Lac+~~	Mal- → Mal+

closer comparison with lower grades needed. Note that both A1 and A2 are reasonably fertile. S^R segregation here? Does it mean that W1678/sm can act as F+ to W1922 (Hfr?) Or is W1922 no longer high level F+?

B)

			D(sm)	D(o)	D(o)$_R$ → Mal	Lac	D(sm)
1	W1895.	1177	++++	++++	→	·	
2	"	1876	+++	++++	→	→	
3	"	~~1678~~ 1903	+	++			R > S
4	W1922	677	—	++++	·	·	
5		1896	1 col. —	++++	·	·	
6		1678	+	++			· S? > R
7	Hfr tet ✓	1876	/	+++	· -7+	· -2+	
8	1903	1896	1 col? —	—			
9	"	677	—	++	← Ca2+	→	
10	"	1802	—	+++			· S > Ca 10% R
11	1678	1177	+	+±	·	·	
12		1876	2	13	→	→	
13		1607	++++ ~~[illegible]~~	~~[illegible]~~		X	
14		1875	~~[illegible]~~	++, ++		→	· R>

(more dupl. D(o) D(sm))

all −/+ ratios agree with F gradient. Note Hfr × 1678 less than maximal yield! Does 1678 transduce F+?

Preliminary tests on allelism using 1 diazotizables.

EMS Lac

3/31/52

	× W1177 Yield	lac	W1876 Y	lac	Cond.
W1765	—		—		background only! λ?
1688	+++	20%+	++	20%−	F < 1876.
1920	++	5%+	±	1+ 5−	$F_{1920} \geq 1876$?

Test vs. 1607, etc.

Hfr crosses

W1895 – W1177

April 5, 1952.

A. 17½ drop daily from gram broth, no fresh broth 5:10 PM.

B. 1/10 each to " "

C. .001/ml each " " (thermotaxis ?)

D Grow together overnight.

B: 20 minute intervals: 0, 20, 40, 60. (ca 2x growth assumed)

Plate at 10^{-6}, 10^{-7} of original cells (~~10^{-5} 10^{-6}~~) (= 10^{-5}, % of susp)

A: EMB lac sm

	−	+−	+
(6)	297	7	
(7)	38	0	
	335	7	

Ca 2%

C: " " 16) 2x 757 ~~3?~~ 4/v ca 1/500. This speaks for non-random contact.

~~B:~~ 0 M. 0+ / 30, >1000.

20 M 0, / 34; all appear. +/ 12/ca 1000

40 M 1+/139; ~~18/ca 1000~~ mostly +/-. 18/ca 1000

60 M. 7: 0/36
1v 133
sm. 1v 139
2/108

6 10
9
13
} 1000

32.

7: 0/32

T16: 3/322

Use 330 as est'n for other plates at 10^{-7}

∴ ca 3%

Dried mixtures may do nearly as well as motile into fresh broth.

B: pick ~~not~~ lac v colonies on EMB lac. Post with 928 KK.

Hfr crosses 931E

April 6, 1952

E. W1895 + W1177 ca. 50 minutes

1 8 plates ca 60/plate EMB lac sm 7 lac+ or +/− (1+ others +/−)

16 plates EMB lac. Picks Lac "v". Also

replica to EMB sm; T1 to check on frequency of lac+ S^R, V_1^R.

+R seriously at junctions of colonies, or at previously picked lacs.

---

13 lacs from EMB lac. On basis of 928K, pick only 1+, 1− from each and check through.

also, plate at 10x, 100x D(0); D(B,).

D(0): 1 100x

D(B,): 25 100x (about half are v. small)

	Lac	Mal		S		V_1		Nutr Require.		
1	+	−	−	−	R	R	R	R	TLB,	HTL ←
E 2	+	−	−	−	R	R	R	R	TLB,	TLB,
3	+	−	+	−	S	R	S	R	BM	TLB, cross?
4	+	−	−	−	R	R	R	R	TLB,	TLB,
5	+	−	−	−	R	R	R	R	TLB,	TLB,
6	+	−	−	−	R	R	S	R	TLB,	TLB,
7	+	−	−	−	R	R	R	R	TLB,	TLB,
11	+	−	+	−	S	R	S	R	BM	TLB, } cross?
12	+	−	+	−	S	R	S	R	BM	"
13	+	−	+	−	S	R	S	R	BM	"
14	+	−	+	−	R	R	R	R	TLB,	"
15	+	−	−	−	R	R	S	R	TLB,	"
16	+	−	−	−	R	R	R	R	TLB,	"

Only 2 colonies tested per ~~plate~~ plating

See also 928CC

Hfr crosses. T2.

April 7, 1952

2:15 PM – 4:50

A. Grow W1177 overnight in T2 broth. + W1895 1 hour 1:10 each.

B. Control 1177/1895

C. W677/1922 Use T1 EMBlac.

A) [To test crossability of W1177 T2). EMBlac sm:

Very high yield!

	lac+/−	−
exc!	5	48
	4	24
	4	32
	13	104
	3	10.6
	prob. underestimate	
	est. 40	500

B

C) EMBlac sm. 60. all Lac+ 14 339

EMBlac T1 Pick and streak out Lacs ~~ab~~! (~~Hfr is sensitive~~) (also 20 lacs pairs: lac+ and −: all V, R ✓ S ✓ Xyl−.

5 lac+ " " " " .

W1922 is clearly still Hfr.

Therefore Lac/T1 selection still leaves only the Mal− segregation.

? Are Mal+ recombinants present in any form? – Malvs T1; sm; $\frac{BM}{TLB}$ are possible.

See D.

D. 4/9/52. = A 5:20 – 8:30 PM. Plate on • EMB Mal, EMB Mal sm ...

E " x 1611 EMBlac sm

F " x 1590 EMBlac± sm

4/10: D– ca 40 lac–... per plate

	plates	#exc.
EMB Mal sm	3	0
" Mal T1	3	0
EMB Mal sm	3	0
EMBlac sm	3	7
EMB Mal	2	0 (no Mal+)

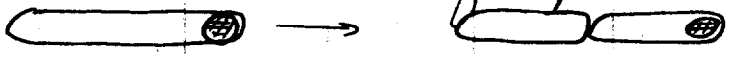
E ca 600 cols. No Lac+ S^R

F ca 700 EMBlac sm No " . EMBlac: 8 lac?/ ca 1000 lac+, – Why? Residuals. None lac+ Gave a few lacs in streaks

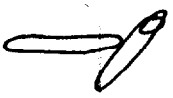
Observations on living cells.

April 6-10, 1952.

A. Use of tetrazolium as tag for parental type. Cells grown in .005% TZ / Penassay broth. W1177: over 95% of cells have 1, usually polar, TZ granule. This does not segregate (observ. on agar-coverglass mounts) PM 4/9. Several cell divisions were of the form:



This does not necessarily mean that the "mitochondrium" itself is genetically discontinuous.

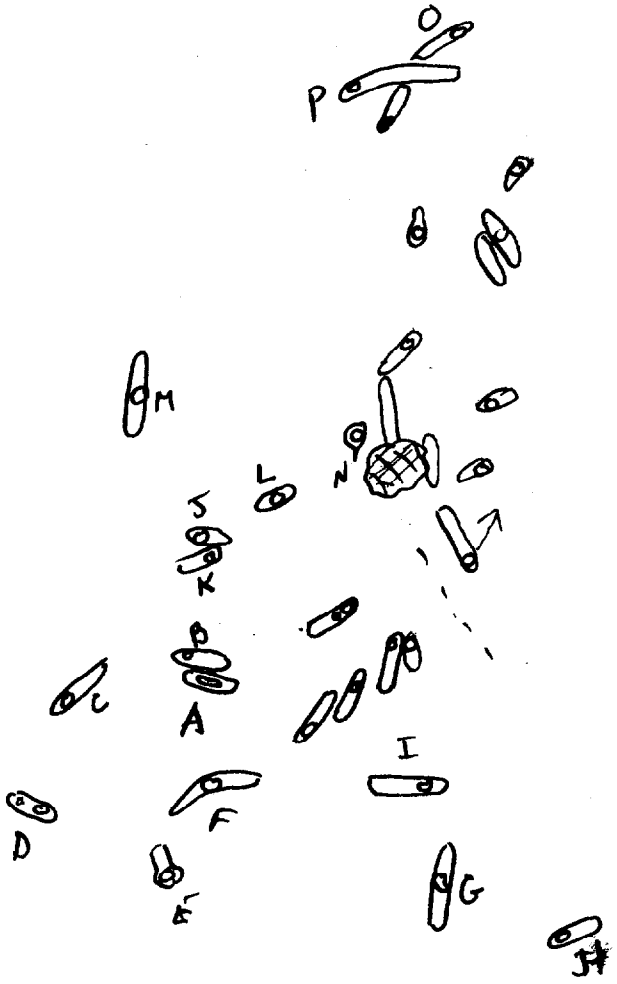
B. In mixtures of W1895 + W1177 numerous pairs have been found  and  These were firmly attached, and in a few cases arose by conjugation, not fission.

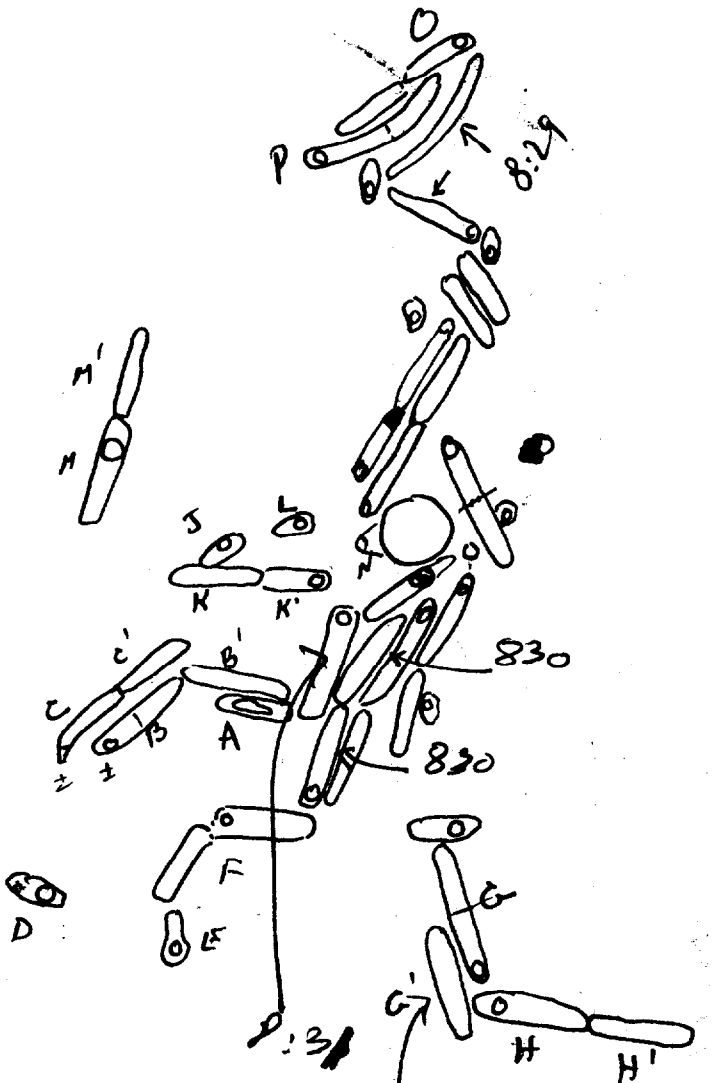
C. Interesting cells  with small bud frequently noted.

108.9
51.8

W1177 TT

5:20





P 8:10
F 8:05
B 8:15
G 8:15

April 11, 1952

EML Irradiated W1895 UV 8 sec. EMB Lac. Obtained ~5 lac-
12 plates <4-8 sec / 8-7 sec. 200-400/plate. Streak out EMB lac-

A	1?	✓	= W1940	most unstable
B	2?	✓	1941	
C	1?	✓	1942	
(1) D	No – noted		1943	slow + at R.T.
×1922 E	No lac – noted		1944	" " "

Bror A-E + W1922 in Penassay 10 AM. Brief streak-out tests.

(2) A	– ✓	
B	– ✓	
C	none noted	
×W1894. D	– ✓	
E	?	

Use W1941 for further experiments on self-crosses. lac- bacs?

See 937.

In allelism tests × W677, EML recorded lac+ recombinants in 1945, 6, 7; 1951. 1940-44 not yet tested.

Recheck ~~1940~~ 1941. Use 1948 in further test.

6/25/52. W1941 × W1956 gave few or no lac+ recombinants. W1940, 41, 43, 44 gave lac+. Recheck 1941, 1948. Use others to search for lac+ balanced diploids vs. lac, –, esp. W1940

~~Hoofbaraldo~~ W1941 was used in some subsequent Hfr (X) expts., but its lac, allelism should be further verified.

Alternative zygote types

April 13, 1952.

A. W1895 + W1177 (overnight no Renassay). Adl. plate on EMB Mal

B. " " EMB Mal T1 $B' = 10\times$ $B'' = 100\times [= 10^{-5}]$.

C. " " EMS Mal T1 B_1. 4 plates. all −.

D. " " EMB lac sm (for "control"). EMB lac = H.

E " " together 1 hour. EMB lac sm and (for double cross arm test).

F " " " " $D(B_1)$ for mapping. $100\times$

G " " overnight. ... EMS Mal SM (BM).

→ G' 1 G'' 3+2. Stuck out EMB lac. 4 lac− 1 lac+

A. 5 plates ca 100 colonies. Pick all colonies that might conceivably be Mal± or Mal+^{s}.

			Mal	Lac
1 + small +^{s}?	EMB(0) Rupl.	all +		
2 ⊙ prob comic.		+ −	1 −	+ − +
3 tiny ±		all +		
4 4 like 1		N.g.		
5 ● but near +.		Mal+; Mal−	2 −	+ − +
6 like 1		+		
7 ⊙ darker + lighter; not −		+ dark+ light	3 +	+ + +
8 med. size, baclags. mottled?		+ " "	4 +	+ + +
9 " " " "	near −.	+		
10 ● prob comic.		+ −	5 −	+ − +
11 like 1		+		
12 "		+		
13 like 8 or 5.		+		

NO evidence of Mal, Lac Recom.

Test on S, V_1, Natr.
↓ Parental

B. ca 30 per plate 5 plates. all Mal−.

B' 200-300? 1 Mal+ ⊙⊙ → all Mal− (uninfected col.?)
B'' crowded 1000? None observed.

D.

	lac−	+	+/−
	31	1	0
	42	1	3
D'	332	6	12

E. 9 plates. Count 1: 109 4 3.

Total 10 plates?

Sleeper.

1100 19 82 / 61

Picks "pure +" 17 Total. But only 8 gave only + colonies in EMB. Very rare — or others. 1 Lac v? #10 → +, − and mostly v Mal-Xyl-Acet.

H. Somewhat crowded for separation of lac S.

(over)

935 E. (lac+ from EMB Lac sm) all are Mal −.

Repl to EMB/Mal T1

"Pure+":

1. S
2. Pure R
3. Pure R
4. "
5. R
6. "
7. "
8. "
9. "
10. R

Note preponderance of lac+ V_1^R in these colonies.

No evidence of $\frac{\text{lac+ } V_1^R}{\text{lac+ } V_1^S}$

Apparent excess of lac+ V_1^R may be due to selective advantage of certain types.

#11 Pure lac− V_1^R lac+ V_1^S.

F. Picks to EMS Lac B_1:

Lac−	+	+and−
9	8	1
5	10	2
7	11	2
11	8	1
8	10	1
5	10	2
45	57	9

H

		EMB	Lac	Lac	Mal	V_1	
1			+, −	+	− +	− S	R −
2 large		EMB. O.	+ rare-maccessible	+	+	S	/
3 tiny	⊙		+ −	+	− −	− R	R •
4 "	⊙?		+ −	+	− −	− S	R ×
5			+ only (rare, macc −)	+	+	S	/
6	"		+ −	+	− +	− S	R −
7			+ rare.	+	+	S	/
8			+ −	+	− +	− S	R −
9			all+	+	+	S	/
10	"		all+ 1−	+	++ +	S	/
11			+ −	+	− −	− R	R •
12			+ −(scarce)		+	− S	−
13			+ −		+	− S	−
14			+ −		+	− S	−
15			+ −		+	− S	−
16			+ −		−	− R	•
17			+ −		−	− R	•
18			all+		+	− S	−
19 tiny	⊙		+ −		−	− R	•
20			+ infr. −		+	− S	−
21			+ −	+	− +	− S	R −
22			+	+	+	S	/
23	 small		+ −	+	− −	− R	R •
24			+ rare −	+	+	S	/
25	 ?		+ −(infrq)	+	− +	− S	R −

18 complete pairs (Lac+, −). ∴ only small colonies are likely.

Recom: 6 are Lac+ Mal− V_1^R / Lac− Mal− V_1^R

1 Lac+ Mal− V_1^S / Lac− Mal− V_1^R

Par 11 Lac+ V_1^S Mal+ / Lac− ~~Mal~~ V_1^R Mal−.

Incomplete pairs: 7 − parental. Recomb other markers?

April 14, 1952.

Mixed 2:30 - 6:00 PM

J. W1941 x W1922. Plate on EMB lac; EMBlac sm for lac s ; lac - s^R.

K. W1922 x W677. Plate on EMB lac sm T1 Plate 10x or (') 100x

L. " " Plate on EMS lac SM TLB1.

M W1895 x W1876. Plate EMBlac; sm; T1 10x

N.

J. ca 150 per plate EMB lac sm: 5 plates

No lac - s^R.

EMBlac 10 plates. ca 4000/1. 4? lac ✓ → lac+ only on EMB sm.

#5: + and -. lac - s^R col. noted. mostly lac+ s^R and lac - s^s 2 lac+ s^s; 1? lac - s^R found. → owing to proximity these might have been secondary recombinants

#5 (5) #6? all +. This 6 n.g.

K. 5 plates. No recomb.

M. EMBlac 5 x ca 150/plate. No lac s

N. T1. 5 plates → 3? lac s ✓ → Lac+, -. Repl → EMB lac Mal sm

1 EMBlac sm → 0.

L. 1 twin colony : 5 plates

all 3 give lac+/lac- ; Mal - s^R

~~Hfr × F⁻~~ Mal / T1

~~957~~
936'

April 23, 1952

W1957 × W1702 (Overnight growth).
Hfr V_1^R Lac-Mal-S^R

① EMB Lac ~~ons~~
② " + T1 100X
3 plates.
Phenotypic delay?

Factors Lac- 7 200

~~+ lac + (Gal)~~

Replica to EMB Lac ~~-~~ T1.
after picking lac+, test 6.

6 tested. - all V_1^S

Replica → no intact V_1^R
3 isolated new colonies, probably mutants

Recheck W1957 / T1. — as recorded this is $V_{1a}^{\ll r}$, unlike V_1^R!

Hfr ⊗ 937

April 17, 1952

A. ~~W1895~~ × W1955 × W1922 EMB Lac

Lac- V_1^R Lac+ $V_1^S S^R$

B. " " EMB Lac T1

B. 7 plates ca 250/plate No Lac+ V_1^R !

A 5 plates. 6 ?? Lac S. Mostly ⊗ types.

Replica streak plates to ~~EMB~~, EMB Lac ± sm.

	Lac-	Lac+
1	S	R
2	S	R
3	S	R
4	S	R
5	S	R
6	S	R

= Only possible Lac/S Recomb. ✓ V_1.

937A5.

	Lac	S	V_1	
1	—	R	R	
2	+	R	S	PAR.
3	—	S	S	

Other parent? Should have tested more colonies!

Check these for Hfr.

all 3 were Hfr (× W1956!) Save ① for further use in crosses. W1970.

C 4/28. W1970 × W1895 plate EMB Lac ± sm. (Appropriate Lac- ?)

L sm. 4 × 150 = 600. 3 Lac V.

C' s sm 2 < 300 2 Lac S

Hfr x F+

April 22, 1952

E. W1922 x W1896 (3PM – 8PM). Plate on EMBlac ±sm
lac.. + S^R
and D(8) → no colonies!

low density plates. EMBlac: ca 60/plate

6 EMBlac sm. No lac – S^R
6 EMBlac 1 ?? lacS. As said.

Repeat 4/25. (overnight)
4 x 150 plates. No lac – S^R

These crosses apparently much less fertile than Hfr x F –.
Return to unselected crosses W1895 x ~~W1875~~ W1876 for further study.

April 17, 1952

C W1895 × W1876 EMB lac sm $10^{30} - 6^{00}$ 4 plates ca 250 lac⁻/plate 2 lac±.

D " " EMB lac 7 plates 500/plate but ½ smeared! 6? lac±.

Rate of recombination much less than W1177 experiments.

4/18/52. $1^{35} - 5^{35}$

~~EMB lac sm.~~

E W1895 × 1177

F. " 1876.

E: EMB lac sm + 10 +/- 16 - 443

5 7

4 3 —

F: + 1 +/- 0 - 1020.

0 2

(0 1) Abandon.

Hfr × F+ is much less fertile than × F−!

F: only 2 likely lac± in EMB lac. ~~Abandon~~

Restreak on EMB lac. add to D.

c. B.2 and 936N. 3. all Xyl − Mal − (S^R). Abandon.

	Lac	Mal	Xyl	Mtl	S		T1			EMB.	TLB.	
D_P	+	−	+	−	+	−	S	R	S	R	P	+ − − + very recomb
P	+	−	+	−	+	−	S	R	S	R	P	+ − − +
	+	−	+	+	+	−	S	R	S	R		+ − − +
	+	−	+	+	+	−	S	R	S	R	×	+ − − +
	+	−	+	+	+	−	S	R	S	R	×	+ − − +
	+	−	+	+	−	−	R	R	S	R	×	− + − +
	+	−	+	+	+	−	S	R	S	R	×	+ − − +
P	+	−	+	−	+	−	S	R	TS	R		+ − − +
	+	−	+	+	+	−	~~S~~	R	S	R	×	+ − − +
Par	+	−	+	+	−	−	R	R	S	R	×	− − + +
	+	−	+	−	+	−	S	R	S	R		+ − − +

control ?

NG 8/9/52

Sic! Source of 1895 × ~~1177~~ 1177? Try W1922 × W1896!

Note crossovers of Mal/S!! Mtl-S-linkage!

5/8/52

2 types:

① Par_1 / Par_2 Mal+

② Par_1 lac+ Mal+ / Par_2 Mal+ !

Further content of these colonies should have been screened!

all showed a Mal+ S^R comp., but zygotes might be S^S.

See 945

E W 1970 x W 1918 EMB Lac + sm
F " Y10. "

E. 4 x 280. Lac sm. No lac +.
5 x 350 Lac crowded No Lacs sem.

F. 4 x 200 1 Lact SR!
5 x 350 3? Lacs.

HN2 — Hfr

April 21, 1952

A 58-161 10^{10}/ml B W1956. 10^{9}/ml ca 25 minutes in 1mg/ml HN2 in D(m) 37° dilute 1:10 in Penassay.

Streak out. Plate and dilute by spreading serial plates.
A. Sterile B. Ca 200 on plate 2. (10^{7}). pS = ca 5.

Replica to D(0) + W1177 for replica-plate test of F+ or + 58-161.

C 4/22/52. As above. 20 minutes exposure.
ca 50 on plate 2 (i.e. .1ml from 1/10 dil to Penassay)
1 Lac- ~~2 addl. lac=?~~ Pick + restreak. W1165
Verne Hfr occ. prototrophs Replica to D(0) + W1177. (spot W1895 control)

(D) ~~B~~ 4/23. As above Plate 2 only.
only 2-3 coli/plate. B. subtilis likes contaminant.

E 4/25 10^{10}/ml 10ml 5mg HN2 in D(m) 20m. 37°.
at 20m, dilute 1:10 in Penassay ~~express~~ dilutions from this as
10^{0}. 2 > 400.
3 60.
~~1 Lac-?~~
This establishes suitable dose level
No Hfr noted this run.

In preliminary HV run, 1 colony 58-161/ca 60. plate was noticeably nibbled. This proved to be λ^{s}_{1} similar to W1655. Discard.

Summary.

A) Lac + I −
B) Lac + S^R I −
} Almost all TL− M+ S^R Mal− Xyl− MtlR. Segregating Lac − V_1 − V_6 in more or less linked pattern. (V_6 − Lac − TL−). Also upheld in C.

C) Prototrophs! → B_1 − > B_1 +. ca 10:1.

In Hfr x F− there appears to be a constraint favoring TL− ... as well as S^R Mal− M+ .... [Rothfels crossed S^R M− x S^S M+ ... Almost all recombinants in his experiments were also T− L− Lac− V_1^R just as here! This was concluded to be based on the M+ .. Lac− .... linkage. It can be reinterpreted as a Lac ... TL ... Y linkage, with Y a hidden selector. But, the order V_6 Lac V_1 TL Y would (if applicable to Rothfels) would give a different sets of auxotroph single crossovers! The conditions of mating do not preclude a limited degree of F+ − transduction.

It may be conclude that selection for TL+ essentially discounts the effect of Y over the Lac − TL region, but leaves this influence at the left end, so that all of this set of prototrophs are Mal−.

① Further Project: Compare Hfr x F+ crosses.

② Study T − L − V_1 more closely

Map W1895 x W1956 940

April 22, 1952.

A EMB Lac . 5 shows. Plate on EMB Lac ± sm and D(B,).
3-4% Lac+ sh 16 Lac s streaked out. Pulk +,- to EMB Lac.

B EMB Lac sm. B' (+ recoverable only).

C D(B,) 100x. ca 50 purplate Picks 40 to EMB Lac B,.

940 A: (Check for possible Lac v true.

B 20 Lac s 3 were pure + (test for V_1 R/S)
20 Lac tor s. Uncertain relationship of Lac -, do not consider these.
isolate Lac + (and -) and test.

B'' 1 pure S V_6^S check reaction V_6
1 pure R V_1^S
R/S. both V_6^R Check nutrition of V_1^S. at bottom of test plate

C. Many of the strokes were mixed +/- on Lac EMSB, despite care to obtain single pickings and despersion of the colonies.

a	+ (sup.)	16	Upon restreaking on EMB Lac, almost
b	–	8	all showed a Lac - component.
c.	+,-	18.	Picks + and - wherever observed. (for later confirmation of nutrition).

Again, 9 unselected Lac segregates were all Mal – S^R.

7 Lac$\underline{s}$ also Mal$\underline{s}$ were parental for every marker

and must be assumed to be trivial clumps.

		Lac	Mal		S	Gal		Xyl		Mtl		T1	T6			BMB, agar		TLB, agar	
C	1	−	+	−	− R	R	−	±	−	−	−	− R	R	S	S	−	−	+	+
D	2	−	+	−	− R	R	−	±	−	−	−	− R	S	R	S	−	+	+	−
	3	×	+		+	S		+		+		+	S		?		+		−
A	4	−	+	−	− R	R	−	±	−	−	−	− R	R	R	S	−	−	+	+
	5	×	+		+	S		+		+		+	S		S		+		−
	6	−	+	−	+ R	S	−	+	−	+	−	+ R	S	R	S	−	+	+	−
	7	−	+	−	+ R	S	−	+	−	+	−	+ R	S	R	S	−	+	+	−
A	8	−	+	−	− R	R	−	±	−	−	−	− R	R	R	S	−	−	+	+
	9	×	+		+	S		+		+		+	S		S		+		−
D	10	−	+	−	− R	R	−	±	−	−	−	− R	S	R	S	−	+	+	−
Par		−	+	−	+ R	S	−	+	−	+	−	+ R	S	R	S	−	+	+	−
	11	−	+	−	+ R	S	−	++	−	+		R	S	R	S	−	+	+	−
	12	−	+	−	+ R	S	−	±	−	+		R	S	R	S	−	+	+	−
A	13	−	+	−	− R	R	−	±	−	−		R	R	R	S	−	−	+	+
E	14	−	+	−	− R	R	−	±	−	−		R	S	S	R	−	−	+	+
A	15	−	+	−	− R	R	−	±	−	−		R	R	R	S	−	−	+	+
F	16	−	+	−	− R	R	−	±	−	−		R	R	R	R	−	−	+	+

P. P. P. P. P P

1–10 show 5/10 segregations. 11–16 4/10

Mal · Xyl · Mtl · S, linked

Lac .. V$_1$ .. V$_6$ .. TL .. BM

meiotic link.

9 total.

4	A	−	+	−	− R	R	−	+	−	−	−	− R	R	R	S	−	−	+	+
0	B											R	S	R	S	−	−	+	+
1	C											R	R	S	S	−	−	+	+
2	D											R	S	R	S	−	+	+	−
1	E											R	S	S	R	−	−	+	+
1	F											R	R	R	R	−	−	+	+

clear

Exc. ? 12, None fail to show Lac+ S^R! Further tests needed for Lac− S^R ... recombinants. But note rarity of recomb. in Lac− selections 1:9

Note rarity of crossovers between M–TL despite Lac, V, syn.

Xyl, Gal evidently unlinked but 12

12 maybe recomb (+/Gal)

① Verify Lac - V_6 linkage

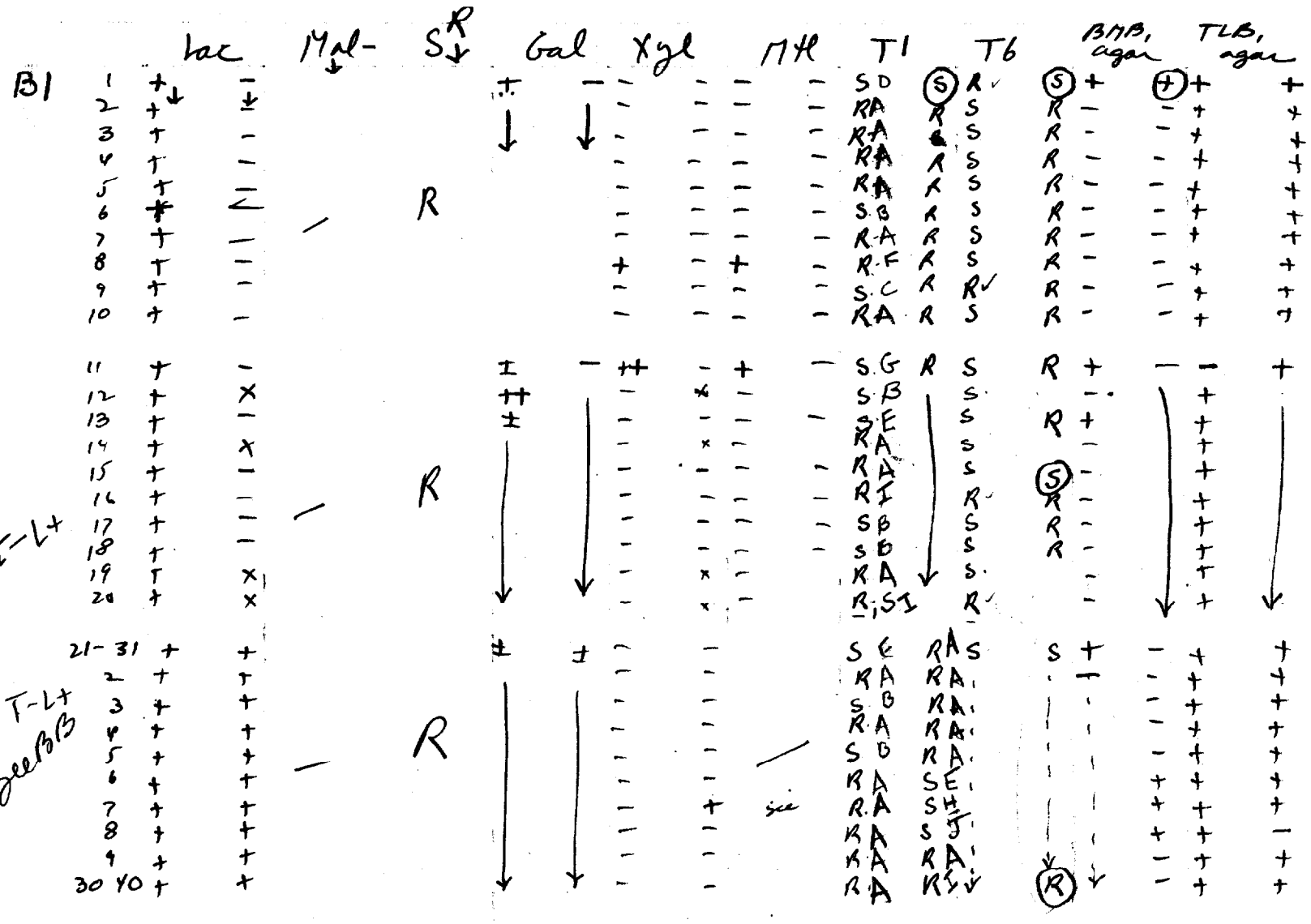
② Probably V_1 - TL linkage (all 7 TL+ are V_1^S)

③ Probable Lac - V_1 linkage (?) $\frac{V_1^S}{V_1^R}$ > in Lac+ than -.

These data suggest a constraint favoring TL- as well as Mal - M+. # 11 (unless coincidence) may point to Xyl - M.

?? Are we missing Lac - zygotes? Try looking for rare Mtl/S recombinants, or S/V_1 [1895 V_1^R x ... V_1^S]

940 B



Among 16 lac- S^R selections, only 1 recombinant. [complent to lac+ V_6^{r2}?]
40 lac+ ... no parental (re mutation ... S^R - mutn linkage?).
Types of lac+ (Mal- S^R Gal+ or ++).

1-20 are paired +, −
21-40 are unpaired +

		Xyl	MH	T1	T6	BMB,	TLB,	
A. ✓	22	−	−	R	S	−	+	standard
B. ✓	5 + 1 (Gal++)	−	−	S ←	S	−	+	V_1
C. ✓	1	−	−	S ←	R ←	−	+	$V_6 V_1$
D ✓	1	−	−	S ←	R ←	+ ←	+	$V_1 V_6$ BM
E	3	−	−	S ←	S	+ ←	+	V_1 BM (TL)
F	1	+ ←	+ ←	R	S	−	− ←	Xyl TL
G	1	+ ←	+	S ←	S	+ ←	− ←	Xyl T1 BM TL
H	1	± ←	−	S ←	S ←	+ ←	+	BM TLB, Xyl BM T1 T6
I	3	−	−	S R	S R ←	− ←	+	J = E + ←

Note preponderance of Mal+!

Prototrophs

940c. Single factor ratios:

	V_6	Lac	V_1
R_1 −	35	32	28
S_1 +	29	32	36

About equal Lac+: − valid unless single prototrophs are not segregating units.

Linkage:

V_6 \ Lac	+	−
R	7	28
S	25	4

V_1 \ Lac	+	−
R	4	14
S	28	18

V_6 \ V_1	R	S
R	14	21
S	4	26

consistent with

V_6 − Lac ——— V_1

B_1 − M 6 B_1+ / 58 B_1− } independent

Xyl − M 2·3 + / −

MH

No Mal+!!

T – L – V_1 linkage

940BB

Compare mutation of (TL) – V_1^R vs. V_1^S. (Lac + generally)
Collect occurrences in following array:

		V_1^R	V_1^S	D( ) TLB₁	TL		TB₁		LB₁		
		a	b.								
A	1	1	14	+	+	–	–	–	–	–	–
B	2	4	3P	↓	–	↓	±	↓	↓	↓	↓
	3	2	6		+		–				
	4	3	9		↓		↓				
	5	4	12								
	6	5	17								
	7	7	18						++		
	8	10	23						++		
	9	14	25						–		
	10	956	1					±	++	± !	++

∴ 16 = B_1 – ✓

Same ~~T-L~~ T-L+. Presumably this is correct order: ~~T~~ L – T

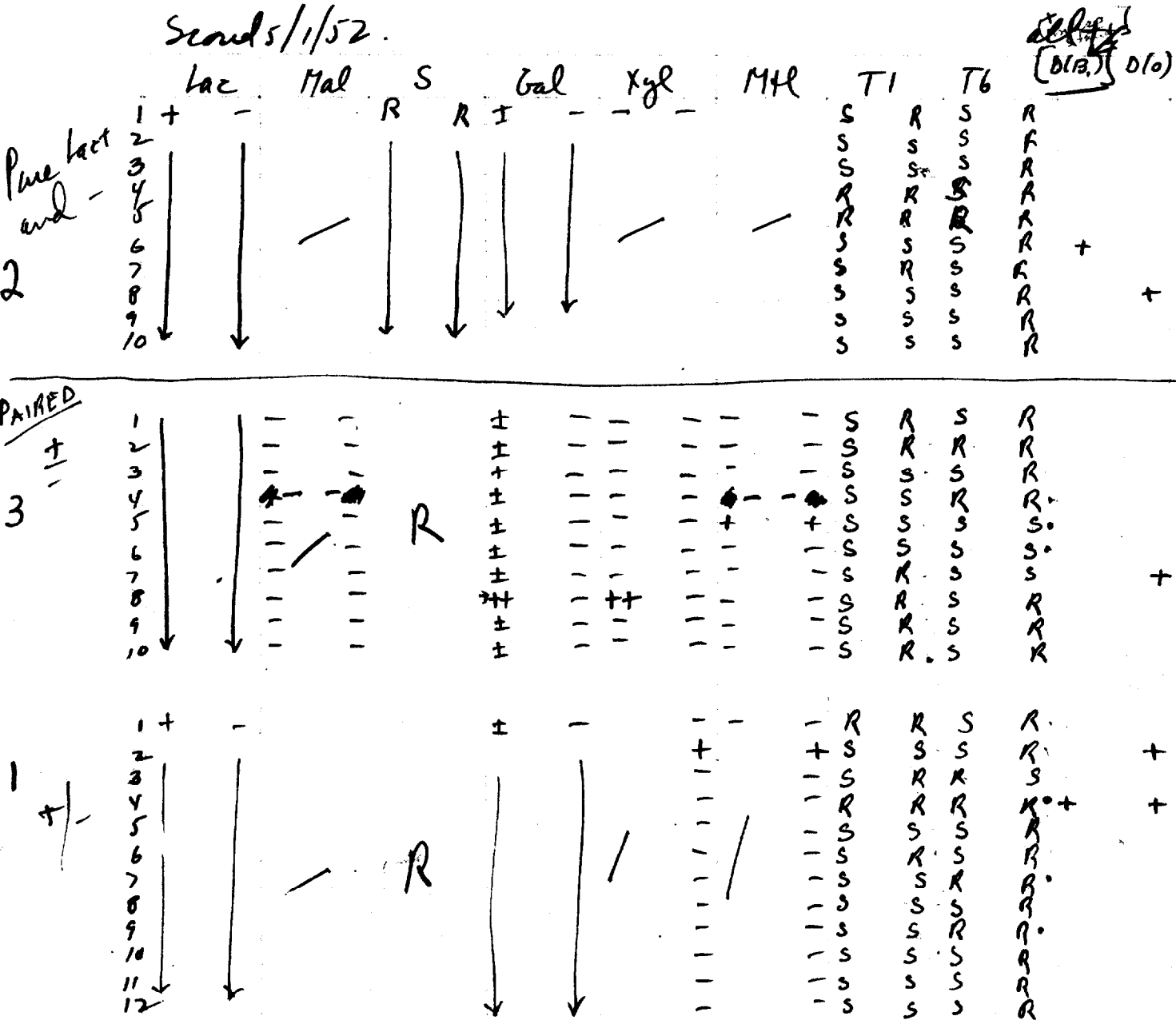
Lac – V_1 – ~~L~~ – ~~T~~

↑ V_1^S ↑ V_1^S ~~T~~ L +

D,± Prototroph recombinants

9/0C

Seeds 5/1/52.



a). Note high frequency of mixed pairs (22 pairs). Some of these might be lac- → lac+ reversions (especially if concordant for V_6) 7 may well fall in this category, and should be checked further. However, remaining 15 are discordant for V_6 also. Remaining pairs are for V_1: SR 8 RS 0 SS 7 RR 1
~~Disregard lac+ of the 7 concordant pairs~~ But no pair was concordant for Gal! all pairs: 8 0 12 2

42 colonies streaked out. 20 were substantially pure, 10+, 10-. Remainder were mixed, pick 1 lac+, 1 lac-.

← over

94@CC

May 1, 1952

Au 943. W1895 x W1956 on D(B$_1$) and EMS lac B$_1$. Incubate 3 days.

EMS lac B$_1$: Superficial appearance. An unusual proportion of

+	−	−(+)	+(−)
34	2	5	7

sectored colonies is indicated. Owing to size differentials, the figures do not show their proportion adequately. Sectoring for texture is also notable on D(B$_1$). Pick well-separated colonies from D(B$_1$) and streak out on EMB lac.

Yields, as usual experience, about 10^{-4} of inoculum.

5/5/52 Picked 32, "random" streaks on EMB lac

	Proportion lac+ (est.)
1	.5
2	.1
3	.5
4	<.1
5	0
6	<.1
7	0
8	.1
9	0
10	.5
11	1.0
12	.9
13	.4
14	.8
15	0
16	1<
17	<.1
18	.5
19	0
20	.1
21	.9
22	0
23	<.1
24	.1
25	<.1
26	1<
27	0
28	.3
29	0
30	1
31	0
32	.8

110/32

Weighted average: 110/32 ca .3 lac+ !

of 32 pairs, only 3 had Mal+ : 20−
22+
31−

as EMS Mal B$_1$, a much larger proportion appear to be Mal+

∥ EMS lac B$_1$, colonies picked to EMS Mal B$_1$: ca 30% +

Effect of EMS medium ??

W1895 × W1956

Various conditions

April 23, 1952.

B. Unaerated. 5½ hr cultures. Mix shown ca 5%?

A. Aerate " " (3 aerations).

A1 —

A2 heat 60° 5 minutes. Plate 10×. No help!

A3 UV 0, 10, 20, 30 sec. on plate

A4 Partially sedimented (ca 90%) mixture. Sediment dil. 20+/268

A5 ↓ Supernatant dil. 6+/264.

A5 appears superficially somewhat less. Should be repeated.

A3): 0 Incomplete counts. ~~22+/350~~ 21/363

10 "

20 "

30 ca 30% surv. 5/184 14/280.

slight effect?

4/24/52.

C. Aerate overnight W1895, W1956 T2. Remove (& air) 10 AM.

Mix and regrow 2 PM.

		(Poor est. of counts) EMB Bac sens.	Total counts:
1.	1 ml ca parent / 10 ml homogenate. Air	3, 6	40, 34
2.	W1956 control "		
3.	.1 ml ca par. No air	0	40
4.	.01 " " "	6	129

Confirms very high relative rate of recombination in diluted mixtures.

1 C1 Stain 3 15 Spread on plate 6 PM
2 "
3 C2 " " plate made to 5 PM before fix & heavily overgrown.

4/25/52

D. 1895, 1956Tz from overn. acc. 10AM - 2PM Region.
Mix ca 5ml each + 5ml Pennassay 2PM — 3PM EMB Lac sen.

1 (+ 90 mins room temp.) < 1/5% lact+
2 supernatant after strong centrif. "
3 Resuspend in saline. Re-sediment : supn't. "

cultures may have deteriorated or undergone added contact.

E ~~Mix~~ Dilute 1:100 3PM. Mix in 10ml: (assume 10^{10}/ml initial)

1 1ml ea (ca 10^8/ml) → ca 1/2% lact SR.
2 .1ml each (ca 10^6/ml) " "
3. .01 ml " (ca 10^5/ml) 0

In view of D these results are meaningful. However, the development of zygotes at extremely low dilutions is confirmed. Competition expts?

Flagellar phages: Salmonella

~~944~~ 942

April 24, 1952

Received this date from Boulgakov

1 "Steam" 372 = ~~H901~~ Sutii - Boulgakov Rough
2 377 H901
3 383 = Felix 6.396 V/3

A & VIII - 113 1936 § had been propagated on H901.
B " "
C " Passage 372
D " " "

3/24. Open 1, 2, 3, A. Test by cross-streaks on EMBLac

	A
1	S
2	S
3	S (later secondary R)
Stanley	R
O-901	S!
LT-2	R
LT-22	R

3/25.

	A	B	C	D
H901	S++	S++	S +	S +
O901	S++	S++	R	R

Apparently C and D fit description of flagellotropic phage. Should be single-plaqued to verify effect of propagation on H901, supposedly the sole distinction of A+B.

Test various Salmonella types on EMBLac vs. C.

1+C cleared after ca 3 hours in Penassay. Streak out for O/N survivors

Restreak 1+C, 2+C. Pick single colonies: restreak; test motility.

1: 3 motile most promising ← like (#1 →). Retest single colony isolates.

2: 3 motile, 1, 2, 4 re tested: show limited motility overnight. Retest single colony isolates 1PM – 0 18 to 10PM (from #1)

H901 controls! — clump of large bodies seen in soft agar!

Motility of H901, & motility of O901 verified microscopically. (over)

O-forms from S. typhi str.

942-2-1 4 colonies retested. #1 did not migrate overnight
#4 ++
↓ should be suitable

942-1-4 " " #2 +
#4 +++

Compare motile + non-motile "mut" for sensitivity to φC

---

check phages 942-1 942-2
added ↓

H901	S (marked)	[culture old, from liquid]
0901	K	
3 motile revts of H901/C	S +++	[from motility agar]
1 NM-H901	R.	save.

			φ A	φ C
1		1	S	P
2		2	S	P
3		3	S	P..
V	(Edw.)	0901	S	R
558	(Kauf.)	0901	S	R
6		stanley	R	S
7		enteri	R	R
8		para B	S	S
9		gallin.	P	R
10		Ty 2V	S	R
11		TyT2	R	R
12		LT2	R	S
13		LT22	R	R
14 K.		13	R	R
15 K		223	R	R
16 K		248	S	R
17		[] + C]	R	R
18	i	SW 519		
19	i	SW 520		
20		SY 79		

Phage C.

	LT2 mediated	
SW 519	S ±	Liq growth with C → motile
SW 520	S +	
SY 79	mediated.	

Inoculate from lytic area to Penassay 10+ AM.

3PM: para B + φA Motile
para B + φC Non Motile → 3/3 Immotile for 8 hours
stanley + φC Non Motile → 1/3 " " " (#1)
LT2 + φC Motile! Recip LT2 + φC also remained motile.

phase?

4/28/ Grow 1 plaque of φC on #1. = φ 942-1 } (Penassay 50 ml)
" " #2 = φ 942-2 } behave alike as H, 0901 contra A, C as rec'd!

Responses of SW519, SY79... phase variation? UV variation?

Replicas of A to EMBlac sm; EMB Mtl.

1. of 31 Lac+, 5 were S^R
2. No Mtl+ Lac were seen in 5 plates (ca. 1000 Lac- Mtl- 31 Lac+ Mtl±)

Owing to distorted ratio of Lac+ : Lac-
and overall low number (5) of recombinants
this experiment is not conclusive

Bordgalsor flagellotypic phage 942

Summary.

4/29/52.

1. Two phages (A-B) (C-D) received from Bordgalsor.
2. AB is essentially a typhi (also paratyphi B) phage, but independent of somatic (842-1 Rough) or Vi antigen.
3. C-D Accords to description of flagellotypic phage. High (?) titre obtained from single plaques either on H901 (942-2 φ) or Sutic Rough (942-1φ) S. typhi. This acts on H- typhi, probably mutant on Vi+ (Ty 2V, SV79) It has given non-motile secondary growth and O-forms of various stability with S. typhi (Sutic R unstable; H901 "stable"), para B and Stanley. Although marked lysis is seen with typhi murium LT2 and SW519 (typhi; i —), secondary growth remained motile (Vi, V antigens ??)
4. Motile "mutants" from S. typhi became again sensitive to φC.
5. Saved for further work: NM typhi H901 (para B; Stanley. Should be subcloned. Also lysates from single plaques.
6. L-forms not of this motility agar. See 944

10/31 - which para B? Stock book records SW533 (=703) but no explicit notation here

4/28/52.

W1895 + W1958 TZ. Overn. Aerate 9³⁰ to 2⁴⁵. Hfr to 4⁴⁵

				lac sm	lac
A	1	1	in 10 ml	21+, 379-	ca =+,-
B	1	.01		4-	
* C	.01	1		3+..;	28 lac+, >-
D	.01	.01		1+ 22-	68+, 17-; 3s

plate on EMB lac + sm.
Again note relatively high efficiency of deleted crosses (esp Hfr << F-).

4/29/52.

1895 TZ + W1958. Aerate overnight. No aer 34 (TZ). 1:10... inocula (no shaking incubation)

				lac sm.	in 10 ml. 2PM...
A	1	+ 1		0	!
B	.1	"		0	
C	.01	"		0	
~~B~~					
D	.001	"		0	

5/1/52. No aer overnight. 1:10 Pen aerate 10AM - 2PM. Suspend air ca 30 minutes to reduce TZ

Incr 1 or .01 ml/10. No air. 2-4³⁰

	1895 TZ	1958	EMB Lac	
A	1	1		ca 20-:1+ !! (lac+ fail to grow in central broth?)
B	~~.1~~	1	lac+ v. infrequent or absent	only 1 lac+ in several EMB lac-: lac- s.
C	.01	1		
D	.01	.01		only lac- seen.

rate → 100% of Hfr cells

Replica A to EMB MK EMB sm.
← (see over)

~~S. typhi H901~~ large bodies

942C.
944

4/27 Many colonies of ~~large~~ L-type growth noticed in course of motility tests in H901 controls.

H901 was inoculated from EMB plate (?) to semisolid agar incubated ca 24 hours. Room temperature 8 hours.
Inoculate to semi solid agar (5 ml ±) + 500 units penicillin.

4/28. – penicillin showed same interspersal of bacilli and spherules
+ " " no macroscopic growth; spherules were prominent.
These are very transparent, practically invisible except to phase microscopy possibly accounting for infrequency of reports on them.

Similar admixtures of spherules noted to varying extent in semisolid agar smears of S. Stanley, pearB, and 842-1 swollen cytolytes of H901!
(Test LT2, K12...).

4/29. — Similar observations without & with penicillin. However the L-colonies are much less prevalent than in H901.
W1895 and W1956 unequivocal in motility agar. Then transfer to meat agar + penicillin 500 u/ml. Occasional L-type, usually not colonial, seen with and without bacteria respectively. Small + very large spheres seen
5/1 Similar structures with B. subtilis and staph. aureus.
Further control examinations showed similar spherules singularly in uninoculated plates, also in freshly poured mediums! Doubtful connection with bacteria!

Hfr x F+ 945

May 3, 1952.

See 938

W1895 x W1876. Grow overnight Parasaay. Mix 12N 1ml 1/10 ml each. Incubate to 1 30.

A. EMB Lac 9 plates ca. 100/plate. 2?? lac ±

B. EMB Lac sm ('10 and "100x). B·2. O+; B':2 O+ B" 3: 3+?

C. EMB Mal sm (1 and 10x)

D. D(B,)

C: 78- 7+ / 75- 8+! / 43- 9+ / 196- 24+ → all lac-. Contact very low frequency of lac+ S^R. These Mal+ S^R appear to be unselected. Possibility of contamination in parents?

Cf 938 D which shows similar patterns.

W1895/1938 "control" - Lac EMB sm. 94-: 6+. Conditions are suitable.

5/4/52. ~~Repeat C and also plate on EMB Mal.~~ Replica A to EMB Mal. As above. Reinoculate ca 5 hours Mix 3 40 - 6 40.

"W1876" streaked out gives 10% Mal+ on EMB Mal sm.

1895 + 1876

E EMB Mal

F EMB Mal sm

G EMB Mtl

These expts n.g. except rare lac+ S^R

H 1895+1956. Spread on D(B,) 3 45. Incubate to 7PM. Examine under phase microscope. Numerous microcolonies. About 1/100 is partly or fully lysed with many granules of various sizes (such as mentioned by Pogt?) Need controls!

Hfr, F+. miscellany.

June 20, 1952.

Resume Hfr studies. Plant of W1895 showed a yellow contaminant. Restreak on EMB lac. Colony #1 was extreme rough. (continued Hfr) Kept as W2041. Retain a "smooth" culture as W1895. Both cultures test regularly Hfr.

Attempt to resuscitate diploid cultures.

777-semi mostly inviable.

81051. OK. MHlv recorded as lac− Mal− Preserve as H-311
~~MHlv~~ lac−.

H245 − U.g. first attempt gave all lac−

6/25. A W1678 × 1607 2 trials No lac+ SR colonies.
B 1956

C W1918 × " 2 trials. No lac+ S^R
D. 1956

Pour papillae in thick streak: A, B, C, D
see 954

6/26. W1590 × W1940 EMB lac. No + colonies noted. 1?
E Hold for papillae. 1? See 952.

F H310 × W1895

G. H311 (Hfr Lac = Mal −) × W1922.
see 955

H267/SH. 7 lac− : all protoclones. 1236 are Mal− 4,5 Mal+ ✓ 7 Mal−

see 953. Save # 2: H312

Hfr Lac+ X Hfr Lac- 952

June 27, 1952.

W1940 x W1590. Mix in Penassay ca 4 hours, streak out on EMB Lac.
24 hours: opp all Lac-, some colonies have a dense center.
A. ① streaked out, gives a few Lac+, apparently not var; but with var. appearance on EMB Mal - ② Lac+ is pure Mal-.

6/29 48 hours: ca 1% Lac S colonies, and typically Lac+ at colony intersections.
B. Picked and streak EMB Lac. (8)

C x W1956. Similar to B, somewhat fewer Lac+. (4)

6/30: Lac+ appear pure, Held for further development. ~~3~~ 3 colonies in B restreak EMB Lac.
no change
some begin to papillate

1 Lac v } Mal -
2 Lac v }
3 Lac + }

Restreak 1, 2 Test nutrition. both TL
1 (BM): - (TLB) + Try B, only.
2 (BM): + (TLB) +++

Use #1 for further tests. Both are mostly rather weak Lac v+.

↓ H 316

Hfr x diploid (H312)

June 27, 1952.

H312 isolated by sm selection from H267 as Mal- lac$\underline{v}$ prototroph.

W1895 x H312 on EMS $\underline{Mal}$ for Mal+ prototroph.

7/30 4 hours. streak on EMS Mal. (v H312 parent. ca 10%v)

Mal+ only as papillae.

---

overnight: streak on EMS Mal.
~~Mal+ only as~~ after 3 days, numerous papillae noted.

7/6 8 streaked out directly. 1 clearly Mal$\underline{v}$. (H318)

Restreaks on EMS Mal → Mal+, Mal- colonies.

Restreaks 4 of these to EMS; EMB Mal. also lac$\underline{v}$ +/+-

---

7/9. 40 tests 2 Mal$\underline{v}$. Restreak EMB Mal, EMS Mal.

#1 ✓ (H319) #2? ↓ Mal+ Lac-. Restreak single EMS Mal col.

~~#1~~

H319 on EMB Mal gives $\underline{\text{almost exclusively}}$ Mal- and Mal$\underline{v}$ colonies. The latter restreaked do the same. on EMB lac, mostly weak+ lac+ and Lac-, occasional lac+. Triploid? or variety of Mal+, lac+ segregants?

H 318 segr. 953A

July 16, 1952.

H318 = W1895 x H312 (H267 Mal- s^2).

8 segregants: 4 Mal+ MH+ all lac-

	S$^+$	D(o)	(Bry)	(TLB$_1$)	S	D(o)	(Bry)	(TLB$_1$)
1	S	−	−	+	R	+	+	+
2	S	−	−	+	R	+	+	+
3	S	−	−	+	R	+	+	+
4	R	±	−	+	R	+	+	+

∴ Mal+ are TLB$_1$− Mal− are prot. (also segr on EMS/Mal see 953)

Note crossover Mal+/mutr.

H310 ~~H310~~ ~~H311~~ x W1895

955

June 26.. 1952.

H310 = non-disjoined (W1895 x W1177) TLB, - Mal-S^R Lac^v Xyl- 8 + Prototrophs picked from EMS Lac. (yield. Lac+ and Mal+).

~~Most appear lac+. Spot on EMB lac, streak EMS Mal, MM (or tgl).~~

4 from EMS Mal.

8 Lac: all pure Lac+ → check on EMB Mal / sm.

↓ 4 Mal- S^R
2 Mal+ S^R
2 Mal+ S^S

4 Mal: 3 pure +, 1 Mal^v ! H313 check on EMB lac.

Prot ↳ S^v Mal^v Lac$^+$ Mtl^v

Reisolate on EMS/Mal; Purify Mal+, Mal- for nutritional and f_2 test.

24 Mal+ streak on EMB Mal: No likely Mal^v.

↙ vs. EMB Mal / sm. ↙

all Mal+: 4 S^R 188 S^S

Pick 8 Mal^v from H313 to purify Mal+, Mal-.

40 Mal+ → no clearcut Mal^v. Restreak 4 on EMB Mal, Lac; check vs sm. not v on either

↙ Others come Mal+ S^S (40)

B) 1 seemed to be Mal+ S^S / Mal- S^R Restreak: appears ~~Mal-~~ Lac+ (2)! Retest vs SM: single colonies and mass.

Replicate single colonies No Mal^v left.

H313 segregant: prepare for outcross

July 5, 1952. 7 pairs:

Mal- Xyl- Mtl- completely linked. All auxotroph, Lac+

	A - - - S		Nutr: D(4)	D(TLB,)	B + + + S	D(M)	D(TLB,)
2	1	R	−	+	s	−	+
3	2	S	−	+	s	−	−
4	3	R	−	+	s	−	−
5	4	S	−	+	s	−	+
6	5	R	−	+	s	+	−
7	[illegible]				s	−	−
	7	R	−	+	s	−	−

These types are thus far seen:

				Repeat	Test EMS Lac, Mal
	(Lac+)	MXM−	S^S (TLB,)−	A 2, 4	✓ 1607 +++ Mal+ > 1177
A	✓	MXM−	S^R TLB,−	A 1, 3, 5, 7	✓ ++ Mal+ > ×3: ++++ ×SP-161: ++
	3	MXM+ S^S	M−	B 5	++++ −>?
	4	MXM+ S^S	TLB,−	B 1, B 4	+++ Lac+ W<
	5	MXM+ S^S	(MTL)? ✓	B 2, 3, 6, 7.	1678

B5 would appear to be ≐ W1895 ⎫ #3 is certainly Hfr ≐ W 1895.
Also test types 1, 2, 4, 5 for Hfr ⎭ #1, 2, 4 are either F+ or Hfr.

7/8. Also mix W1607 + 1, B, 4 4 hours. Streak EMB Lac SM. (for recomb + F+ transduct)

B. 1 Scanty Poorly?
3 Scanty Poorly
4 Scanty Poorly

C. Repeat 1956
1345: 4PM−9PM in broth
all gave 1-5% Lac+ S^R
∴ Hfr.

cg 2%+ on repeat, 1, 3, 4 gave 1-5% Lac+ S^R.

D) Resolate Lac− from B 1, 3, 4, 2.
Plate × W1956: all F− ✓
∴ 1, 3, 4 are confirmed Hfr, not transducible

#2 is only one in doubt. It reacts with intensity of F+ (non Hfr) with W1607.

Is #2 Hfr? 8/20, test H310 ✓
Lysed colony noted in plating of B 2 (W1607) EMB Lac → λ^S (acc EML).

July 13, 1952 ff.

H310 (mass culture) x nO(o)

7/16. 58-161 +++ ∴ H310 is presumably Hfr !
W1607 ++++
58-161 x 1956 ++±
1607 x 1956 −
1802 x 1956 −

and W1895 x H310 is not especially significant.

(cf. 955D).

7/16. Test Lac+, Lac− from H310. compare with from haploid W1895 x W1956.

1 E.R.L. 7/10/52 Single cell - Zygotes yr. Lac+ SR #7 TL W1607
2 " " " " Lac− SR #7 BM
3 " " " " Lac− SR #11 TL
4

5 - H310 Lac+ (TL+)
6 " Lac− (TL−)

+ what does this mean?

All gave 0 prototrophs x W~~16 1956~~ 1607!, #2 x 1956
Repeat H310, Lac+ and Lac− x W1607; #2 x W1956.
(F+) F− probably F− but cant.

Repeat H310 and Lac −

H310 x 1607 +++
H310 Lac− x 1607 − ~~+++~~
" x 58-161 +

∴ at least two segregants of H310 are not Hfr.
H310 itself appears to be.
Try crossing H310 x F− ! Streak out H310 x W1607 on EMS, EMS Mal
= ~~955E~~ 964

7/21/52

a. 8 Lac+ S^R from W1895 x W1956. 5 were also associated with Lac- S^R. Check nutrition: 2 Lac+ were B_1 -; remainder were T L B_1 -. Test these 6 Lac+ and 5 Lac- for F states.

1-8 = Lac + 11-18 Lac -. x W1607.

	x1607	
1	-	++ x 58-161.
3	-	
4	-	
6	-	
7	-	
8	+++ → ~~bac~~ (3) 2 types of colonies on Lac	→ A Lac ++ → ~~MM+ and -~~
		→ B, C Lac+ weaker sl. mucoid

12	-
13	-
14	-
15	-
16	-

all appear to be compatible ♀ F-.

Restreak #8; The others appear to be F -.

b. Isolate Lac -, + segregants from individual H3/0.
Use early after checking purity.

Lac+		Lac-	
21	-	31	-
22	/		
23	-	33	
24	-	34	
25	-		
26	-		
27	-		
28	-		
29	-	39	
30	-	♀ 40	

all were incompatible x W1607.

---

955 F 8A = MM- but MM+ papillae and one + colony noted!
Restreak. Test MM+, and MM- W2068
MM- papillae after several days. ♂ W1956 stuff?

~~H311~~ x ~~W1895~~
H311 1922

956 ~~956~~ 956

June 26, 1952

H-311 = Het diploid: Lac- Mal- MtlV. prot. EMS lac. Picks 8 lac+ → lacV.
A) Prot. mostly lac-, occasional lac+. (Maybe revertants of H-311?)
8 picked: all are Mal- S^s MtlV (exc. #1, #8 Mtl-). Restreak these on EMB lac. (prot. not diploid) (Should select for Mal+ prototrophs)
✓ : these two are lac+ Mal- Mtl- not diploid.

? Are the lacV reversions of H311 or recombinants with W1895?

Restreak from EMS lac (ctg. lacV) to EMS Lac., Mal. B)
This streak is ca 30% lacV but very few if any Mal+ on EMB.

C) Reni EMS Mal.
~~Almost all Mal-. 2?? Mal+ prototrophs. Restreak to EMB Mal.~~
4/5 show a +V rx on EMB Mal. Possibly Mal+ lacV? Restreak on Lac, Mal: all 4 are lacV Mal+V, as surmised.
These must be results of H311 x W1922, and presumably hemizygous for Mal. Het diploids may not bypass elimination!

Test additional
Test #2A for Mal hemizygosity: 8 Mal EMS papilla → Mal+ on EMB. Restreak on EMB lac: all were lac- → lac-
~~Hlac+, Hlac-~~ 7/10: 3 add'l. ~~sequential prot. All were Mtl-!~~ See 956A.

D. 40 lac+ tested: 23 were clearly lacV.
Spot on EMS lac, EMB Mal: all 23 are Mal-. Do not save

7/15/52 Segregate (H320) A2. 4 Lac+, 4 Lac− (all MH− mfreist test) (Hfr × Het dyploid MH u)

	lac	D(o)	D(BM)	D(TLB)
1	+	—	—	+
2	+	—	—	+
3	+	—	—	+
4	+	—	—	+
5	—	—	+	—
6	—	—	+	—
7	—	—	±	+
8	—	—	—	+

Which are MH+ ?
Pick on EMB/MH.

Btypes: #1, #5, #8.
TL− M− TL−

All should be S^S Mal−.

[Test for Hfr by Lac S cross. (#5 and 8 are lac−)]

H320: Select for reversions on EMS/Mal. Pick ~~88~~ 48 papillae to EMB lac.

1 was still lac u → lac u Mal+ ∴ H320 is hemizygous Mal−.

λ / W1827

957

June July 1, 1952.

Seed plates of:

lysis = +

Spot	W1827B	W1485
λ	+	+
K12	+	+
1485	−	−
1827 ABC	−	−
B	−	−
518	−	−

no background plaquing

Cross-streaks on EMB Sec.

	B	λ	1485
1827A	−	−	−
" B	−	+	−
" C	−	+	−
1827B + λ	+?/plaquey	(B: + !!*)	+ pl.
K12	− ?	−	+
1827B + K12	−	+ , +	+

* prob. used 1827B.

No evidence that 1827 (= 1827B) is lysogenic vs. 1485 or B.

Include B/λ ✓ B is λ^R.

7/2.

B/seed plate:

1 λ

2 (λ + 1827B plaques)

3 (λ) + (1827)

4 1827

Plate ca 100 particles of λ on

A) 1827 → 100 plaques, clear,

B) 1827 + B → no 100 turbid plaques

Pick plaques A, B To 1485, 518, B. None grew on B

all others equally + on 1485, 518.

No gross evidence of phage modification.

Use 1027?

EML now reports that W1055 was responsible for the modification.

H245 x W1922

958

July 2, 1952

7/3. 40 Mal+ colonies streaked EMB Mal. Mostly Mal+ or +v Sarelikely Malv. Restreak EMB Lac, Mal; EMS Mal.

#2 3 6 78 are Mal+/- v?

1 2 6 78 Lac+/- prob. Lacv. #3 are -+

1,6,7 certainly Lacv 6 certainly Malv. #4 pure -

Retest from EMS, single colonies: 1, 2, 3, (4), (5), 6.

1-5 are Malv Lacv #6 Malv Lac+

At first glance #2 appears Lac+/- , 1,3,4,5 Lac +-/-+

Repurify Lac+/-. H321

H295 secondary. ....
H324-325

June 26 ff. 1952.

Plate H295 from D(lac) on EMB Gal. Replica to Lac. Pick apparent Gal-lac$\underline{v}$ and test for λ...

1 Gal-lac$\underline{v}$ secondary isolated. It is apparently still Lp$^+$/Lp^s. = H317

Later proved to be Gal$\underline{v}$ still, although with slower expression of Gal+.

Difference between H295 - H317 obscure. May be primarily a shift from segregation ratio + > − to − > +. Lysid colonies (Gal-) are very prominent.

H324-325.
June 9, 1953. Plate out from D(lac) to EMB lac, Repl. to Lac, Mal, Gal. 3 plates each. >95% lacv(+). ca 150/plate

possible lacv Mal-

1. H325
2. H325

lacv Gal-

3. H324

See 1056

4. Galv lac- H324
5. Malv Gal-lac- H324

H316 x W1895

July 4, 1952.

EMS Mal.

v. high yields. ca 20% Mal+. Picks smallest colonies, restreak EMB Mal

16 tests: 3 likely Malv. Hold to pool with further tests.

+ 40 tests } . No Malv noted !

Some possibly Mal+v but no Mal- segregants seen in estimate.

F- via sonic disruption

7/7/52. A) B)

1 plateful W1895; W1678 in ca 15 ml. H_2O.
Subjected to Raytheon machine at full power, 15 min.
Then filter (14 lb Mandler).

4:30 brot 1 ml filtrate (+ 1 ml W1607) in 5 ml Penassay.

9A8: Controls clear. Plate Exp. with W1177 as D(0) or EMS tac.

	× W1956	as EMS Gal.
A = 1607 + 1895 fil.	· 0	· 15
B = 1607 + 1678 fil.	· 00	· 7
C = 1607	· 0	· 13
D = 1607 + 10^9 λ (K-12 UV)		· 315 (confirm ML/Rose).
E 58-161 × 1956	$+10^2$	

48h.

∴ Ultrasonates lacks both F+ and Gal+ transduction activity

Streak out papillae from C, D (8) (12) to confirm Gal segregation; test possibility of F transfer coincidentally.
"D1" was a very dark papillae. C papillae do not show such clear fermentation generally.
E (v) are areas between papillae for control use re F+ transduction?

In addition to streaking-out, cross-bucsh pushby pulled papillae from EMS Gal; all uniform spot on D(0) - all auxotr.
Broth pooled samples to Penassay to prepare pulsum. F+ test.

C 1-8 D 11-20
D 9-10

8C: all appear to give pure +

12 D: at least 9 show Gal v, ~~some~~ possibly all.

Repurify from single (selected) clones.

Pooled Gals (C, D) and Gal- (E) remained F- (× 1956)

7/14/52

Restr single colonies.

C (sport rev.) 8: all pure Gal+

D. (should be 19/20 Galduced). 12 tests:

1	+	−
2	+	−
3	+	−
4	+	−
5	+	−
6	+	−
7	+	−
8	+	only
9	+	only
10	+	only
11	+	−
12	+	−

4.5 | 3.5

∴ 8 | 0 8 is comparison. ~~No serious doubt of effect.~~

6.63 | 5.49 | 12

11 | 9 | 20

For further study, Restrials single colonies of # "1" for acquired stability, and 8, 9, 10 for persistent states.

7/15. In restrials, #8 showed 1 Gal− ? (Reduct). 9,10 were entirely +.
but restrials single colonies → all Gal+

7/15 #1 showed 3 +,− 1 "pure +". A. (but background has poss− ?) Restrials

7/16. All colonies were +,−. Restrials + and v → 7/17 all +, −, v colonies.

RESUME: (W1607+ λ(K-12))

9 | 3

Gal v | Gal +

Purification: ||||||||| ↓ | ↓↓↓ all +

rabbit v... | ↓↓↓ all +

7/15 all v

7/16 all v

7/17. all v

↓ 12

7/18 all v

↓ 8

7/19 all v

Stability of Gal v seems to be persistent. No fully stable Gal+ noted; if interested by further studies.

Save as stocks: 1v, 8, 9, 10+.

Restrials to look for + from v

---

8 sport. mutants from Gal− from #1 were all stable + for 2 transfers

July 18, 1952.

5 plates DNase harvested to ca 20 ml. Ultrasonic (10,030 cps) 2 minutes. K12, W1895 (H)

A) Survival ratio (measured 7/21/52). Plate .1 ml 10^{-8}. } Initial counts
B) untreated / treated – estimate density at ca 5% (early cat.) } ca. 10^{10} /ml untreated cells ca 10^{8}/ml treated

Filter through 14# Mandlers: Highly opalescent filtrates.

#1: K12 2: 1895 . test sterility 1 ml samples. add 1956.

Sterility affirmed in 4 day incubation.

Reinoculate samples to fresh broth for F tests.

(~~#1956 – of K12~~) ~~2~~ Test × W1607: Both F– !

∴ strains inactive re F

A 1895 / 5 ml Primassay + 0.1, .2, .4, .6, 1.0 ml of 0.1% BT
B 1956 " 0.1 5% Tetr. (blue tetrazolium)
blue coloration by BT, but inhibition of small marks

ca 7/15-16. ERL pulled a single long cell from 1895 x 1956. This divided to 3 cells → microcolonies.
Only parental combinations were recorded, however. (no detectable crossovers or plasmogamy). Save
-A1 (1895 type) -B1 (1956 type).

F+ transduction via λ

Lysate # EML 769-9

A	1 ml	in 5 ml Penassay	① clear	② turbid!
B	.5	" "	+ W1956	
C	.2		"	
D	.1			
E	B	1/5		
F	D	1/5	"	
G		0	"	

1st testing at ca 6 hours.
2'd " at ~~30~~ 24 ".

Lysate may have a "dormant" contamination. Streak #s on Gal, NA.
① → Mal+ Gal+ (very likely the W1506 Gal+ used to prepare lysate)
∴ Experiment above is confounded. Do not pursue.
Ex this's morning suspensions of treated W1956 showed some Mal+ as
EMB Mal: #9 and #10.
(750+) (1939)
Tentative conclusion: F transduction is due to "dormant contamination".

4P20: 8hrs 1 ml lysate #10 in 5 ml broth
P 10 A 21: clear
P ~~10 A 78~~ " .

Repeat ⓒ #10.
1. 1 ml / 5 sterility test
2. 1 ml + W1956 F- transduction test 48h. CLEAR. Add 1956
3. 8hrs test of 4P20 ⓒ W1956

2 and 3 were both F− (×1607).

✓ #1 : F−

July 21/1952.

Lie 955E. EMB, EMS/Mal.

Repeat cross EMS lac 7/29.

8/1 24 lac+ → EMBlac.

8/3 Repurify 6 possible lacv.

	Lac	Mal	EMBlac	
1	V	+ V		Mal+
2	+?	+		
3	−	−	N.G.	
4	V	−		Mal−
5	V	?		Mal+.
6	+?	−		

Save for hemizygosity tests on Mal− lacv.

H322

H310 Revertants! EMBMal.

#1, 2	Mal+ Lac−	no test.
6 addnl	Mal+ Lac−	" "

see 1057

Phage tests on W1... 965

Sleeves

7/15/52. Check phages: (small vial stocks)

	T1	T2r	T3k	T4	T5	T6	T7	λ-2	Bacty. X
B	✓	+	±	±	✓	✓	✓	—	
K12	✓	+ v.sm.pl.	—	+	✓	✓	✓	✓	

T2 T3 T4 should be received.

					K12	B	K12	B
T2	1.	T2:	6/8/48	Nozicks? ✓ ✓	R	S	S	S
	2	T2r	12.3.46	Lu? (contam.)	R	S	S±	S
	3	T2k	6/8/48	Nou?	R	S	S	S
	4	T2r	6/8/48	K12	R	S	S±	S
	5	T2r	7/17/48		R	1 plaque	S±	S
	~~6~~	~~T2r~~	~~7~~		R	S		

T2 should be further grown on B. EOP probably reduced.

					K12	B	
T3	1	CSH 10/23/51	✓	✓	R	S	K-12 is
	2	7/30/51 (13)	✓		R	S	
	3	9/24/51			R_s (typ. pl.)	S	clearly resistant
	4	T3C	11/2/51		R	S	
	5	T3K	7-17/48		R	1 plaque	to T3 proper.
	6	T3K	7-18/48.		R	S	
	7	T3	6/8/48	A.N.	R	S	What is T3K? Test
	8	T3K		A N	S	S	

various stocks for history. Possible host-modifications?

T4	1	6/8/48	A.N.		S	S
	2	"	(11.12.)	✓	S	S
	3	7/18/48.			S±	S
	4	7/17		✓ ✗	S	S

HT5" A.N. 6/8/48. what phage? : not T1, 3?4? or mod?

	T5 AN	T5 ✓	
~~B~~	S	S	Our present T5 stock seems
~~K12~~	S	S	ok. Identity of T5 from
B/1,t	S	S	Nozicks?!
B/4	S	S	
B/347	S	R	
W1955	S	R	
W1956	S	R	
W1957	S	S	
W1979	S	R	

cf. T3 - T3K. B(S), B/3,4,7(R); K-12(R), 58-161(R), W1678, W1590, W1485(R), W1918, 1956, 1802

	T3 S	T3K	plaque.
B	S	S	
B/347	R	R	
K12	R	S	
58-161, 1678, ~~1590~~ 7	S	S	
1590, 1485	Plaques	S	
1918 1956 1802	S	S	

Note: T3K ≠ T3H.
Plaques on 1485!

T3 strain is evidently mutant on K-12 ....

It shows a limited e.op. on W1485 full activity on W1918 (Y10.... acc'g Bouvier).

T3K was selected by growth (on K12?)

9/15... T3, T3K spotted on E coli B, 1918.

Repeated and tested:

	T3/B	T3/1918	T3K/B	T3K/1918
B	+++	+++	~~B~~ +++	+++
K	−	±	—	± (1pl.)
1918	+++	+++	++	+++

Question of possible host modification should be studied more explicitly. T3K originally gave +++ on K as well as B.

For testing WG series, use T3K initially; then T3 for T3K ˢ types.

Prepare Salmonella FA. 966

9/22/52.

(A) Stock "PLT22/LT2" 10¹⁰ 3/52 found quite moldy.

(B) 10AM. In 100 ml Erlenmeyer. Incubate in aeration

1	1 ml LT2	+	1 ml stock PLT22
2	"		.1 "
3	"		.01 "
4	"		1 ml SW435 (LA22)

2PM. Heat shock #2, #4 60° 1 hour. Sediment. Treat supernatant i CHCl₃.

Test .1 ml "4" / SW435 EMB Gal

(C) Gal+ transduction:

9/21 ca .1 ml SW435 ± .1 ml stock FA (A22/LT2.

9/23 SW435 — 1 papilla → Streak out 16
SW435 + FA — ca 30 Gal+ papillae → 1st plating: apparently clean Gal+, 2d "(+)": all apparently pure +. Repeat a few lightest (mid ? −): 3d plating: inoculable (Cover)
FA streile — 0

(D) Assay FA #2 on SW435/Gal: papillae [redacted] 5
.1 ml
D(0): colonies. 6

Throw out ~~#2~~ FA: streile

(E) 1 ml each SW603 (para B 0 ca 10¹⁰/ml); 966-4
→ trailes and swarms small loopful 9/26 (48+ hours)

(F) SW603 5 ml/petri dish UV { 0, 20, 40, 60, 120 } — [redacted]: isolate
No trailes / no swarms

(G) Assay 4: 800×10⁸ phage/LT2; Gal+ papillae/ml/SW435
∴ phage/FA = ca 10⁸! 61/.1 ml = 610

Note — SW552 "Group D Rough" — not fermentative by PLT22/2.

966 C 16 tests of SW435 transinduced Gal+
all stable

9/29/52. Second run: SW 435 + .1 ml 966 B 4. (see C)
Picks 8 papillae (smallest or aggressively mottled)

↓

↓

all stable cf. 9/23.

---

24 tests total

UV-induced O → H 966F

9/27/52

Hg vapor lamp, 20 seconds gave a few swarms. Repeat on a larger scale.

b— = F1

9/27 2 plates SW603 / 10 ml.
irradiate 5 ml aliquot 25 seconds. (F2) 5 ml — (F1) 0
Concentrate to ca .5 ml Plate .02 ml / plate

9/29 UV-0. 5 plates: 3 sh over swarms (late develop; no tracks)
Inoc to Pen. & purifying = F2, 3, 4. = all b

UV-25 secs 5 plates 0 swarms 0 tracks

Recap. (late letter to Irving)

① WG 5,6,7,8 found to ~~be~~ grow poorly on D(o)

② Indole-negative citrate positive (presumably slow)

③ Crossability tests (= 776-34 = W1395)

a). C : O (× >300/plate but all pairs. (certainly not dextrinitus!))

(acetoin-positive intermed. (cellobiose-not gummy))

b)

④ Auxotroph hunt. Grew poorly on D(o), apparently resistant to penicillin. Should be repeated with D(succ)

A. Repeat SRP (interim growth)

			Mal	Lac
9/24	K-12 x 2058	+++	+, −	
1	wg6 x 2058	5	all+	
2	wg6 −	5	all+	all +
3	wg6 x 1956	9	"	
4	wg6 x 1970	1	+	
5	wg6 x 1817	9	all+	

no suggestion of fusion!

B. Penicillinicum. ca. 3×10^8 /ml. 3:15 – 930

	succ	penic.	Turbidity	
1	+	−	+++	
9/26 2	−	−	+++	
3	+	100	++	
4	+	300	+	
5	+	500	±	} plate: ca 300 / 10^{-2} ml
6	+	1000	±	
7	−	500	±	

→ But poor growth when replicated to D(o) + spread succinate
1? mutant

(over)

~~SRR~~ + SRP test do not support putativity of wg 6

Similarly wg 7 never gave a clear response.

ca 9/26 Try crosses:

W1987 x 1817
1978 x 1987
1978
1987
1978 x 1956
1978 x 2058
1978 x 1817
1987 x 2058
1987 x 1956

} on D(o)
all barren

~~Lab~~ Observations on recombinants Hfr X

TCN expts.

9/23/52.

TCN IV recomb. 23 lac$^{\pm}$ on EMB Lac. All tested also had lac+ S^R, ... Constant streaking, all appeared to give pure +, − (no nondisjunctions).

9/26 Restreak ± to look for possible persistent lac ± (like H3 10) all pure lac+

9/28 VIII TCN. Pick Lac ± colonies from EMB Lac various times to EMB Mal.

		Mal −	Mal +
1895 × 1956 Penassay	45 min	3	0
	90"	19	0
	120"	19	1 *
	180	14	0
	240	7	1 *

Replicate to EMB Lac sm to verify. Restreak * to verify whether they are actual zygotes or trivial conjunctions.

→ on streaking and replicating, very few Lac + S^R. ~~These~~ These two are probably trivial sectored colonies.

FA from other Salmonella.

Sensitivity tests and productions

~~188~~ 970

9/26/52.

1. Test sensitivity of var. stocks by cross-brush of broth cultures on EMB plates

	PLT22/2	PLT7/7
LT2	S	S
SW 435	R	~~S~~ S
SW 603	2 plaques	R
stanley	sens sR	R
dublin SW 553	S	S
eastbourne	R	R
Boyd 4	S	S
" 11	S	S
typhi H901	S !	R?

LT 7	S	S
coli 2	R	R
coli 3	R	R
Boyd 4	S	S
Boyd 11	S	S
altendorf	S	R
typhi Bo	R	R
typhi H901	S lytic	S lytic!

Phage (continuum)	FA (SW 603)	
~~1A~~ 1A	—	—
~~1B~~ 1B	—	—
~~2A~~ 2A	+++	+++
~~3~~ 2B	+++	+++
7B	+++	
8B	+++	

Thousand

PLT22			
LT 1	S±	12	S
3	S	15	S
4	S	typhi V,F,	R
5	S		
6a	S		
8	S		
9	S		
10	S		
~~11~~			

Note host specificity of PLT22/2 on para B as previously remarked. PLT22 remains the phage of choice. Grow in broth and on plates on

upper = A, broth = B	① stanley	② SW 553 (dublin)	③ altendorf	④ H901
	B d 1,20	D gp	B c 1,7	D d

⑥ enteritidis	⑦ abony	⑧ sandiego
D gm	B b enx	B eh enz,15

	22	7
6 enteritidis	S	1 pl.?
8 Sandiego	S	S
7 abony	~~S~~ - few pl	R
stanley	R	R
derby	R	R
montevideo	R	R
coli 5	R	R
coli 1	R	R
539	R	R
~~typhi~~		

	PLT22	7	
SW 609	R	R	spont b
618	S + few pl.	R	transind b
623	2 plaques	1 plaque	transind i (PLT22/2)
H 901	S	faint S	
SW 664	R	R	
SW 623	1 plaque	R	

Although H901 is readily lysed by PLT22, no satisfactory lysates have been obtained in liquid culture. Adapted plaques should be used. Ditto for stanley

B g - derby carbohydrate

D b marinum

D e eastbourne

= Prepare PLT22/603 609 618 623 lysates from single plaques from PLT22/2. SW 603 and derivatives are evidently poorly sens. to original PLT22/2

phage - lysogenicity tests on

stanley LT4 / plaque. PLT22++

H901

eastbourne LT10 several, (LT1, LT3) probably severe lysog.
PLT22 very slight

typhi 60

Boyd 1104 eastbourne++) but filtrate acted on neither

A B C D Layer plating test for lysogenicity: did not work too well owing to overgrowth.

Plaques:

	1	2	3	4	5
A	stanley	London	derby	eastbourne	
B	LT1				5
C	LT6				10
D	PLT22+	+	+	+	

FH / Salm. misc. 970

B
d 1,2 ① STANLEY, typhi ...
Repeated efforts to grow PLT22 on stanley, typhi H901 or 60 have failed, despite plagging on agar.

~~④ S. typhi H901~~

⑤ heidelberg 5a LT-7 5b LT-22 n.g. Repeat: o.k. ... Jre 971D5 ...
PLT22 / heidelberg

? eastbourne
coli 1-5
london derby montevideo

SW694 — resistant to PLT22, PLT7

not A against SW666.

Motility transductions

9/29/52.

A SW603 / motility agar

B SW435 / " + typhimurium serum 1:500 (~~mitigate, typhoid~~) (OK) .

C stypht H901 / d - antiserum 1:500 ~~[illegible]~~ (inadequate) ; 1:200

A! pre SW666

1 + "FA 970-1A,B". motiles no swarms (FA: no phage)

2 FA 970-2A,B numerous motiles and swarms. Streak out and keep num as 971A-1,2 ~~+ SW663, 662~~ ✓✓

b 971A-3 test single colonies : Streak 2A, 2A' < 2A: 1 b+++ ~~[illegible]~~ later shown
2A': 1,2,3,5 b:+++

Check non-b on available pcrs: b, i, d, e, 1,5; 1,7, 1,2 Inagglutinable gP but motility o/k under scope

9/29. 7. Swarms + motiles. 2 plates Test a b, e.
a } 16 isolates all b. = 971A 4
b } = 971A 5

2/29 8. swarms + motiles. 2 plates.

		b	e
a	7 isolates all b		
b	8 both b 2 are ? (#5,8.)	[illegible] SW667 / ebony	+	+
		A7: A8, [illegible] sandijo	[illegible]	+
		possibly IX XII enx - ?		
	react i enx 1:100	para B	+++	
	abnormal mostly b, very faint enx 1:10 motile i #1, etc (dangue ?)	e serum?		

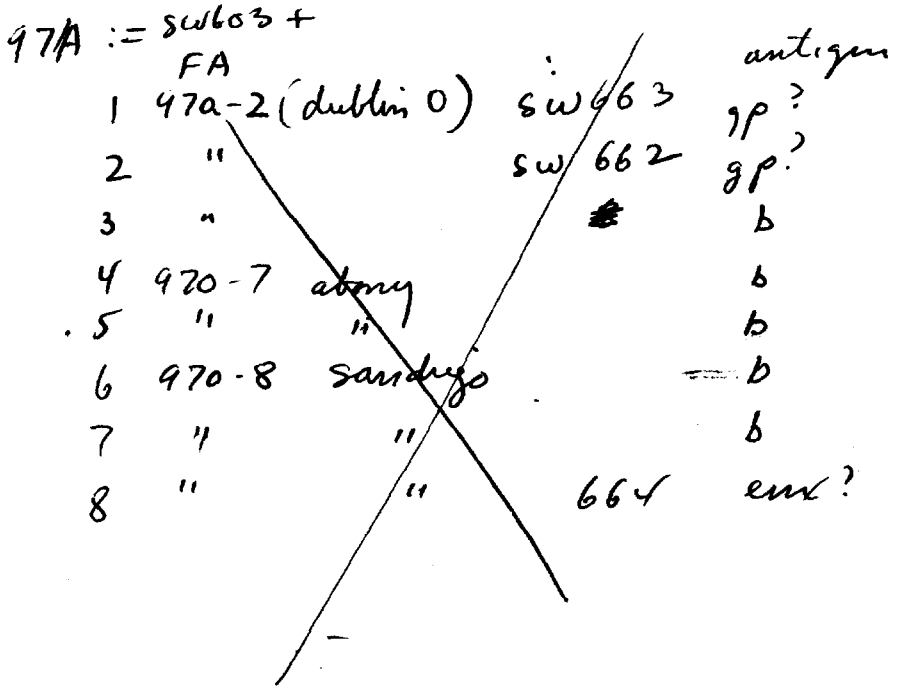
Repeat 7 for non-b

3. few motiles; swarm

6. " "

Notes: SW603, A1, A2 are strong Xgl+ SW553 is v. weak ±.

recount for stock tab.


97A := sw603 +
FA
1 97a-2 (dublin 0)
2 "
3 "
4 970-7 atony
5 "
6 970-8 sandiego
7 "
8 "
"
"
"
sw663
sw662
664
antigen
gp?
gp?
b
b
b
b
b
enx?

Recap.
10/7/52

A SW603 [= para B/o]
B SW935 + typhus serum
C S. typhi H901 / d serum 1:200
D SW666 = SW603 Gal⁻

A. 1. FA 970-1 Inactive

(dublin) 2 970-2 2 plates, 5 colonies each. 1/5 : b 1/5 : b
D gp ✓ others presumably gp to be sent to Chamblee to verify. Save 2 var-b as SW-663,662 = 971A1, A2 resp. Save 1 b as 971A3.

altendorf 3 970-3 1 plate: several fractions and swarms. Streak out and
B c; 1,9 test single colonies. 10/10 b

4 970-4 H901. No satisf FA to date

5 S. heidelberg
B r; 1,2,3

entiritidis 6 970-6 1 plate: as 3. 6/11 b, save 5 var b
D gm ✓ [illegible] t. See D6

10/2/52

971A

abony B b enx

7. 1st test: 2 plates many T+S's (tracks + swarms). 16 isolates all b.
parent culture is largely in b phase [anti b serum would be invaluable].
2d plating 10/2/52 4 plates. 8/8 single colonies: b. save 2 b as 971A-4 and 5
Inoc #1 (strong b) #7 (weak b) and mass into b agar. Also about to isolate enx phase.

10/4. Both 1 and 7 were blocked on b agar, gave 2-3 swarms = 971A7-1B, -7B.
[about swarmed out dimly (single cols. should be compared). also cf. sport rev.]

B: orig. 1 b, 7 b
3 swarmers. not b, 12, enx ... ??

T.O. all of previous cultures (probably ≥33)

8 2 plates many T+S. 7/7 all b 6/8 b 2/8 reacted i enx at 1:100
This serum aggl. para B at 1:10. Rx not very strong but confirmed microscopically. ~~Save~~ 2 b as 971A-6, 7. 1 presumptive "c" as 971A8 = SW664 (✓ ch: —)

San Diego B ch enx15

Antigen transductions to typhimurium 971B
SW 435

Sept 29 - 1952

B 2 9/30 2 plates no buds 10/3 2 buds replant to i, 1, 2
(dublin)
D gp #1 did not grow out well on i. #2 1 maggl. i, b, 1,2 (had rough i each) Presumably gp. Phasicity?

↙ not Salm on EMB ↘ (Gal -) SW674

B 7 9/30 4 plates no buds PM 1 swarm. Crew poorly. probably contaminant
abony
B b enx 10/3 2 buds. Pick to broth #1: b #2: maggl: b, 1,2, enx, i

↳ isolate as SW672. Plant on b ↓ contam (not Salm).

B 8 9/30 4 plates no buds 10/3 no buds or swarms
san diego 1 plate: filmy growth contam?
B eb en 2,15

B-0 Immobilization OK on 1:200, 1:400 1:800 H-typing antiserum in 1:500, para B grew well However, buds are growing out very poorly!

Antigenic transductants to S. typhi (H901) 971 C

Sept 29, 1952

C - 0 9/30 H901 not completely suppressed: dense spreading growth may obscure some swarms.

C - 2 9/30 1 plate no buds PM 1 bud-swarm → maggliet b, d, i
D of Isolate as SW-~~668~~ 667

C - 7 9/30 2 plates 5 buds 1-5
B beer all 5 are b (abnormal pred. this phase)
Isolate as SW 670

C - 8 9/30 2 plates no buds PM. 2 buds? ✓ → swarmers
B ch en315 both react with ent (a?) Purify and isolate as SW 668-9

C - 15 (abnorm 2) cf. C7. 10/25 - v. faint turbidity away from moc. P, clot + test

C - PLT22 1 plate 2 (or 3?) buds. → 8 swarmers later
B i 123 3 isolates: all 3 are i-, not ~~b~~, d; 1,2 = same as 971 C 22

no tails associated with the buds. Moc buds to fresh plates, broths

C5 (anti D inadequate) but no kidsor swam

transductions to paratyphi B
SW-666

971D

~~#~~ 10/1/52

D7: many swarms pooled: all Gal− n others. 5/5 b

Bacteria to b agar. → several buds. Pick for test (D7B:1−)

10/4 B. Plant individual colonies from original pool to b agar. (Two kinds of dysbiosis??)

3 swarms: inagglutinable b, 12, cmx

1 ~~sw~~ does not swarm on b agar 2 swarms slowly.

D7A2 gives weak reaction after swarming!! cmx ?

Rev D7A1 A2: 4/6 b strong = D7A1 = b; 2/6 b weak! D7A2 b

and B0 give weak b SW671

B1 maggl (2d phase?) 678

of A7

streak and save D7B1 Others.

D3 10/3 From swarm from transplant to b agar. → non b, i. 1,7 ?

c 1,7

streak out and save as SW675.

z 33

10/5. Second run, plant swarms to b agar and save selection: slow out growths

(probably second phases again) < 1 − still b; 2 − non-b

D3: ~~a : c~~ ~~equify as SW 903~~ z 33

a, b : not c. (probably "j") 3/5/53: D3b = b

T.O.

D5 10/3 − FA mg. 10/?.. 3 buds → streak and test single colonies. see SW683

v; 1, 2, 3

D15. 10/24. Numerous single swarms. Pick + test : see 979-4

D18 (LT-2II) 1/8/53. FA18 × 666. 5i 52b Note concordance with 22 × 666 see 986. 4/5 were Lp3.

D6 10/7 3 swarms. 2 non-b 1 b. Save non-b as SW 669

FA39 (Sendai) 12/4/52. 30b : 2a (22? : 4/ mixed) swarms may have been mixed. SW & some rough

3/4/53 "weak" and "strong" b have not been carefully studied. May represent features of b? Throw out

#3

533 → 534 ⟶ 588

AraB 0 sp+ (1,2)

note: 588 still sure. like 533

5436+ are resist

S W. cultures.

RACK 1

SW			
351	538	548	566
414	539	549	567
435	540	550	568
530	541	550R	569
531	542	552	573
534	543	553	574
534R	544	556	576
534	545	558	577
536	546	563	578
537	547	565	579

RACK 2

SW		
580	600	628
584	609	629
586	618	630
587	619	
588	620	
595	621	
596	622	
597	623	
598	625	
599	626	

RACK 3

SW			
653	669	680	696
654	670	681	697
655	671	682	698
656	672	683	699
662	673	684	700
664	674	685	
665	676	687	
666	677	688	
667	678	694	
668	679	695	

RACK 4

701	770R	829	LT-2 I
703A	771	834	Stgobli Vi FI
703B	774	837	LT-2 II
704	775	839	2nd PRE TO
704B	776	840 A	LT-15
706	787	840 B	SY 79
715	791	842	LT-12
721	803A	862	
760	803B		
764	825		

RACK 5

901	913	923	933
902	914	925	933
904	915	926	934
905	916	926 mm	935
906	917	927	936
908	918	928	937
909	919	929	938
910	920	930	939
911	921	932	940
912	922	932 b,2	941

RACK 6

942	954
943	954 +
944	956
945	979
946	
947	
948	
949	
950	
952	

FA56 (SW960) appears inactive re Fla, (SW666; SW967)
FA55 and FA57 —× SW666 give b only
~~FA~~ —× SW967 " swarms:

(57 —× 967 swarms immediately above)

3 trials each FA54 —× mainii (d, a, 123 strain) to secure Z_6 phase gave only 1, $\frac{*}{5}$; $\frac{T}{1,2}$ resp. ~~FA~~ 54A is designated as $\underline{Z_6}$ but should be rerun.

missing:

A	B	C	D	D
1394	112	1827	1728	618
1177	125	1969		
1895	1368			
1922	1606			
1918				
#310				

pulled for re-makes

8848
1979
1895
1896
1570
1635
518
1325
1452
1618
884
96108
760
1015
1022
1023
1024
1033
1327
2069 H+
1674
1666
1649
1688
1517
1529
1541
1585
2069 P+
1832
1852
1846
1903
1920
1939X
1970
2020
2019
1957
Y 28
Y 679
1801
383
1742
1813
1730
1734
1729
543
513
404

X-phage

9/29/52

host
Survey range by cross-streak on ET4/Bac:

1	typ [redacted]	R
2	S [redacted]	
3	[redacted]	
4	Boyd 1411	
5	coli 3	
6	LT2 [redacted]	
7	altendorf	
8	eastbourne R	
9	san diego	
10	albany	
11	SW541	R
12	enteritidis	R
13	Boyd 1404 Y	R
14	coli 5	R
15	Stanley	S
16	derby	R
17	london	R
18	SW553	R
19	montevideo	R
20	eastbourne	R

D

1	coli 2	R
2	coli 1	R
3	H901	RS
4	SW435	R
5	"coli 4"	R
6	SW539	R
7	typhi 6 F1	R
8		
9		
10		
11	LT15 #292	R
12	12 114	R
13	1 i, 1, 2	S
14	3	R
15	4	R
16	5	R
17	6	R
18	8	R
19	9	R
20	10	R

check motility of S, RS. 9/30

A

1	971 "A2"	R
2		
3		
4	=A1	
5	A3	
6	"A2'"	
7	=A2	
8		
9		
10		

F	1	R
	2	
	3	
	4	
SW569		S
	653	
	582	
	581	
	577	
	588	SR
	547	

SR

C

634		R
635		
636		
637		
638		
639		
641		
	2	
	3	
	4	

645		R
	6	
	7	
	8	
	9	
650		
	51	
	52	

reported also tested to adapted X

B

1	592	R
2	594	
3	546	S
4	609	
5	610	
6	611	
7	12	
8	13	
9	14	
10	15	

6	16	R
6	17	
6	18	
623		
	25	
	26	
	28	
631		
	32	
	33	

Records on SW543 D'?

Notes

Stocker reported that SW 588 was more susceptible to X grown on SW 592.

H901, H901 i both S

S479; S479 i both R

History of 588: [redacted] 544 (tyrmer (O form)) spont → 588 ∴ originals strains was. [redacted]

10/7/52

Compare X 942-1 and regrown on SW592

	X_{942}	X_{592}
609	R	R
610		
~~673~~		
617		
618		
623		
625		
633	↓	↓
LT1	S	S
LT2	R	R
SW435 ~~LT22~~	I	I

No improvement.

SW543. Lwi seems resistant.

Typhi derivatives:

Later, recheck of X/SW592

		942-1	X/592
	703	+	±
	588	++	±
	633	±	—
(typhi)	537	+	+
	976-5H.	-	—

A SW541 5 EMB Gal 3 Xyl 8 sec UV (°K).

1 each?? — Gal n.g. Xyl — lookasok. SW665

excellent mutant: no transductions, papillae are clear in 48 hours.

Apparent rather high yield compared to SW435. (should be compared closely)

Plate c PLT22/2. Pick papillae + streak to test stability

12 papillae. Streak to give large but poorly terminating colonies!

Compare 541, 665 and papillae! (possible a Xyl+ weak allele, in this background).

In various tests, inconsistent scoring. Best result: incubate at 37, thermostat up. No signs of segregation — not many tests

B SW603 5 Gal → 1 sectored col. → good Gal — SW666 | 1 Xyl

considerable dimorphism on gal, especially after UV, not remedied by repicking large colony.

excellent mutant colour. Pick papillae & streak to test stability. +PLT22 → plaque ridden (e.o.p. ca 10^{-4})

8 papillae. 1 appears Gal v = 973 B1 Restreak several colonies.) All others appear stable.

10/9 Pick addnl. 16. (all large papillae only). All + appear stable on 1st streaking. Restreaks: no — segs, but + ≠ 2c shows some mottling. Review this as 973 B2: repicked + and + mottled (phage?). Repeat once again:

appears to throw stable, mosaic types. Repick B1A to look for new stable, "stable" ✓ 1 colony is stable.

Streaks of B1A mosaic Gal v → −, v, + . Check +, restreak v. → pure + → −+v

∴ This unstable transduction segregates pure + and −.

~~Can~~ (over) Isolate pure + and −

973C PLT22 + SW665

973D ϕ SW541 + SW665 Xyl.

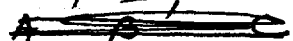
973E PLT22 SW435 Gal. – 20 : all pure + !

D) SW541, transductions of SW665 behave very peculiarly on xylose. Sharp, Xyl+ colonies give – restreaking; SW541 itself gives inconsistent responses on EMS xylose, but not to cause a false negative reading. Only one restreaking gave a typical + reaction. 24 tests 22 – 1++ 1 weak + !

These colonies gave typical + reactions on original plating !

Possibility : interaction with SW655? Try mixture.

Save 1 plate which carries 1++, 1+, 2 –.



Efficiency of transduction and effect of serum.

973 B

FA is PLT22 966-4.

Mix .5ml ca 10^{10}+ cells/ml with .5ml FA (or dil. as ind). 966-4
Let stand 5-10 minutes · plate .1ml.

1 = ~~SW665~~ as EMBXyl Note self plaquing!

2 = SW666 EMB Gal

3 SW435 EMB Gal

---

papillae / ml FA →	.05	.025	.01	.025 + .12 serum .02 ml	0	
SW665/Xyl	477	218	40	31	1	self plq.
SW666/Gal	15	6	5	~~4~~	0	

.5 + .02 ml serum.

Nearly plaqued all plates. Obvious reason for non-linearity — less heavy plaquing 16

SW435/Gal. 53 50 31

why non-linear?

8000 / 8000 x 10^7

JAN 26 1955

This calculates at only 10^{-6} papillae per phage of BADS claim of

10/4/52

= FA22

Streak out PLT22/2 on SW666.

Pick single plaque to broth = FA9

streak out FA9 on SW609 = FA10 (washed plaques)
618 = FA11
623 = FA12

10/2 974-1 SW666 + PLT7/7 → 1 trace-swarm: i

Purify again and keep as SW671. No swarm on i, 1-2 agar. (i clear??)

10/5/52. SW666 + FA as indicated

			grow on glyc. b	i	plant on agar anti b	i
9	No T or S	(o)	→ no swarms			
10	Many T+ S	(b sp.)	+		−	++
11	"	(b transd.)	+		−	++
12	"	(i transd.)	+		no swarm on i agar	−
22	"	(i)	+		" " " ++	++

why PLT22 × 666 different on i agar than FA12 × ? (test further for full single?)

(over)

Pick swarms to broth. Streak out on EMB Gal to get joint transductants. all Gal−.

Plant on i and b agar to screen for diverse types

After 48 hours, second phases appear i 10 and 11.

22: 5/7 i
2/7 o

Do not keep

B.

1	FA12 + SW609		grow together on agar; plant on b motility agar.	many buds	5 tested → all nar b, i
2	+ SW618	b	6 buds/plate → 4 nar b, nar i		
3	+ SW673	b	many buds → i. Streak out ✓ SW682		
4	FA9 + SW623	i-12	no buds (ca 10/7)	2? (10/10)	not b or i
5	FA9 + SW435	i-12	no buds ...	solid	
6	FA10 + SW623	i-12	no buds	0	
7	FA10 + SW435	i-12	no buds ...	0	
8	FA11 + SW623.	i-12	no buds	0	
9	FA11 + SW435	i-12	no buds ...	10/10: solid growth	

Repeat series using larger amts. of FA.

i-12 always negative

Repeat 12.

10/5 4+ swarms from 2 addnl. plates
to i,12. no swarm.

10/7 4 addnl. plates: 1 soil bud. 6 to i,12
2 showed slight movement (moisture mvmt?):
both are b
others remained stationary.

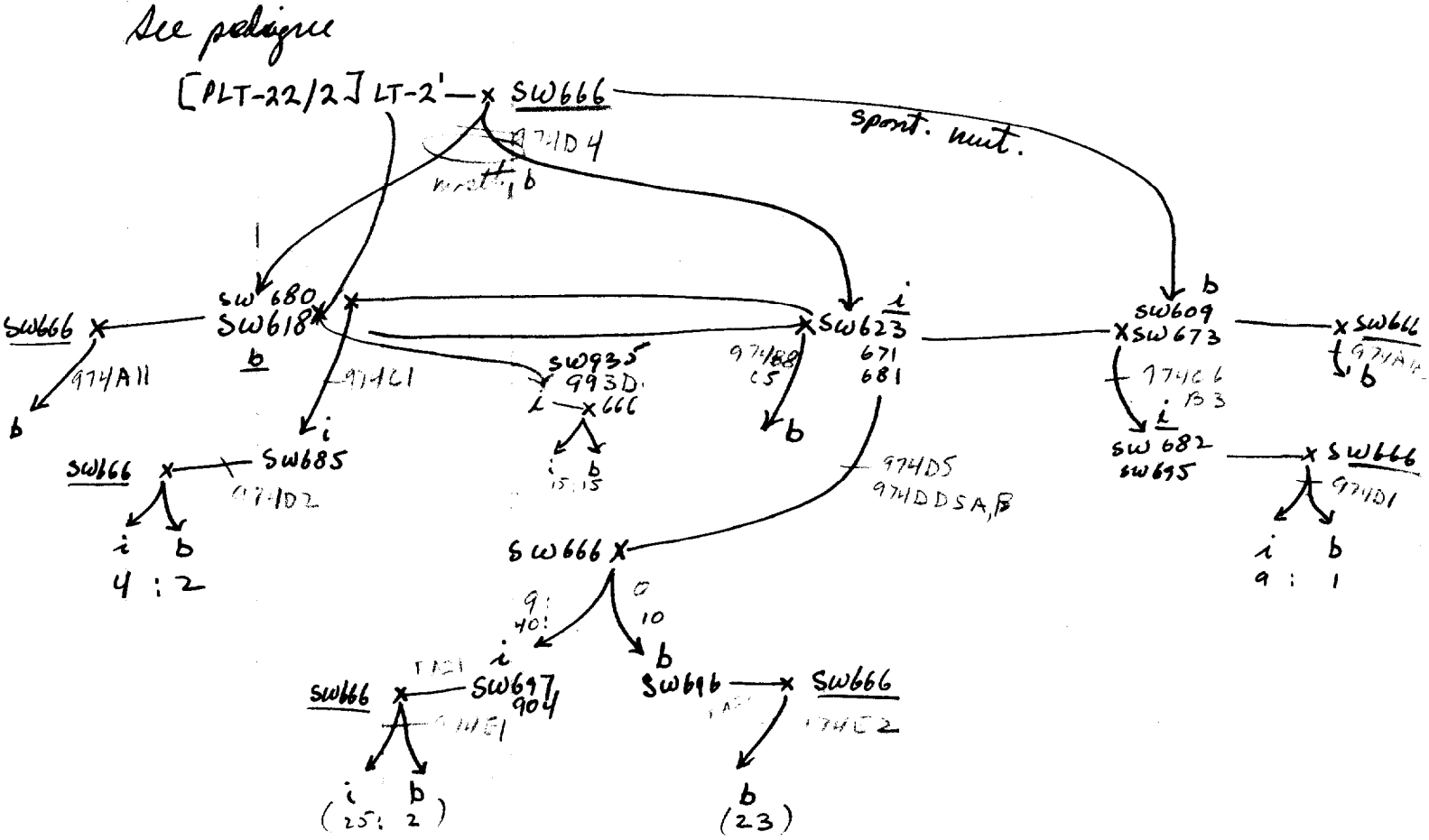
10/8 18 addnl swarms from 2 large plates (including pooled swarms)
all stationary as i'ages.

Total 30 swarms: all i. ?

Progeny tests on SW666 x — i

974

See pedigree



Note: 974DD5B

① seems to show purity of individual screams, but controls may be indecisive.

② In three cases tested, failed to isolate O-forms from initial flare. Treated themselves not tested. Established growth seems to be inhibitory to reinoculations. Maynard both methods: see 680

974/3C

	FA	SW		Mate in	
1	12	609	$A^i \rightarrow H + A^b$ sp?	b	no buds. of B3
2	12	618	$A^i \rightarrow H + A^b$	b → c	✓ pur. SW 685
3	22	618	$A^i \rightarrow H + A^b$	b → i	SW 686.
4	11	435	$A^b \rightarrow H + A^i$	i	no buds! → 1,2
5	11	623	$A^b \rightarrow H - A^i$	i	swarmed: → i ✓ isolated colony i
Repeat 6	12	609		b → i	SW 695
7	22	609		b	no buds i.e. "swarm" is still i
8					
9					

C5. 10 buds 9/10 b 1/10 still or mixed.
naturals.

974/C3. Repeat 11/21/52. Use relatively light bacterial inoculum: this seems to work much better. Numerous swarms (not well separated when finally seen). Includes 2-3 flares. Picks these as possibly representing segregation of b.

C3A flares (fundamentally from contam. by blocked b not certain.) Grow in broth assay, test probably and select on i agar. : all i

C3B Distinct swarms for all Lp^s i: 16 - all i., Lp^s Prepare FA. See 993.
(#10, #15 may be Lpt)

4/5 blocked i 1/5 → b. (possibility of contamination).
In further experiments use added R cells as a contamination marker.
(e.g. 666R).

after pul. test have #1, #4 as SW9

PA from two-step transductions 974D

10/13/52

1. FA16 (SW682) + SW666. only 2 swarms → both i
2. PA (SW685) " plant on i, b agar
3. " (686) no "Tot b!" "
4. " PLT22 " many swarms: pool and streak out
5. FA 12 (623 = trans d i) " individual swarms pulled out

1 Repeat: mixed swarms - no motility as i - agar.
test pool growth: (2)
a no agglut! → b, i± as subcult.
b: wanted b, (i ± ?)

2. " as above (2): do.

i and b!

although no swarm on i.
Perhaps medium n.g.
Would acct. for failure of
C4-5.

✓ Immotile as before on old (i, 12)

5 i i b b i b i i i i i i i b i i i i b

12 i
5 b

4

This experiment was recalled to be confused, and these results bear it out! From previous experience, 4 should give a mixture of b and i, 5 i only.

REPEAT 10/17.

this is actually mixed b, i

4 b+i?) pool was streaked out. Test colonies: 14/14 b
7/7 b pool rx b, not i
7/7 b test rx b agar

migrated on b agar

D1 separate T+S 7i 1b

D2 1 plate T+S pool - to both: reacts i ++ b + . Colony tests from strain: 4i : 2b

D3 No T or S need new PA

D5 ~~both~~ individual swarms: b and i ~~8~~ Total

	i agar	b agar
b	swarmed.	8 19
i	swarmed.	

previous difficulty not reproduced.

D2 affirms F-2 progeny test of limited transduction

10/21: Reisolate SW623 single colony (b) and prepare FA (FA9 + ...)
FA(SW623) + SW666 → many T+S. (very well defined on large agar plates)

5A Pick reasonably individ. swarms + retest b or i:
10: i 1: b (weak, maybe b + i) → purify and retest

5B. FA12B + SW666 on motility agar, well separated swarms and tracks.
Count: 14 lateral swarms (mostly i tracklets) 46 tracks. In many cases, the tracks flare out into swarms. Pick well isolated swarms for later selection test. Pick flares + streak out to reisolate presumed O component.

at least 2-3 mm from streak "FA" from SW686 (2 batches) – no T or S. (check sensitivity to PLT-22).
SW666 controls No T or S.

see over.

→ Spot on i (or b) agar to test purity. 5/5 i immotile.
6 add. tests spoiled by bad agar, but spike. Also single b stopped around and retest → all are immotile. i. Each swarm was pure.
To check, spot i on b plate and v.v. 11/16 swarms. 5/i grew spont. (plate dried out?)
Repeat test by serial inoculum.

6. FA18 (PLT22 2/2 b. / LT2 phase I + 1, 2) + SW666.
16/16 b.
Total 18/18 all b
no 1/2 ?!

Spot 16 on b agar: no swarms. typical slow spr. growth, some mutual inhibition.

Conclude: these are morphs b identical with SW618.

(over)

see 979

5B: 3 flares streaked out.
15 colonies tested from each: all i no O forms!
(45 tests!)

Perhaps the flares are not actually O microcolonies!

---

12 isolated buds tested: 11 → i
1 → b (#12)
inside from rear source

(as over) Spot on NSA, transfer to homologous serum agar.
all uniciliged. ∴ each swarm is pure

This agrees with 15/15 individual tests on each of 3 swarms.

Note 11/8. SW688 (Boyd "I rough) gives very coarse flares on motility agar, prior to later "smooth" swarm. Reisolate and compare morphology, swarms & original unselected.
This may be the basis of flares. see 978

progeny tests on SW666x — i, b

974E

FA21 → SW666 1st test: agar too sloppy 2d test activity?
Repeat preps: try FA9, LT7.
new FA9 prep. apparently lysed but activity still poor.

---

new PLT7 prep. inactive — v. few Trailers. 2? swarmers.
but might be carryover?

Test other hybrid i's for assoc. to PLT22. cf. 697 which is app. resistant
e.g. 974DD5b: 1–11 and SW697. All show no lysis on plate (but hybrid?)
with FA9 except #6. = SW904. However in broth 697 does seem to be inhibited by FA9!

FA21A (904) → SW666

27 swarms. 25 : i 2 : b → 1R / 1S

Note: in 974DD5b of 11 i, 10 are LP22R 1S / 4S
were of 25 28

31 R : 5 S

2 FA20 → activity? / a number of T+ swarms developed later. Maybe contaminants: grow poorly on NSA
as above. 1 swarm → b.
new prep (FA9) → T+ swarms fairly separable.
22 → all 6 (not i) ∴ 23 total
Test on FA9: 1S 21R. of 986

Serum effect on transmotilityation.

~~974~~ 975

October 4, 1952.

Many fewer swarms occur in antigen substitution experiments (e.g. typhi x typhimurium) than in "transmotilityations" (eg. SW543 x typhimur), and it seems unlikely that this is due to a lower frequency of occurrence. The SW543 transductions allow a comparison of the techniques.

10/3. Plant D22 (SW-666 + FA P4722/2) on motility agar ± b, i 1 2.

No serum: many tracks and swarms (ca. 60 tracks, diff. swarm)

i: 1 bud } A4: transplant — to ~~E~~ i

b no buds, no tracks. }

Could this effect be due to O-antibodies? Absorbed sera should be used.

Also of. 971D.

D3: 19 ~~buds~~ tracks. many swarms D3/b: no buds, swarms or tracks.

Repeat P4, D3/-, /b, /i (to test specificity of i effect)

A5: D3/- swarm; ca 15 tracks

D3/i 1 sw ca 12 tracks.

D3/b No tracks or swarms.

D3/d T+S as above.

In this case, b is specific of 974

$D3_0$/b 1 swarm. See D3. Agglutinable.

NOTE. Agglutinable phases might come either from FA or alternate phases in SW603. Occasional buds appear in each plate of SW(666)(HH)/b, e.g. SW673. → agglutinable by b, i, 1.2, enx ... Furthermore abony/b (occurs very readily) = enx shows very weak reaction with our enx serum!

Second phases.

10/5/52

Plant indicated steams on serum-motility agar

1. SW671 /i,12. (Insuff. antibody to prevent slow mass diff.) No buds or rapid swarms. Growth out not entirely even, but still i.

2. SW673 = para B/o sport b+ /b. See 975. After 24-48h., four buds/plate. Test: b, enx, 1, 2, i : 4/4 swagglutinable. Purify 1 as SW676 may show trace reaction with b. (pur? or phase ⇒ variation?) = z33

3. SW670 = typhi H901 x abony b enx /b. 3/4 buds. 3/4 still react strongly i b (=3-1) #4 swagg. streaksout, mesolate, and retest on b agar. (=3-2?)
#1 very weak b.
#2 swagglut.
10/22: /b slow spread. rough ← T.O. j ?= z33 slide test by 3/5/53
10/22 /b slow spread

4 SW672/b. (=typhimurium x abony) → 4 days: non b, i, 1,2, enx
SW435 itself is relatively poorly motile! (Auxotrophy? - 435. moves very slowly through kept transducers) i but 1,2 phase appears
623, 435/"I" - apparent buds still i.
LT2 & LT22 → swarm 1,2

5 SW703 - 4. 704/b (spice absorbed) → 1,2
703 k, r, a, gm ⇒ swagg. Repeat immediately on b agar
✓ 703/b swagg. b, i, 1,2, 1, T, enx. swarmed / [glu+ lac−]
see over ← 703 itself = b. Streakout: occasional more opaque colony, non-b = 976-5A
6. SW618. Swarmed /b → streak b! (Insufficient serum?)
→ slow spread /b → b

7. 623/i 12h. slow spr. LT22/i 3 isolated buds (subsequently to slower & main stock)
612/b " " " → b SW569/i no swr. → later heavy swarm.
623/i → slow spread → i
(over)
slow spread

Note: "b" serum inhibits SW546 (phase II) and thus contains 1,2
do. Seastone's "b" Absorbed SW546 Check SW435/I" OK! ∴ alt. phase of SW609... may be a third "j" phase

Check Spicer's sera

- 618 swarmed on i, not on b.

974D5 b - sw. i not b
i : sw. b not i

SW435 is relatively immotile compared to LT22. This may relate to behavior of SW672

703 (purif.) /b → 1, 2 (3 bands)

SW 672 /b (spun) ⟶ 1, 2 (very infrequently)

a) Rechecked single colonies: some seem to react both 1, 2 and ex Regrow: 1, 2 only

Second phases

~~976~~
976

1 SW671/i, 12 No.

2 SW673/b → agglut. SW676

3 SW670/b → " ; very weak b. save

4. SW672/b(1,2) → agglut. cf. SW435 - poorly motile as tested and gave no buds / i
/b → 1, 2

5. SW703 orig. → contaminant, isolated later s serum = 976-5A
SW703 pur → 1, 2 Separate phases
SW704 → 1, 2 " "

6. SW618 No

7. SW623 No ("sw" = i)

8. SW612/b "

9. SW569/i " → d (1/2)? ~~but may be migrant rough~~ Not distinguishable from typhi. How about Entam?

10. LT-22/i isolated buds 1, 2.

Unstable transductions

10/13/52.

SW 684 is Gal +/-. Transmotility ± PLT22: ca 50-100 tracks and streamers. Pick more or less well separated buds, streak on EMB Gal to look for a superimposed transduction. 15 streamers 0 = 1 trans & stationary

A. All streamers were substantially pure + or - 12 -, 2 + 1 mixed but no V.

B. SW666 + ~~FA 437~~ FA(SW684) only 7 papillae.

6 gave -, pure + 1 had +, (IV)? ? Restreak

(Cf. 986D - This one not saved) ⇒ this has phage - mottled appearance, not +/-

New transducing phages;
R forms.

10../52

1. R isolated from aged bottles of eastbourne, Boyd 110.4 typ. minimum . . .
SW688.
688 is sensitive to φ2715 (Dasroudreau) although this is described as an O phage!! 6885 original appears resistant
It is highly lytic on R but may pit transduce to S?

SW688 is resist. to PLT7, PLT22 S is sens. See over →

2. LT-10, LT-1 a) aged b) grown with Boyd 4. No phage activity is noted
seperate either on original parent or Boyd 4. LT-10 in penicillin ~ 1/unit,
no plaquing in ~~streak~~ streak-out.

3. Cross bunch LT-1 to 10 / SW688 LT10? on SW688. SW688 shows
O-10. discolored plaques, velocities with O 10 (spin)

4. O-10 / H901, Thompson, abony 1, gall, montevideo, heidelberg, stanley, altendorf
SW666, eastbourne.
No action – probably too dry.

LT-1 to 10 all R.

5. SW~~#~~665 self plq. Cultivate, ✓ broth.

6. Test mixed φ on SW435, 666. (Select for R?)

	435	666
HP 18		
PLT 22		S
7	S	SR
LP 30		
LP 36	S	S
φ 2715	S..	
HP22		
S 21 φ	1 pl.	plaques.
HP20		
HP13		

Streak out survivors, pick to broth: none were clough!

SUMMARY

O-10 (Thompson φ) has a component v. weakly active on SW688 (rough).
wait for Spicer to build it up

Like PLT10 - need to find other hosts to build it up

"10" phages attack rough phages. O-10, PLT-10 inactive on Bovis monbifucans
strains here. Wait for Boyd's material.

	φ2715-43	942B
Boyd 1184	R	R
" SW688	S	S±
Typhi 1+90	S	S
~~SW540~~	~~S~~	
LT-1	S	S
thompson	R	R
montevideo	R	R

These are not strictly R or S phages.

SW-688 is motility agar - see 974DD
motile selection appears "smooth" - first i phage
still resistant to PLT22. (check somatic antigen.)

Phase variation.

of available material, abony^{B} (enx - b) and typhimurium^{B} (i - 1,2) seem the most appropriate to study phase variation, as FH and sera are available.

In previous experiments, SW672 was made by SW435 ↔ abony^{I}. ~~[redacted]~~

In preliminary tests, SW672 appeared monophasic b. SW435 however is itself suspicious in its motility behavior; in addition sera previously used may have acted as b-1,2.

(1) Test SW670, 672 in b agar.

(2) Repeat, but ~~[redacted]~~ LT2 ↔ abony^{I} and ^{II}. (Use LT2 to improve possibility of progeny tests).

A	LT-2	FA14	→ b ⇄ 1,2	SW699	
B	LT-2	FA15	→ enx ⇄ i	SW698	
C	LT-2^{II}	FA14			
D	LT-2^{II}	FA15			

partial

NOTE: i-enx is found only in group C (baienses) but i is often a rare bird in general.

(3) Try enteritidis as recipient for transductions.

(4) See 971D SW666+ FA15 (abony^{II}) 17 swarms: all b, not enx

✓ Gal-.

~~Purify and test vs. b.~~ Purify 4. Test all directly on b(1,2) agar.

4: b → 6: ✓ nonmotile of SW678 ~~[redacted]~~ small buds from #4, b. pick, 2d plate is nearly pure. Pick center (guess)

(5) Phases 1 and 2 of SW703.

979-5A = FA23 → SW666

979-5B = FA24 → SW666 (several thousand from several swarms)

test pooled from various spots / b.

15 spots (probably tests >100 swarms): none swarmed directly out on b agar

3 gave what may be buds. pick these: 5B1-3 : still b. Remember

Repeat 5A + B: number of swarms is phenomenal.
Test Cal+ transduction?

---

streak out pooled swarms on EMB Cal: all –

Also, test on b serum: no swarm from A or B

5B: 27 single colonies: all b, none 1, 2. (2: may show equivocal reaction in 1, 2)
+ 8 added. all b → Recheck all b. or maybe sl. rough
(sw981)

5A: ~~20~~/20 b.

10/26 Repeat ĉ deleted FA...
1/100 no trailers or swarms

10/27 FA 1/10

---

6 ~~FA~~(sw~~5~~88) → sw666 6 1, 2 FA streak: –
BADS 2 mixed
more or less all isolated swarmers 14 b
streak out on EMB Cal.
b, 1, 2 – both Cal – sw699, 901.

Note sw533 (= 703?) → 534 ~~588~~

But ~~533~~ 703 behaves differently from 588. Recover NZ culture of 533

Recap + summary — Phase variation

10/28/57

② mantis-i

A. LT-2^{I} × abony^{I} gave 1/swarm SW699 b → 1,2 (slow) → b

B. LT-2^{I} × abony^{II} gave " SW698 enx → i → enx

C and D not successful in initial trial (LT-2^{II} × —)

④ FA15 (abony^{II}) → × SW666 17/17 b.

⑤ A. FA23 (SW703') → × SW666 15/15 b on bagar. Very high rate!

In second gen, streak out pool (>>100 swarms) and test colonies.
20/20 ⑥. Pool does not migrate on b agar.

B. FA24 (SW703") → × SW666. spots of pool b on bagar
27/27 ⑥ Pool does not migrate on b agar. ~~b~~ at furthest.

⑥ FA (SW588 — B.A.D.S.) → × SW666 14 b pur. + save SW699
2 mixed
6 1,2 " " SW901

979DD 6: FA18 (LT-2^{II}) → × SW666 18/18 b no alt. phase could be selected

Consideration of ②, ④... leads to hypothesis of phase variation:

~~i*~~ i^{*}; 1,2 ↔ i ; 1,2* where * is a locus-fixed activation (or suppression).

$\dot{A}_1^i \; A_2^{1,2} \longleftrightarrow A_1^i \; \dot{A}_2^{1,2}$

④, ⑤B, 979DD-6, and previous expts also ~~typhi~~ lead to hypothesis that $\hat{A}^{1,2}$ can not be expressed in monophasic strains. Apparent paradox:

SW703 $\overset{?}{=}$ SW533 $\xrightarrow{K}$ 534 $\xrightarrow{sp.}$ 588

↓ → b only ↓ → 1,2 ; b

∴ SW534 / may have a unique $\hat{A}^{1,2}$. Also SW546 = SW867.

(over)

11/2

LT-1^{I} reacts both 1 and 2

LT-1^{II} " predom 2

699 " " b

abmy^{1} " " b

abmy^{2} " " enx

E abmy 1 FA22

F " 1 FA18

G " 2 FA22

H " 2 FA18

11/3 Repeat. LT-2^{I}. Rx predom 1.

A LT-2^{T} FA14

B I 15

C II 14 Bud: ? aggl. ✓ single colonies → 1,2 + (?) → maggl. (abcdienxgm 2 1,2

D II 15 ✓ lysogenic for LT2^{1} loc-. Save as 979-C for later identification. May be poorly motile.

(Last weekend of Boston Trip)

E^{-} abony 1 22 —

F 1 18 2 b

G 2 22 2/5 i? + 3/3 b 1: i → enx. PLT22^{R}SW924
pur: 2: i " "
3: i " "
4: i " "
5: i " "
} 4. S.

H ✓ 18 6/6 b ~~3/6 b 1,2~~? 1/1 b 2/2 b (enx?)

No clear-cut buds. E-H mostly b, although G-H were originally enx. Purify single colonies, and test alt. phase

(moi ✓ b++ enx±)

1-2 plates each. all negative except as indicated

A-D in i, 1, 2 serum ; E-H in b-enx.

serum probably more antibenx than b.

J abony 1+2 × FA 22A
i : 12

Numerous swarms (10-20/plate).

Of #1-16, purif. 11 are i
5 are b (cultured by HLB)
17-33 (not purif.) all but 18 and 29

Later, i isolates from three unpure kinds.

Confusing variants probably with H.

appear i: #29 i+1,2?? 18b?
29: < 3 col A: i
1 col B: i b±?

18: single colonies < A i+++ 12+3
B i± 12+?

K. × FA 18
i : 12

possibly over-inoculated.
1+1? /4 plates. (Repeat!)

Note: FA 18 × 66 gave only a few swarms. Melka new preparation of FA 18 A (see below).

L. SW 698, 699 × FA 14, 15 i, enx or b, 12 Neither 1/plate each. No swarms many (parental & special overgrowth)

(K)
1. 1,2 ↔ b Lp^R
2. 1,2 → b Lp^S
SW 932

(J)
18A i 18B enx++
18A' (enx)? 18B' enx++?
29A i 29B i
29A' (i and V. unk. 12.) 29B' enx

i : i

Conclude: 18A i : enx, 18b : enx : (b)?
b/cols of 18A are enx+++ 1,2 - 1/5 i+++ 12±
/5A 29B i : enx

11/30/57.. 29A = 34 is i. Repeat 34/i: enx!
.. nothing 29A' = i (v. weak).
.. odd one.
#14 appears agglutinable. All others are i.
See over for /i..

Repurify 18A before further tests are made. 18B may be preserved contam. from abony. 18A' is enx, but also shows trace 1,2? /Lp^R 18A "as 18.

29B as 29

All 34 are Lp^R!

There's no clear cut 1,2 × from FA 22.

979-AA Note: phase variation in S. abony. Stock culture labelled abony II, isolated recently from single colony is about 20% b! Many individual colonies react both b, enx. ∴ the phase of FA 14-15 and cells abony' and 2 cannot be regarded as under control. This suggests enx → b. Not known whether b → enx likewise. In a parallel check on LT-2 II (from Salmi susp.) 6/10 were strong 1,2 4/10 were scarcely agglutinable (i?): regrow these 4 (see over).

Reisolate phase 2 cells for new FA pops

LT-2 II

4: These colonies when regrown give only trace agglutination in 12 (smi? i?)

In broth, #4 is very sluggish motility, again only trace 1, 2.

#5 (strong 1, 2) very mot.

most cells immobile; some quite active. Reselect on motility agar then gives a strong 1, 2.

Streak out present suspensions of LT-2, above. (H.L.B. tests)

b ⇔ enx frequent

above I: b: 7 enx: 3

above II: enx: 2 b, enx: 2 (see above)

HLB

LT-2 I: 16: i 1,2: 1 ? 5 (both or weak?)

→ 18 i 1: 1,2 other R. retest

LT-2 II: 0: i 1,2: 20 weak 1,2: 3.

Thus, FA22 - FA18 are fairly reliable, but

FA14 - 15 are not, but nearly equivalent.

9795 second phase: select on/i serum. Test swarms: (i blurry) all isolates swarming on i agar.

streak out pool of 9795, pick colonies test Lp(22)^s: undetermined proportion of Lp^s. Purify and test SW941 as Lp^s; i:

979K...

12/1/52

K. FA18B × abony 1,2: doubtful outgrowths, very late: ~~3~~ not Salm., 4.

4 1,2 → [redacted] b
5 i → enx
6 1,2 → b
7 1,2 → b

11/30. FA18C, D. 12/1. 3 on C 0 on D. 5, 6, 7.
12/2 ladd. c. 1 D 8, 9

(15 colonies of A5 tested all i.)

8. 1,2 → b
9. 1,2 → b

12/4. FA18D (canc) × abony 1,2. 5/4 plates.

10. 1,2 → b
11. 1,2 "
12. 1,2 "
13. 1,2 "
14. 1,2 "

12/6. (15. 16.) inagglut. less s.c. notably medium. (occurred v. late)
react v. weakly with b: presumed "j" phases.

8/3/53 see 1025 J14 is (i enx)
K1[redacted] enx:
K1[redacted] enx:

J: 12/1/52 FA22 × abony. Crowded swarmers. Pickpost and streaks (for ij s).
(Previous isolates 4p^a - not suspecting as separate isolation was delayed)

typhimurium — x above two step

979 L.M

Selection i b.12 serum.

L. FA22 → SW932 [FA18 → above] Phase 1,2

M. " " " Phase b.

= ants FA22 (but excess) and appear = cells.

Swarms are fairly heavy in both L and M but appear considerably more numerous in M. Phase control not yet established. Isolate 4 each.

(not 1,2 - mutely)

L 1	i → 1,2	
2	i "	
3	i "	
4	i "	

M 1	i → 1,2	
2	i "	
3	i "	
4	i "	

Lp tests? all appear +. save L1 = SW 983

Thus, i: 1,2 can be reconstituted from i: 1,2 → b: cnx in two steps.

J. abony 1+2 x — FA 22A

33 swarms. #34 = 29A All are i except #34, inagglutinable.

Select second phases i anti-i serum. All 34 are Lp^R.

18, 29 straightened out.

In view of variability of abony (I = 7/10 b II = 20% b), make purposeful mixture of phases for these experiments. LT-2 $\frac{I}{II}$: H.L.B.

count 33.

Second phases of #1-34 (14, [redacted] not included) are all enx.

In later test, #14 = i. Second phase: + 1/1

Probably roughness conferred same of above. ∴ 32/32 are i → enx.

33/33

K. 16 swarms total: 1 cont. (not Salmonella)

2 inagglut (viable? ≈33?)

12 1,2 → b

1 i → enx

Summary: In this experiment:

Input / Output	i - 1.2	i - 1.2
i → enx	33	1
b ← 1.2	0	12

add 9796: total: 38 1

0 12

There can be no question of a significant difference in the FA of the two phases of LT-2

Input phases:

① 18 i : 1 i,2

② 20 1,2 : 0 i.

Estimate purity ca. 95%, but may be slightly altered from inoculum used for FA

Note: no i : 1,2 or

b : enx

∴ no linkage

Selection of O- and H

10/23/52.

A. SW 603 $^{G+H-}$ + SW680 $^{G-H+}$

~~B SW603 + SW 618 $^{G+H+}$~~

C. SW 666 $^{G-H-}$ SW 618 $^{G+H+}$

B. SW 603 $^{G+H-}$ + SW 666 $^{G-H-}$

D. SW 618 $^{G+H+}$ 10: SW 666 $^{G-H-}$:1

Work ē D (10+ : 1−)

Prepare broth mix cultures as indicated.
Except possibly in C, (+ > −) initial ratios are maintained in a passage through Penassay (rough check estimate).

① Motility agar center still 10:1
periphery pure + (perhaps slight increase −).

② In soft agar - gelatin tubes: Mix top and recover after 24 hours
− glucose ca. 10:1
+ glucose + ca 30 : 1 −

Should use fully non-nutrient medium.

③ In broth + b serum .02 ml/5
a. inoc from top of broth
b. sediment lightly − top of broth
perhaps allowed to grow too long.

Heavy agglutination.
ca 5:1 ?
ca 10:1
Repeat ē growth 11:35 − 3^{30} (recover)

E. Owing to its resistance, the SW543 line is commonly used for selection by χ phage. In pilot experiments, make up mixtures of SW666 and SW703, and determine what proportion of χ are the pre-introduced Gal− H−.

But SW703 showed virtually no response to χ! of SW533
Phage n.g? Repeat ē χ/SW592 : now all are susceptible (incl SW537)
∴ 942 − 1 has lost some activity
Repeat ē new χ

9803: short serum passage:

unsedimented: ca 6:1 +:−

briefly sedimented: ca 20:1 +:−

control: too dense to read, but very low-apparent.

∴ brief sedimentation appears to be harmful.

Repeat i second pass:

control is another tube from original 980 mix culture serum (ca 1:300) mix i pour into serum tube after light sedimentation. Compare no, light, strong sedimentation.

ca 20:1 heavy sed.

180:1 control

D4 ½% agar. ± glucose

	Val ratio +	−
Broz: washed cells, 980D sec. passage:		
+ glucose	100	<1
− glucose	100	<1

∴ this method is unfit for concentrating O forms.

Methods for concentrating O forms.

10/29/52

1. SW666 has a selective disadvantage in broth passage with SW618
2. Dilution by migration through agar (± gelatin ± glucose) does not conc. the O forms
3. Asher's method may be slightly efficacious — needs to be worked up.
4. χ phage not yet properly tested. Of 588 as H^+G^+ + H^-G^+ 666. start fresh inocula

---

F. SW603 (H^-G^+) and SW680 (H^+G^-). Wash, conc. to ca 10^{10}/ml.

~~Mix 100600 : 1680~~ 100680 : 1603 Add b serum to titer of 1/100.

~~let stop in ca 1½ % salt.~~ Put at room temper. 5:25 — N.G.

Repeat in original broth (mixed & dilutions in saline). (poor agglutination)

Initial	ca 100 - : 1 +
4½ hr. no aerate.	ca 100 : 1
" heavy aerate.	>> 100 : 1

Broth culture here labelled 603 agglutinates ~~ineffective selection.~~ in b. (Mixup?) Test slants

G. χ selection. Make up SW666 (H^-G^-) 1 : 100 SW588 (H^+G^+).

Cross-brush with χ SW592 on EMB Gal, Lac. Compare ratio before and after. Note: SW666 is lysogenic for SW588 : grows plaques on 588

		Gal+	Gal−
χ(Lac)	[dry agar, poor phage action]	~~H+~~	ca 1 : 1
		→ ca 8 : 1	
χ(Gal)	marked phage action	ca 1 : 1	
	Initial	> 100 : 1	
	Final & phage	100 : 1	

∴ χ phage probably is effective to extent of 100 : 1. But how can it be used to study flares?

* recover result may be influenced by lysogenicity. Should strain out from area of mixture arrays from phage action.

In some ~~con~~ control tests,

SW666 reacted weakly i b when grown from broth,

but not the saline suspensions from agar.

Retest both cultures viaz from → v + but very weakly b not i

	Broth	Water
A slant	±	–
B broth culture as above	–	–
C saline susp	–	–
D motility plate (negative)	++	++, not d, i, 1, 2

microscopically: nonmotile

Limit test from cells on D gave wk ± b – d, i

603 broth also v b.

This may be related to the "weak b" reaction seen in earlier experiments.

Restreak D, spot on motility agar.

Conc Ox formed

11/4 G. Y solutions 666+588.

Initial ca 100+:1−
2:30

6:30 Control "

Y ca 50−:1+ ∴ ca 5000 fold solutions!

4 Col+tested: still $H^{1,2}$: all are now X^R (slightly rough?)
Residuals and retest.

F. SW 603 ($H^- G^+$) + SW 904 ($H^+ G^-$)

Initial 4:30
room temp. { + i serum (.2%2) 8:30 } { ca 50−:1+ each
Ct. emt.
Heavy emt. } despite heavy agglutination! (why?)

11/8. F2. a 904 1:1 603 6:30 PM

b 904 100:1 603 added .2 ml i serum 1/10 spec.

9 AM. a: heavy aggl moder. turb. Too much serum? — O-reactive or nonspec. aggl?

b: heavy agglut., lightly turbid supern.

603 "spont aggl?" — not as heavy

904 heavy aggl.

These strains not entirely suitable

Quantitative comparisons of tracks, swarms and Cal+ transduction.

Mix .2ml SW666 & .2ml FA24 ('/10 or '/100 in broth)
Incor areas of con & ca. 0.01 ml each Count swarms + tracks overnight.

	.01 ml ×	EMBCal total tracks	total swarms
		exp. 3 days	24h
30° 1:100	7	0 0 ✓	2 3 ✓ (...)
1:10		15	3
37° 1:100	5	3	0
1:10	5	20	5

all b

T. may not have developed sufficiently at 37° in 24h

each swarm had a distinct flare, several hundred microcolonies. Picks of one distinct to repeat effort at isolating O-forms: (with only 1 does not have an adjacent track)

→ 15 colonies from regions of flare, all b++.
(Total now 60).

~~30° 1:100 5 0~~

	Cal+	
1:10	5	2
1:100	5	2

} Note non-linear response also seen in previous experiment

Background papillae & satellite effect! Restreak these → possible slow Lac fermenter. ✓ in ferm tube.

Acc 9796B

Pedigree of SW534.. 548 etc. 546 982

10/31/52.

According to stock list,

SW533 $\xrightarrow{x}$ SW534 $\longrightarrow$ SW588

||

SW703

However, SW703 $\rightarrow$ only 6 to SW666

SW534 } $\rightarrow$ 1,2. Also, SW546 $\rightarrow$ 1,2.
SW588 }

In view of change of phase of SW534 cf. 533 and alteration in the behavior of its 1,2 antigen question arises as to identity of 534 and possibility of its derivation from SW546.

Possible differentials:

PRE: ?	533 = 703	546 = 857	not "706"
	IV V XII	IV V XII	IV V XII

When were cultures received? 703, 706 are already listed in stock list therein 857 is not

"533" recorded as misc. Body A and C. first mentioned

	Xyl(F.T.)	Rhamn	Inos	P942B	P942C	P666/588	P2114-43	EMB/MR
SW546	+ less yes	− ✓	− ✓	+	++	+	++	++
SW588	+	− ✓	− ✓	+	++	+	++	++
SW942		−	−					
SW703	+	+ ✓	+ ✓	±	÷	−	+	+++
706		+ not	+					

✓ 48 hours

Most differences are slight but all tend in same direction.

SW588, 534 must derive from SW546.

Note: 534 mor+ SW666 on motility gives swarms; parents alone do not.

Attachment: 2 were Gal+ 1,2! (Anomaly in direction of transduction)

(see over)

Retests ± PRE original stocks (via NZ inoculum)

	Rhamnose	Phage A1	A2	A3	
#3	+	R	S	S	
#4	+	R	R	R	
#6 ?	−				Salmonella did not grow out
#157	−	S	S	S	
SW534	−	S	S	S	
942	−				
807	+				

857 = culture out of Edwards' slant

546 = culture maintained here, presumably identical ± above.

546 and 534 should be compared with PRE #6, which has evidently become contaminated here. It appears almost certain, however, that 546 = 857 → 534.

Note 3/1/53 SW703 contam. (see 976-5) now proves to be −1,2, and may be ± Edw 157. This would explain origin of SW534 from stock culture of 703. Some of contam. ??

Additional phage tests

11/1/52

Smyth cross streaks on EMB Lac. S. typhimurium

↓ \ →	Boyd 4	4R	LT1	2	3	4	5	6	7	8	9	10	FA26 PT22	FA26 688
Boyd 4	Boy		–										+	
Boyd 4R													– –	
LT 1	–		~~+~~										–+	±
2	–		–										+	
3	–	+	–		maybe surf pl.								– +	
4	~~+~~		++										– +	
5	–		–										– +	
6	–		–										– +	typ pl.
7	–		–										– +	
8	–		–										– +	
9	–		–										– ±	
10	–	±	–										X +	surf pl.

∴ ~~FA~~ LT4/LT1 LT3/Boyd R should be grown out: hor mix. to both

---

✓ in homologous parents: PLT4/1 → overgrown lysis on LT1. FA 27

PLT3/688 → full lysis on SW688. FA 28

build up against; mut's to be "adapted" ← FA26/LT6 → ~~no lysis of LT6~~ scattered plaques in LT6, 588.

6 sens.

	FA27	FA28
LT1	+	–
2	±	–
6	+	–
7	–	–
10	–	–
22	–	–
Boyd 4	–	+ (maybe res. R?)
688	–	++
666	–	–
909	–	–
Dov. mon. 1	–	–
Landau	–	–

Transmotil.

	26	27	FA 1	28
906	–		+++	
908			nearly P+S	
909	–	–		–
666	2 Ts., res.	–		–

		27	28
11/13	LT-1	+	–
	912	+ (lysin?)	±
	688	–	++
	688 Mot	–	++

of FA 28, prob Boyd 4 688 688 mot

titres of FA27/28 may be inadequate

FA 28 is certainly a "rough" phage

Misc. phage tests

11/12. Prepare Boyd A1, A3 (grown on 912). Test on Boris-mod. strains 1-8 (2 spicer): no action. ORT on 912, no action on 6887. Same results obtained with the bacterial cultures in lysogenicity tests. (Write Boyd for his boris-mod. indicators.)

	A1	A2	A3	
703	~~703~~ −	−	−	later +
666	~~66~~ −	−	−	
905	−	−	−	
546	.. −	..	..	(inadequate cells)
534	++	++	++	

None of these phages appeared to transduce ~~S. para~~ 666.
Recompare 534 i cultures from NZ of available "S. paratyphi B".

	A1	2	3
LT2	R	S	S
7	Rs	S	S
22	R	R	R
927		R±	RS±Id?
abony		R	R
coli 1		R	R
2		R	R
3		R	R
(Hac-) 4		S	S
5		R	R
eastbourne	R	R	R
stanley	R	R ±	R
typhi 757	R	R	R
~~22~~			
558	S	S	S
senftenberg	R	R	R
duisburg	↓	↓	↓
montevideo			
florida			

Include LT2 ± UVLT2/A2, A3.
558 seems to be most satisfactory general indicator. A1-3 not advantageous for other Salmonellae (cf. abony).
A2-3/ S. coli 4 ?

1st preps.		+ SW435 EMP Gal	+ SW666 Gal.	+ SW666 Mot
	A1	2	0	—
	A2	5		—
	A3	5		—

φSW666 transducing ability

misc.

11/1/52

SW666 seems lysogenic for SW588. See 983 for tests

φ666/588 = FA26

FA26 + SW666. No swarms. Numerous tracks: 18, 10 tracks

Retest, of 37° and 30°

FA26 + SW906 (minute): 1 track, no sw.

FA26 + SW534 several tracks no swarms.

FA26 + SW666 2 plates < 1: 2(1) swarms. both b, Gal−, 35 tracks
+1
0 swarms, 29 tracks.

2 s. 64 swarms
in EMBGal − 0.1 ml → no papillae

SW534/+666 → 2 s[illegible], Gal+, 1, 2 ! (i.e. SW666 → 534.!) 0.1 ml/plate

θ-20 + SW666 - not. und Gal : No +, T, or S

see over for host range of FA26:

FA 26 tests for lysis (~~Enterobacter~~ EMBLac

Resistant: London (self-lys.), marlow., duby, easth. stanley florida
poona, senft., sundai

E coli 1,2,3,5, ~~Boyd 2~~ Boyd 4, SW704, LT-2, abony.
(9790-1-5) SW905-908, SW666.

sensitive gallinarum 774 and SW558 → A few plaques on "coli 4."
very faintly

b - agglutinability of SW-666

985-

Cultures sitting on desk
appeared to show b agglutination (Shiki tests at 1:100 serum)
Streak out: numerous rough colonies. These are non-specifically agglutinated from colonies. How explain specif. broth?
Prepare both cultures from isolated R and S colonies.

		LP22	FA-mot	
1	stable	R		
2	sed.	R	–	
3	sed.	R	–	SW910
4	stable	S	++	

Resuspension of #1 streak-out gave two kinds of colonies, rough and very rough.
"VR" gave stable susp. "R" gave aggl. growth in broth, but mixture of R, VR colonies.
Repeat in broth, restreak.

		FA9	FA26	md. cols.	broth on EMB
985-1- 1	stable	R	R	VR	R
2	aggl.	R	R	R	VR
3	stable	R	R	VR	R
4	aggl.	R	R	R	VR
	66	S	R		
	5V6	S	S		

Res restreak (transpar) each of two types now appear stable
These cultures are not specifically agglutinable i b!
Repeat #1 = SW909
Assume that "b-agglutination" is spurious.

Lysogenicity and b/i transductions

Nov. 5, 1952

Summary of transductions/ratios. — × SW666

974 E1	SW904	25 i	LP22 (FA9) 21 R	4 S
		2 b	1 R	1 S
974 DD5b	SW623 (12B)	11 i	10 R	1 S (SW904)
		1 b		1 S ? (SW696)
5A		10 i		
D5 *		1 b 19 i	8 b	993 E 9 i 0 b
974-12		4 i	2 b (18 i — ?)	

Note heterogenicity	other data
* 12:5	
* 19:8	35 i : 2 b
31 13	∴ 12, 12B ×

974 D1	SW682 FA16	2 i + 7 i 1 b	
D2	685	4 i 2 b (colonies from pool)	
974 ..	PLT22/2	5 i 2 b	

Bruce's data:

971 D ?	32 b : 10 i !	(but may be phase 2 FA)
(588 →×) 979-6	31 b : 13 1,2	
573 →×	18 b : 2 i	
546 →×	5 b : 1 1,2	
971 A (dublin)	5/10 b	
A3 altendorf	10/10 b !	Contrast typhimur!
A6 (entutibis)	6/11 b	D6 1/3 b
A8 sandiego	13/15 b	

but check rates of phage

There is some indication of differences in the transducibility of b/i in different cases.

Collect addnl. data

A. 27A → (FA from purified phage 1) ~~20~~ (plates kept room temp overnight prior to incub.)

FA9: ! 19 b : 1 i — swarms relatively close together
19^S 1^S
A2 A1

B. 21 → 666. — swarms very crowded!

Plated: — but all reactions clearcut except as indicated. (But FA21 stock ?!):

9 : i 2 : b 1 b+i

FA9: 8^R : 1^S 2^R 1^R
B1 B2 B3

→	↓ LT-2	FA 9	FA 22
A1	−	S	S±
A2	−	S	S±
B1	++	R	R
B2	−	S	S±
B3	++	R	R

C. Lysogenicity:

→	LT2 ↓	SW666
SW697	+++	±
904	−	−
LT-22	+++	±

∴ SW697 has become lysogenic, presumably the basis of resistance

D. Streak out lytic area SW416/FA9. Test single colonies: ~~9^R : 12^S~~ Restreak 1 and retest
(Should use instead the stationary part of a ×SW616!)
1 rough 2 now smes. 1 FA9^R, not lysog. may be rough.
Repeat plating 666 + FA22, FA10 on EMB Cal. (correlate lysogen; transduc.?)
Cand. Cal+ from FA10, no visible ϕ act.; FA22: 2 Cal+/plate ca 300 plaques.

Note: A was treated with PLT-22, B with FA21 (adapted to typhimur. and para B resp but both untested with FA9. Also test ± PLT-22

! → EXPS with FA21 doubtful unless marked st.! Re-streak FA21!
A+B all Cal−, as is FA21/cart. A preps OK.

E FA22 or. → 666 37°

F FA 22A → 666 37°

G. FA 21ST → 666 37 Uniform outgrowth. Sterility of FA21? Redo G: 1st exp no repeat!

H. 22A R.T., then 37

I 21ST R.T., then 37. 12 swarms moi P11.

J

G. 72 sw. 4b 18i (#i-4=b) FA9: G(b): 4R. i 12R 6S Total: 16R. 6S

I 12: 1b 11i all S 12R

F 11 sw: all b. ! : all S all S

E 24 sw: 23b: 1i ! b: all S i: all S all S

34b: 1i for FA22

5b: 29i for FA21

No obvious correlation b, i, lysog. except that FA (thymus) → > b, S

FA 21 → > i, R

No drastic temper. effect either.

K. 21 → 603 (to refute sterility problem) finally! 18 swarms: all bal+

2b 1b+i 14i

all R 5 FA9S 9 FA9R

in full agreement with G-I above.

Totals: FA21: (count b+i as 1 each)			9R	9S	FA AEF 22		9S	9R
	b	8	0		b	53	0
	i	32	11		i	2	0

Does frequency of i relate to host adaptation of the phage or to the previous selection for an i transduction? See 993

986 D

P- FA10, streak out Gal+ papillae ~~(all) (only 2 papillae)~~.
a) papillae: 20: all plus +
b) recover Gal- assoc. c papillae
c) pick inter-papillae areas: (visibly phage free: always possibility of invisible plaques)

1 PLT22 × 666 (only 2 papillae)

E: cross knusle LT2 /PLT22 and 666 /FA9. (1) (2)
streak out and test colonies for lysogenicity on LT2

1. 10/12 are lysogenic 2. 1/12.

Pertinent to isolate = 986~~E~~1, 986~~E~~2

R = lysogenic S = sens.

a-b: D1: 1^R-a both band/a ^{S}. D2: →

c: D1: $18^S : 3^R$ D2: $16^S : 3^R$

No obvious correlation a and b, the latter appear to be random.

Note: Gal+ are $13^{R+} : 7^{R-}$

D2 Gal- (adding b+c) are $24^S : 5^R$

13	7	Gal+
5	24	Gal-
4/5	knelp	

Sufficiency of phage?

(over)

a	b
−	
+	+
+	−
+	−
−	−
+	
−	+
+	
−	
+	
−	
+	−
+	
+	
+	
−	
+	−
+	−
−	−
+	−

13+ 7- 2+ 8-

11/23

a	b
+	−
−	−
−	−
+	−
−	−
+	−
−	+
−	−
+	+
−	
+	+

5+ 6- 3+ 7-

c: 25 all- !

(over)

a/b comparison

	Lp+	Lp-	
Cal+ a	18	13	31
Cal- b	5	15	20
	23	28	51

a/b+c1

a/b+c1+c2

D3 - Lp+? colony is 4/23. Apparently throws stable Lp(+?) more frequently than D2
- most regular colonies Lp~~+~~ -
  irregular " Lp+. same as D3

SW905-6-7-8

As received from W. Hirsch

905	b (1,2)	Rapidly motile	} in first selection. Mailed slime wall, especially 908, also 905...
906	b	Non "	
907	b+1,2	" "	
908	0	" "	

Isolate "pure" b phases 905-906

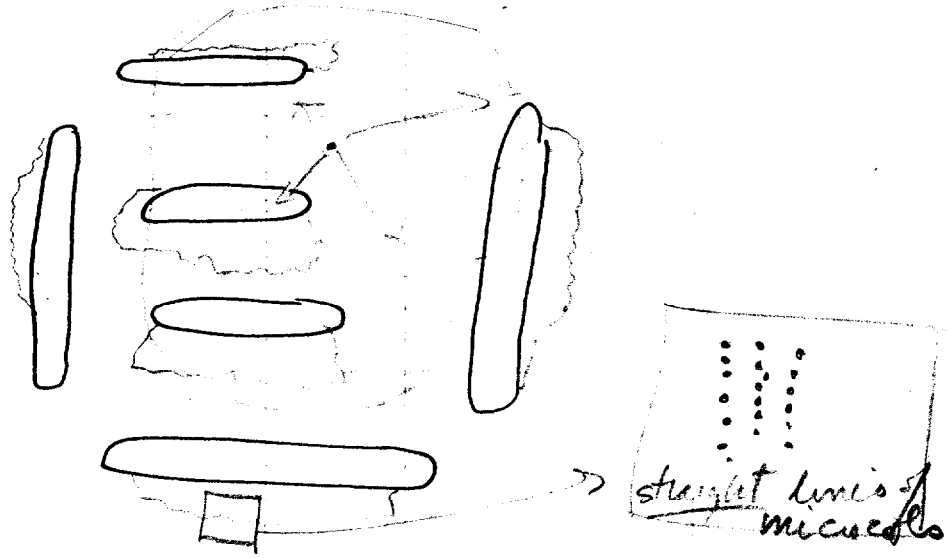
907: 3 single colonies: pred. 1,2, also b! Restreak

7/7 colonies mostly b as well as 1,2. Pick #4, least reaction to b as SW907 (1,2). Either very high mutation rate or b ↔ b,1,2

Note. Contaminant spread on 908 plate. As it did so, there was a filmy, limited spread from edges of non motile 908:

A).

One repetition,
SW 908 and SW 554 responded very strongly;
SW666 and 550 gave no appreciable spreading.
Swarming is confined to the surface.



Reisolate the contaminant for further study: inhibited on NA (gent. wilt) and EMB., but apparent pure culture in broth. It is a spore former, not actively motile. The outgrowths are not the spore terms but not actively motile.

B Phage tests: all 4: FA 26^R; faint sens. to FA 9.

908: gave a spont H^+, no plate / 3·4 (no flare).
+ FA 9 gave a good yield of offtarts and swarms, occ. flares in these

SW905: b → 1,2 → b ca 20% per streaking! Most colonies grossly mixed!

a) note very high rate of phase variation.

C b) FA21 → 908; numerous T+s. (tracks unusually long + prolific).

14: 6 & 1,2 mutants (ca ½ each predominate!)

2: 1,2 + 6? (or i?) Restrict these for further test: definite 0, no; mutants.

phage unprocessed to FA9 are indistinct. However, 10 (or more) appear to be still sensitive to FA9.

L

O-form incorrect

4/1/53.

in SSagain

① SW616 + SW967. Controls — . ⊗ 4 extinct trailers. ·1 Gal -)

O-form crosses

11/12/53.

666 × 550
666 × 551
666 × 554
550 × 551
551 × 554
551 × 554

No motile. sw550 sends innumerable very short hairs into agar. (Cf. sw

906 × 550
906 × 551
906 × 554
906 × 666
906 × 534
906 × 909
534 × 909
909 × 550
909 × 551
909 × 554

1st trial: bad agar: ruled repeating

A sendai 771 × abony^2 (15) 11/9. 2 buds 4/11 3d bud (2d plate)
a, 15
not saved 1. cur ⇒ a SW925 ferried not tested
2. cur → a
n.s 3 cur → a
11/13 4 a (not transd.)

Purify swarmer
Isolate single colony
+ select on homologous
serum for alt phase
B 11 × abony' (14) 1 bud — not transd.
a, 15

C. eastbourne 770 × abony^2 1 "bud"? — cur+. not transd.
cur
15

D × abony'
cur
15

J × FA 22 typhimur. → 1 "bud": weak cur Re ← cur++ i+3 i0.
maybe mixed: streak out and look for i
12/12 cur+.

E SW557 (typhi 901) × SW588 (FA25) 2 plates, 1 bud each •
d 10/13
save #1 = SW930 1. 1,2 → - 3. 1,2 → -
Purify and test alt. ph. 5 isolates 1,2 not d. 2. 1,2 → - monophasic 1,2.
Control: ~~46~~ 546 /1,2 → no mot.

F SW ~~5~~ 546 × FA typhimurium FA22 4 buds: all i
1,2

G 546 × FA11 3 buds → b

H 546 × FA 15 1 b !
2 b !
3 cur
Does this mean heterozygosity for b in 546?
See H, supports latter.

Note 546 on motility agar shows no flare.

Resumé

A. SW534 ← FA22. 30 tracks: 2 swarms (no flares obvious)
534 maybe heterog. rough: necrotic smooth.

B. FA21 → SW666.

	Tracks	swarms
~~+5~~	13	4
	28	6

Pick free tracks. 5/7 are Lp+

C. Stick out from non-metabolized regions: 3/11 are Lp+.

11/20../52
See 989

test pleurisy 5 Lp 1 cave as SW...

F. FA22 → ×546 11/11 : all i 1-4 tested as anti i : i : —

11/24, repeat. very numerous swarms 16/16 all i. Purify and test /i ; Lp ~~=2~~ 9
In first few hours, none swarm. ~~8~~ 8/16 are sensitive to P22.
Overnight : no swarm. (#1,3,4,6, 13,14,15,16) Save F1 as SW-934

G. FA11 (SW618 b:-) → ×546 3/3 : b: — (all Lp^R)

T.O. 991G1A ~~eventually~~ isolated. Save for z33 test. ✓ prob. z33 by slide test
991G1 = SW939 Lp^R

H. FA15 (b:"enx") → ×546 1.-2. b: —
3. enx ⇔ 1,2 = SW926

H3 tested after several passages and 3 single colony purifications.
In first tests, enx phase also reacted with 1,2., but purified version was pure enx on slide agglutination.

H1 eventually gave "inagglutinable" H1A. Save for z33 test. ✓ z33 3/6/53 ~~Save~~

H1 ~~&~~ Lp^S, H2 Lp^R. Save H1 = SW937 H2 n.s.

pass SS 3/5/53 → both 1,2 T.O.

K. FA18 → ×546 (cf. F) K1 ~~&~~ pan agglut? reacts b, enx, ... ? ~~save~~.
K2, K4 still 1,2 K3, K5 "inagglutinable" but slow 1,2 ? 3/5 ~~save~~

C. 546/1,2 control 2 "buds" < 1. 1,2 2. inagglut. (cf. K3,5) save! C2 3/5/53 inagg. pass SS

M. 926/1,2-enx " . 1 "bud" - still 1,2. Not ~~saved~~. n.s ↓ still inagg. save

not Lp^S L. FA22 → ×SW926/1,2; enx → Many T+S. 8/8 i → enx
3/8 are Lp^S. Save #1 as SW933
n.s. others

N FA22 → ×937 numerous sw. Pick 12 : all i : —. ✓ Lp ~~#3.5~~ others: R. do not save

P FA18D → ×937 (rare). ~~Not plated~~. 14/4 plates inagglut! Reselect as b.enx (and control as SW937): 2 grew more than others, but inagg. Select /i motility agar. 6 added; also inagglut : inagg. (weak b?) cf. H1A!

Q = 937/b control: ~~lots of enx overnight~~ 1 sw/plate after 36 h. T.O.

(see over for conclusions).

a. N25 → 157 presumably a spontaneous mutation
(b) (1,2) see Edwards 1946. b → 1, 2.

b. #157 carries a allele at H_1, as shown both by ×546 and 546 × experiments

c. The H_2 allele is either null or the "j" phase that occasionally crops up.

N25 = SW942 should behave as $H_1^{\ b}\ H_2^{\ o}$

a) b: homologue of H_1

b) $H_2^{1,2}$ × 942 should → $H_1^{\ b}\ H_2^{\ 1,2}$

see 1000.

See 989E.

E. 546 → typhi H901 537 : 3 (1,2), (a²) Purify : single colonies pure 1,2.
On 1,2 again:
1 1,2 → — SW 930
2 1,2 → —
3 1,2 → —

A 546 FA25 → abony' 11/19 : 4 blues — all 1,2 1 1,2 → enx 5 12 → enx
2 " 6 "
3 " 7 "
4 "

B 546 FA25 → abony² 11/11 : 3 blues — all 1,2 1. 1,2 → enx → 1,2 [redacted]
2. 1,2 → enx
3. 1,2 → enx
4 "
5 "
6 "

3 s.r.

Study B1 in greater detail: ←.
1,2 phase does not agglutinate (shikes) in enx
enx phase shows same (1,2).

all are 4p except A7; same as SW 938

~~C. 546 1,2 control~~ (see 979 for abony' ≠ abony²)

~~D 18 → 546 1,2.~~

Host adaptation of PLT22 -...

b/i determinations

SW686 unfortunately is Lp+

A. FA31 (= FA9/LT-2^{I}) → × 666 Many T+S.
2i; 11b.
In cross-streak tests (~~no plaque count~~) this phage appears to have retained its specificity for ~~parental~~ strains. Ca. half the numbers are phage-plaqued. Phage character should not be determined s prior streaking out of the culture.
This result would indicate that host adaptation of the phage does not determine b/i ratio.

B. FA21 → × 928 Fairly distinct swarms: 4b: 16i

C. FA22 → × 928 Many T+S (confluent). Pick plaques. (Lytic area noted in swarm!
(Pick and test for Lp^{s} or Ph. mutant.) ⇒ no evidence of either; clear area may have been single stabbing infection.
Swarm Not true will separated. 14:b 1:i 2:b+i. Real total as 16b:3i

∴ lysogenicity of transducee does not influence b/i ratios.
SW686 is unfortunately not feasible for FA (Lp+). This remains the basis for a final test of mechanism of ratio. In addition, other i's from FA22 → × 666 should be examined. See 973C3

11/24

D1	FA 36	A	×	666	T+S	9i : 9b	sic!
2		B	×	666	T+S	7i : 7b	"
3		C	×	666	No	7i : 3b	
4		D	×	666	No.	6i : 2b	

(SW935 / SW936)

Test 36A → × 666, 36B → × 666 for Lp^{s} i — A 6/8 are Lp^{s} (one is " " n.s.)
(vague opportunity for verification during isolation) B 3/7 are Lp^{s}

E FA12 → × 666 Relat. few swarms, but 9/9 i

(over)

11/28. 628 → x 666 7i 4b 1b,i

8i : 5b

This appears to be quite distinct from 623

11/28 FA12 → x ✓ 5i : 0b

FA12B → x 12i : 2b

Using pools from
12 → x 15i 5b
12 → x 14i 6b
12B → x 16i 4b

poor agreement with other data -

625 → x 7i 3bi 3b (swarms must have been confused, from overlaft again)

680 → x 14i 6b 1bi

15i 7b.

note: ~~sup~~ suspicious sum of b's as I did up on Possibility that there are hidden cues for b/i on the swarm plate?

plate:

i i
i i
i i
i i
i b
i b+i
i ~~b~~ i
b
b
b
b
i
b

Rx verified!

b:i determinations
summaries

12/5/52 → × 666.

		i	b	
A. FA 31 (= FA/LT-2^{I}) →		2i	: 11b	Need more data on parents, B adaptation.
B. FA 21 → 928		16i	: 4b	
	cum. total	60	12	
		13	11	
C 22 → 928		3i	: 16b	
	cum. total	7	80	
D 1 36A (SW935)		9i	: 9	
see 2 36B		7	7	
973 3 36C		7	3	
C3 4 36D (SW936)		6	2	
(SW623)				
E FA12 → 666		9	0	cum tot 61:4^{b}
" "		5	0	
12B "		12	2	
pods, colonies :	12	15	5	} discrepancy?
(HLB)	12B	14	6	
	12B	16	4	
625		10	6	(3 were mixed)
626 FA37		49	21	(+16 mixed)
628		8	5	
680 addnl (HLB scored)		15	7	
		22	5	16+i } heterogeneity?
		63	7	
		100	19	
18D		[redacted]	24	
18 see 971D18.		5	52	

Number of classes is doubtful.

SW935 ca. 50% each

623 61i : 4b

680 100i : 19b (heter?)

626

?? Selection discriminating b, i?
Difference might be controlled by selective effects of the desired markers. For further study, more efficient scoring is essential!

11/25 FA10 titrated as 2150/ml* on SW666. 2 plates each

A. (.05 ml FA10 → SW666)

B. SW609 (= Gal⁺ H⁺) 177; smeared

C SW609 + SW666

D .05ml + SW609 + SW666.

control on φ⁵ of 109 Lewis!

24 hours. Counts in C appear > D. D noticeably but not greatly ahead of A

11/5:

		control H⁺	transduc H⁻		
Totals: type	Lp⁺	~~8~~ 3	18	3	18
	Lp⁻	43	43	43	3

After 24 hrs: Rate of lysogenization fairly low.

This experiment would be improved if lysogenization were limited by known factors (insufficiency of phage)

D:

	Lp/172	H⁺	H⁻	Not	2	3
C 1	−	+	+	+		
C 2	+	−	−	−		
C 3	4 −	+		+	+	
C 4	11 +	−	+	?	−	
D 5	−	+		−	+	
C 6	−	+		+	+	
C 7	−	+		+	+	
A 8	44 +	−	−	?	−	
C 9	−	+		+	+	
C 10	−	+	+	?	+	
C 11	−	+		+	+	
C 12	−	+		+	+	
C 13	44 −	+		+	+	
C 14	−	+		+	+	
C 15	−	+		+	+	
C (16)	4 +	+	✓ +	(+)	+	
C 17	−	+		+	+	
C 18	−	+		+	+	
C 19	−	+		+	+	
C 20	−	+		+	+	
C 21	−	+		+	+	
C 22	−	+		+	+	
(5) 23	+	−		?	+	
− − (24)	+	+	+	(+)	+	
C 25	−	+		+	+	
C 26	−	+		+	+	
C 27	−	+		+	+	
D 28	+	−	+	?	−	
C 29	−	+		+	+	
C 30	−	+		+	+	

	Lp	H⁺	H⁻	Not	2	3
C 31	−	+		+	+	
C 32	−	+		+	+	
A 33	+	−	−	−	−	
C 34	−	+		+	+	
C 35	−	+		+	+	
A 36	+	−	✓	−	+	
C 37	−	+		+	+	
C 38	−	+		+	+	
C 39	−	+		+	+	
C 40	−	+		+	+	
A 41	+	−	+	−	−	
C 42	−	+		+	+	
D 43	−	−		−	−	
C 44	−	+		+	+	
C 45	−	+		+	+	
C 46	+	−		−	−	
C 47	+	−		−	−	
D 48	−	−		−	−	
C 49	+	−		−	−	
C 50	−	+		+	+	
A 51	+	−		−	−	
C 52	+	−		−	−	
C 53	−	+		+	+	
C 54	−	+		+	+	
D 55	−	✓		−	−	
C 56	−	✓	+	+	+	
C 57	−	+		+	+	
C 58	−	+		+	+	
C 59	−	+		+	+	
C 60	−	+		+	+	
A 61	+	−		−	−	
C 62	+	−		−	−	
C 63	+	−		−	−	
C 64	+	−		−	−	
C 65	+	−		−	−	
− (66)	+	+	✓	+	+	
C 67	−	+		+	+	

		1	.01
FA10	SW666	215	18
EM/Gal	SW928	360	29

FA22 .1ml 928
.01ml 978

A. Goldstein strains

995

Received 11/2[illegible]/57.

W2148 = "MSFB"

very rough, apparently homogeneous on EMBLac; MH. grows well on D/0).

2149 = Harvard (Goldstein)

dimorphic, especially on EMB MH.

A: smaller, darker (lytic ?) ~~or gummy~~ colony

B: gummy, ~~[illegible]~~

poor growth when streaked on D/0).

11/24. Separate A and B for further test — character persists on EMB MH.

Replica plates: A: no growth on D (0) or EMS Lac in 48h. Eventual reversions ?

B: all colonies grow (ca 50 tested).

For further work, need to run down requirements of A.

In subsequent plating of B, ca 2/300 auxotrophs. Pick as 995B 1-2

4/14/53 EMP

Subtypes W2149 observed

homogeneity W2148

Both R to 2-2

Srp with W1926 (F-) and W-1177 (F+)

on $Smal_{sm}$ + $Slac_{sm}$.

Dienes' L forms

Acc 1319 8/56.

11/16. Bought tracts from Boston slants of "Proteus 52" and Salmonella typhimurium "3". In addition, a vial of L-growth of the Proteus (Dadey shaken in flight.

(A) Plated in Penassay ~~agar~~ broth; no growth here for two weeks. 11/30 – 12/1 surface films noted in 1 of 3 tubes.

12/1. Transfer to fresh tubes: Penassay; Penassay + horse serum 10%; Penassay + cotton; motility agar; motility agar + SW66: Good growth in 2–3 days, especially on penassay broth. Very poor growth on agar. Note a curious film of material in the course of L-form growth: fatty material? in phase microscope.

(B) ① Proteus 52 heavily inoculated in agar layer with ca 1000 u penicillin ca 11/18

ca 11/21 ② cut out block and smear over surface of fresh N.A. + penicillin plate.

Both show L-type colonies especially in depths of agar.

③ Transfer to broth / agar slant + horse serum + penicillin.

12/15: Dense growth on agar over lysis! No bacteria noted.

12/16 Remoculate to wet slant & horse serum + penicillin

12/17 a Broth; motility agar: typical motile bacteria

b Agar + serum: mostly bacteria; some granules + fragmentation; irregular hyphae in microcols.

c Penicillin serum agar: no marked growth.

12/19

(C) Transfer cultures of 12/3... to v. small inocula.

P24

1.	Broth	–
2.	" + antiformin	–
3	" + collodion (boiled)	++
4	" + mineral oil	–
5	D/nic)	–
6	D/nic) + a.f.	–
7	" + oil	–
8	Mot agar tube	+++
9	" 1/2 & penassay	+
10	" 1/6 " "	–

12/23 11 Streak out on ~~mot~~ motil. agar.

12/27: Motility agar, in tubes seems best
~~Emulsified~~ Emulsified inoculum, however, does not grow well. Heavy inoculum grows well in broth

Protues 52 pour goute vol in 0(0) ? impair ? mi ?

12/20 996 B: grew very well on motility + penicillin; no swarming.
Microscopic examination: large spheres + small granules.

D Salm sw 4/6: smaller sph + gran. ξ as Mother culture.
E Pr sw 4/7: like B

Test for recovery of bacteria; try to float Ba broth.

12/28 C, E all gave turbid growth. Carry on penicillin broth. Transplant D to second penicillin-mot agar.

E gives faint hazy turbidity in penicillin broth. Microscopically, only swollen forms were seen. Titrate on EMB lac and plain broth penicillin.
D gave clear broth. Plate out E → Broth ± 500 u/ml penicillin

E: 10^{-1}, 10^{-3} ml: crowded on EMB Lac 10^{-5} ca 100 (size v. variable)
10^{-7} 0 on EMB.

12/h.: Passage Turbid ± penicillin 10^{-1}, 10^{-3}, 10^{-5}.
10^{-9} clear 10^{-7} - penicillin turbid
+ penicillin turbid but settles granularly.

These are generally more turbid than above inoculum.

Microscopically
a. Inoculum spheres and granules
b. 10^{-7} - penic. motile bact
c 10^{-7} + penic " "
d. 10^{-1} + pen swollen bacteria and many spheres
e $^{-1}$ - pen. spheres and " "

note degree of granularity → different type of bacteria!
Subculture → normal bact!

try 1 ml inocula

(over)

c8, stood on motility agar in frig., and rewarmed back and forth to Chamblee gave a desposie growth in broth 3/10/53. Resembles paracoli ° °° under microscope. Presumably contaminant — fails to grow on EMB lactose

1/14/53 D - (Saline) has shown no turbidity or growth of any kind

E. ②. Had been turbid throughout medium; has now also developed a film on surface. Transplant to fresh penicillin broth + penicillin + colloidion = E3 a, b.

F. Centrifuge E③. Assay supernant and filtrate of same for bacteria on EMB lac. Resuspn sediment in 1 ml (ca 5×) H_2O — forms a gelatinous mass. Under scope, bye clumps of L-type 

Put on plain agar 1:15.

after ca 6-8 hours, small microcolonies of plump pictures appear in conjunction with clumps. Bacillaform could not have been present in comparable numbers but detailed origin was not determinable.

filtrate: sterile on fresh broth and on plate. ~~Unfiltered~~ Unfiltered supt had ca 10^4 colony formers/ml. (1000×10^1 but 0×10^{-3}!)

Osmotic Shocks

998

12/4/52 Osmotic Shocks.

A. add .25 ml PLT22 (961.4) in broth to .75 ml saturated NaCl.
Add 10 ml H_2O. Add 2 ml broth.

Est titer $\frac{1}{4 \times 12} \times 4 \times 10^{10}$
= ca 10^9

B. as A, but add NaCl last.

Assay plagues; .01 ml samples on sw666 / motility: too crowded.
.1 ml on sw435. < 50 on either! see 999
for host adaptation peculiarities.

A: plague: 17×10^7 B: 33×10^7
525×10^6

No marked effect of shock on plague titre

~~Repeat 12/9 using diluted PLT22~~. Dilute in sat'd saline
~~to an estimated titre of~~ of Diluted ca 40:1

Assay for plagues ~~on sw666~~;
for prototrophs on sw435 D60)
Salt " " EMBsal

use NaCl more dilution?

A. no salt
B. salt added to diluted
C. salt added, then diluted 10:1 for shock.

0 counts
A 45×10^7 B 53×10^7 C 138×10^6

The shock has had only a negligible effect, $pS \ll 1$.
Set aside until more details on conditions are learned.

assay on sw435: .1 ml	SALEMB D10	A	B	C
		3	0	3
		8	1	3

435 has been behaving peculiarly lately.
Probably now largely lysogenic.

UV - FA21; FA12

December 4, 1952.

Preliminary experiments

Assay FA21.

SW666 21×10^8 219×10^7 LT2 1×10^7 !

$= 2.2 \times 10^9$

(also 18D = 192×10^7)

Place 1 ml FA in Petri dish. Expose to UV 120 seconds.

12/5/52 ~~Add 4 ml H.D broth and cocci~~. Assay survival. ~~and titer~~

~~SW666~~ (SW666): 15×10^7 Plaques smaller?

Control: 128 $\times 10^7$ SW666 253 $\times 10^5$ LT2

... $\times 10^5$

✓ 22B may be ill adapted to LT2. (to cells u.s.)

Recheck; FA/435 D/O). FA22 .1 ml 325

FA21 " 0

FA21UV " 2 Resistant thereplagues.

Swarm induction

FA21: .001 samples:

Σ	#	# swarms
	6	0
6	6	1
8	2	2
9	3	3
4	1	4
23	18	$= 1\frac{5}{18} =$ ca. 1.3

999: .001 ml samples:

No zeros. Estimate ca. 5 / sample but not properly countable! Cannot count tracks either. Tracks also still prominent.

Relative activity: $\frac{1.3 \text{ transductions}}{128 \times 10^7 \times 10^{-3}} = \frac{1.3}{1.3 \times 10^6} = 10^{-6}$ per phage.

(For future: Use 120 × no. plaques., more dilute media.)

FA12 maybe more satisfactory.

b:i ratios:

control (FA21)			999		
b	i	mix	b	i	mix
4	13		4	18	2

12/7 Pool FA12, 12B for further studies.

Assay at UV = 0, 120, 240. Plate .001 ml samples on SW666; motility.
1 ml / 10 ml plate on SW666

A	0	666	swarms		
B	120	"	too numerous	355×10^5 / LT2	105×10^7 / 666
C	240	"			185×10^6
D	0	918	15 sw / 9 x .001		430×10^5
E	120	"	18 sw / 10 x .001		

More dilute platings needed.

		phage / 666.	Gal+ / 666.
12/8.	UV 0	[redacted], 87, 100	
	360	130×10^5 — $\times 10^4$	

Swarms: 0: ".001" ca 5/ea.
".0002" 28 (+2 a 3?) / 19 [redacted] $\frac{1}{6} \times 10^{-3}$
".00004" 3/20 $\frac{1}{36} \times 10^{-3}$ =

Fastaders, 1 plate shows 29 T 0 Sw
9 T 3 sw (denser agar)

UV 360:	.001	ca 2-3/ea	27/10
			31/10
			31/10
	.0002		14/10 + 13 faders discrete. plaquing notable!

est. ca 50% reduction in Fla+ FA action. cf. Gal+ ratio.

Note, UV 360 has phage "activity" of 1.3×10^7, Fla+ of [redacted] 1.4×6000
6×1000
= ca 84000/ml.
= ca 1/2000 phage particles!

Phage inactivation considerably faster than FA? Still larger doses needed for specific inactivation studies.

12/8/52. ① UV·0 φ/666 BT; 100 $\times 10^7$ Gal+/666 .1ml: 376
FA12
② 360 130 $\times 10^5$ 280

SWARMS. Samples of 1/10 .01ml, $\times\frac{1}{6}$, $\times\frac{1}{6}$. A B C

$\frac{FA}{\varphi}$ = ca 10^{-5} sw
= $.4\times10^{-5}$ Gal+

1A. >> ca 5/
B. ca 28/19
C. ca 3/20 < 29 trailes 0 sw
later, about 11 additional sw. appeared. 9 " 3 " (dense agar)

* The 3 early are 3/3 i. The later swarms are 7/11 b!

$\frac{FA}{\varphi}$ = ca $\frac{1}{1300}$ sw
= $\frac{1}{5200}$ Gal+

2A 27/10 31/10 31/10 10/10 i
B 14/10 : 12i : 2b (+13 trailes) discrete plaquing (predicted ca 300 plaques/trail)

12/9. ③ ~~600~~ sec.
~~Gal 2 sat'n~~
~~comp~~
some evap. noted (ca .5ml recovered)

φ: 172×10^3 ; 13×10^4 0×10^5
Gal+: ca 1500 on SW666; 28 on SW928
per .1ml (count 1/4)
Swarms: .001 ml samples: (12 hours): 19/23
ca 1/sample
trailes still evident even on soft agar.

$\therefore \frac{FA}{\varphi} = \frac{1002}{172\times10^3} = \frac{1}{172}$!

$\therefore \frac{Gal+}{\varphi} = \frac{15\times10^3}{172\times10^3}$ = ca .1 see E

Non-confluent plaquing is obvious on this plate.

---

* C1B: 26 i/2b includes 3i/1b classed as "small swarms"
C1C: early 3i/0b 1-3
← delayed 4i/7b! 4-7, 8-14 motility of these should be compared!
C2A: 10/10 tested: i hold remainder
C3: 10 tested: 3b, 7i interpretation?
see 1001

* Evidence for selective differentials of b, i, presumably owing to linked factors rather than the alleles themselves.

UV - activation of lysogens? 999D

12/10 1. UV = 0 2 UV = 120. (dil 1:1 prior!)

A	35	A	54
B	99	B	(155)

A = 666 B = 928
.05 ml samples.

12/12: no FA: 0

Does dose partially inactivate? FA/666 activated FA/928.

ca same activation at this dose

Linearity of FA, UV = 0.

Calf	666	928
.1	534	16
.05	291	7
.02	113	2
.01	52	2

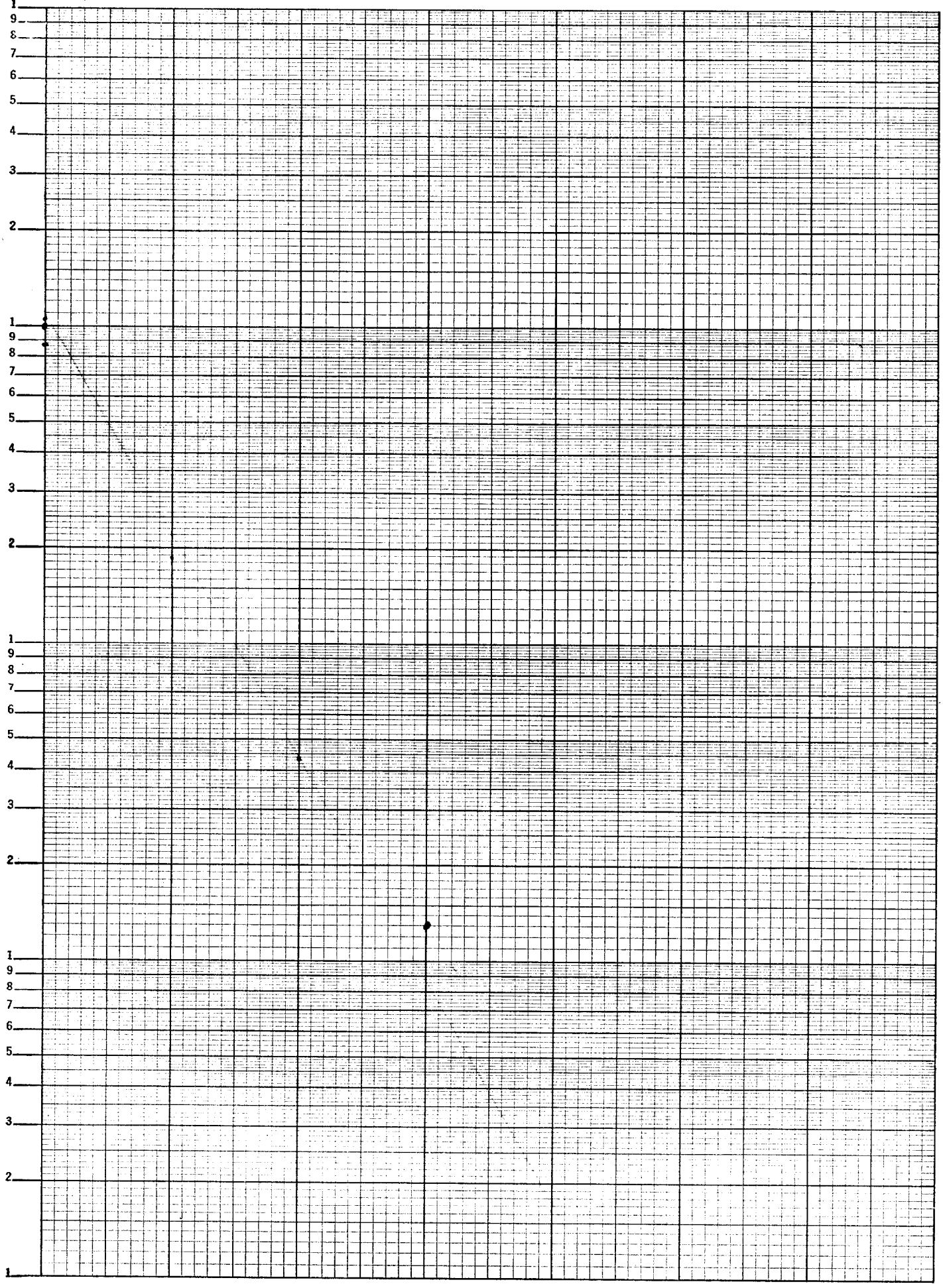
satisfactory linearity but of 999C1 (somewhat less)

ditto, but why lower efficiency? Of.

different batches of 666 cells may account for < heroical efficiency.

FA12/666

999



10⁹

KEUFFEL & ESSER CO., N. Y. NO. 358-81
Semi-Logarithmic, 4 Cycles X 10 to the Inch, 5th lines accented.
MADE IN U.S.A.

180 360

999E

Limiting inactivation of FA ~~11~~ 12. UV 15 minutes 50 cm.

① Dilute 2 ml FA 12 with 2 ml H_2O. "

② " FA 22 " " "

③ " FA 12 UV 5 mins ca 15 cm distance.

12/12.

		SW866		SW928 Gal+	
E1:	$\frac{1}{2} \times .02$:	Gal+ 65	φ 100	11	∴ ca = plaques and transductions.
	$\frac{1}{2} \times .1$:	Gal+ 380	φ 360+	68	
now UV	E10		,05 φ	91	Pick transductions away from plaques.

E2: Residual phage uncountable at 10^{-1}.

E3 φ 16 / .1 ml 16 Gal+ (streak out 12)

E2-0	SW435	,01 ml:	18
		,05	71
E2		0	3
		,05	14

Repeat c LT22!

note slime on D(0) at RT!

ca 5-fold decrement in FA.

E1-3 (1-20 are E1 21-32 E3).

#3 lysogenic: (scattered plaques)

19, 20, 21 lysogenic

all others are non-lysogenic; sensitive to FA9.

These are presumably fortuitous. Lysogenization does not occur typically c transduction by mediated vents.

Restreak all on EMB Gal. All pure + # 19, 20, 21 show some colonies s sheen (λ^+/λ^s?) Restreak: 19 normal of 20, 21

of E3 #21 showed overt evidence of lysis in streaking.

Spot pur. test of 1-20, none were lysogenic.

Aug 1966

SW 942 (b: —) homologies

1000

12/7/52 Edwards N25 = S. paratyphi B java, source of SW857, recd. Prepare FA 42 (FA9/942)

12/8 A. FA25 (SW588) × 942 V. Numerous swarms. Isolate and test 4: 1,2: — (stable > 3 days as 1,2)
~~#1,2, 3 are Lp+~~ save #4 = SW945

~~10/9 B. FA18 × 942 → b: 1,2~~

~~C FA15 × 942 → b. enx~~

~~D FA42 × 944 (1,2 enx) → b. enx~~

~~E FA42 × 946 (1,2 enx) → b. e. not studied.~~

F 18D × ~~H~~ SW609 (H_2?)/b. 8? sw / 3 plates ÷ 8, either b or maggl. Not i or 1,2 of # × 546 but may be due to much greater rate of potential phase variation in the latter.

B. FA15 × SW945 1(1,2)

C. FA15 × SW942 1(b) → 1 sw. 1,2 (see!) 1 bud maggl?

D. FA15 × SW609 1(b) 1 sw: maggl. 1 v motil 3/5/53: 233 T.O.

E FA15 × SW857 ~~1,2~~ (1,2) (note rarity of enx in pur.)
2 buds. both stiel 1,2

4-5 plates each as indic.

C 1: stable 1,2: —
2: not but maggl.) saved E

This expt. gave nothing except ?? C9, recurrent 1,2 from SW942?

D ① stiel b.

B no buds
D only 1 maggl bud.

B ① stiel 1,2 partly rough ?
② " " colony reacts ⑥ and 1,2 ?
Re-select 1,2 : no swarming. T.O.
several maggluninable

Descrepancies in b/i ratios. 1008

999C1C .. seems to point to a bias among i : b transductions correlated with earliness of swarms.

Hyp. 1. i's start earlier for unknown reasons, but move at same rate as b

2. trans ductions start at the same time, but there are diffuses in effective motility

2B. Followed by selection which results in comparable rates!

Repeats unpurified resp of 991C1C:

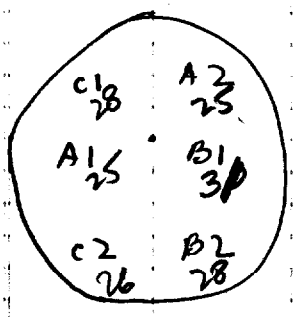
Lysogenicity/LT2.

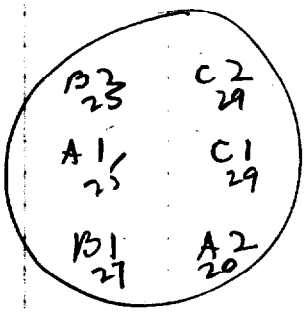
1001 A 1-3 = C1C early swarms, i all +

B 4-7 = C1C delayed i all +

C 8-14 = C1C delayed b. #1? 2,3,4,7 + (5,6 −) of the b except?

From resuspension inoculum compare movement on motility agar 2PM – 8PM; duplicate plates, 2 from each.





– drain in mm

A	25-25	25-20
B	31-27	28-25
C	28-29	26-29

If anything, B, C > A rather than the converse! These were isolated from a different plate, and any diffuseness may be irrelevant to the present issue. Hyp. 2 may be excluded, but 1 vs. 2B cannot now be determined.

Next see also 1030.

Lwoffates - Salmonella. 1002

ca 12/5/52 Prepared by M.L. Morse
Label as Lw.

922 Lw 1 } had some Fla,+ activity & SW666.
927 Lw 1 }
928 Lw 1 no Fla,+ as expected.

~~same character~~ same chancy.

Titrate 928 on SW666, 922 and 927 on LT-2.

922 $< 10^7$

927 $< 10^7$

928 $4 \frac{1}{2} \times 10^9$ /cc. 10^{10} by loop del.
4×10^9
unfortunately, few suitable

12/12 927 Lw 2. 94×10^6 /LT2

			Gal+/666 /mc	Smears
999-16 = SW944	Lw. MLM.	ca 10^{10}; 40×10^8	625	+++
999-24				
999-66		$< 10^6$ but many mejuta plaques!		
SW686	Lw. "	ca 10^{10} 30×10^8	397	+++

Retitrate at

10^{-7}	A. SW927	No UV	on SW950.	
10^{-8}	B	25 s	200×10^5	
10^{-8}	C	40 s	100×10^7 (927 Lw 3)	Respectable lysis!
1/8.	D	No UV as A	10^5	
	E }	No UV Sup't and 24-48h. culture	10^4 ±	plates smeared;
	F }	Try tryptan doses	3×10^5	cassys ± 100×

Serum inactivation

12/12/52 Strain's anti FA serum.

add .1 ml to 5 ml ca 10^8 PLT22 in broth. Incub. at 37° 10 minutes. Assay on LT-2.

	control	serum
3	++	82
5	182	3
6	21	

poor counts

∴ ca 2 decades in 10m. at 1:100

∴ k = ca 50/minute.

Do a more careful test in this range:
Quantity of serum needed to prevent plaquing (and presumably, secondarily segregation).

12/19. Mix ca .1ml 2×10^6 PLT22 + .1ml 10^{10} LT-2 + 10ml ~~broth~~ broth

After 15 minutes add to equal volume of various serum dilutions, plate on EMB Lac.

1	1:1	Plaques evident, small, may be reduced in number.
2	1:5	ca 10^{3+}
4	1:10	"
8	1:20	"
16	1:40	"
32	1:80	"
64	1:160	"
128	1:320	"
256	1:640	"
0	0	ca 10^{3+}

Note: clear plaques noted: 3 as "0"
1 as "2"
no others!

Very high serum conc. will be needed to prevent cross-infection!

Copper / Weed

1004

Dec. 15, 1952

12/14. Inoc ca 5×10^7 cells in 10ml D(o) + Cu. A = W1485 B = E coli B

A

	$CuSO_4$	A		a colonies	b	c	B		a	b	c
1	0	+++	+++	+++			+++		+++	+++	
2	5×10^{-6} M	+	++	+++	+++	1% min ?	+	++	+++	+++	
3	10^{-5} M	±	++	+++	+++		±	++	+++	+++ 1 min !	
4	2×10^{-5}	±	+	+++	orc mm?		±	+	+++	+++	
5	10^{-4}	±	+	+++	+++		±	+	+++	+++	

6PM. 1st reading 9:30 AM 12/15.

a) Streak out on N.A. 9[20] AM. Remainder.

b) 1[40] PM

c) 6PM 12/16.

Restreak 1004 A 2-4, B 3 — normal size colonies in both.

c: few smaller colonies in B5, A2, B2, A3, A4. Restreak ⇒ (normal size)

12/18. Repeat expt. in T(o) as above [redacted]

B

	$CuSO_4$	1485 48h	colonies	B	
1	0	++	++	8	++
2	5×10^{-6}	+	++	9	+
3	10^{-5}	−	few ++	10	±
4	2×10^{-5}	−	−	11	−
5	10^{-4}	−	++	12	−
6	D(o) 10^{-5}	±		13	+
7	D(o)	+++		14	+++

No dwarf colonies from any plating!

(D(o) "citrate? - detergents Cu)

Streak out at eg 2864 no secondary!
Restreak 1485.

C.

Restreak W1939:

	T(o) colonies		T(typ)		D(o)	D(typ)	Penn NB
1939	1 +		5 ++	+++	9 +	13 ++	++ 17
1939A	2 +		6 ++	+++	10 +	14 ++	++ 18 +++
2042	3 ++	+++	7 ++	+++	11 +++	15 ++	+++ 19
2090	4 +	++	8 +	++	12 ++	16 +	++ 20 +++

12/18 PM

~~front~~ Note almost = growth 18:20 on 1 assay. Also Note Both from Penassay; streak out. Note lack of strin. key tryptophane for 2090. 2090 seems to do relatively much better in both than on agar! (over)

Try B-12 in broth, again. No effect.
$Na_2S_2O_3$
2090 – mannic sl. less turbid than the others in Renessay.

cf. aerated and unaerated Renessay 2090, 1939A: 2090 Aer: mucoid
in density at same slow rate as anaer
but reached higher final titre.

despite poor growth use inoculum from C2 for MNQ expt.
in T(0) 4P19 E coli B + W1939A, incor. per Colwell
D) 25, 12, 6, 3 ppm MNQ to T(0) liquid.

	"1939A" tube density overnight	36h.	"B" tube density.
MNQ 0	+++		++
PPM 3	+++		++
6	+++		– ++ normal colonies only.
12	+++		– ++
25	– n.gr.		– +

12/21. Restreaks. A25, B
A25: sterile
B6,12 normal ++ only
B25 some minute colonies
& numerous ++.

Restreak these.
all normal size!

∴ these expts. fail to confirm Weed, Colwell mutation
of 2CV

As received from Colwell (1st and 2d time) W1939A is a mixture of Mal+ and Mal–. Both are highly stable, but Mal– after purif. did give after papillae → blue and +. (Mal+) + W2090 did not give normal Mal–.

Strains for phase control. 1005

S. Sendai SW 771 is being considered for ~~st~~ studies on determination of phase. However, it appears to be unduly rough. Attempt to isolate a smoother strain.

All isolates, incl. from lyophil, are at least partly coarse in habit on nutrient agar, making H-scoring difficult. Gradually H- is rapidly attenuated on solid medium. Ditto for SW 925.

∴ Not at other Salmonella diphasics

see 1024, 1035

Multiplicity reactivation

FA12: ca 10^9 299E3: "160/ml" Does this depend on multiplicity?

SW666 ~~[redacted]~~ 12/17. Estimated bacteria = 2×10^{10} FA12 ≐ 10^9

① Mix .1ml E3 + .1ml undiluted bacteria. Plate .1ml on EMBLac + ~~[redacted]~~

② " " bacteria 1:10 + ~~[redacted]~~

③ " " " 1:100 + ~~[redacted]~~

no light

After 10 mins RT. add .1ml saline to ①, .1ml undilbac to ②, ③ and plate .1ml samples on EMBLac., after that put also on

	EMBLac		Cal: plaques. Cal +
1	6,7	(.075ml)	2
2	2,8	(.05ml)	4
3	1,5	(.06ml)	1

Multiplicity reactivation probably does not account for the residual plaques.

"Lytic variant" 1006

12/22

See 1003: Streak out plaques 1-4 to isolate pure lines. An initial streak of #1 was mix. of clear and turbid plaques, both small; 2 + 3 also mixed, mostly larger clear plaques, 4 small and large clear plaques. For first purps. pick well isolated clear plaques, grow on LT-2

Imp'l tests, 927 and 928 was not lysed by any plaque. LT-2 was grossly lysed. 666 showed only few weak plaques on #4.

Grow plaque on LT-2/2 in broth. All but #3 gave v. clear lysates.

a) test vs LT-2, 927 etc.

b. Add ca 10^5 cells LT-2 to eq. vol [redacted] LT-22. Plate c .1 ml 22V4 (1/2 dil). at various intervals, incl. 0. Then there is a final plating = 1/4 × .1 ml of diluted cells. All platings equiv volumes

		ca. log survr.
1	No PA22, No 22V	10^3
2	No PA22, 22V	(1 or 2)
3	60M FA22	10^3
4	" + 22V	10^3
5	1M FA22 + 22V	10^3
6	~~25~~M FA22 + 22V	10^3
7	25 " , no 22V	10^3
8	45M FA22	10^3
9	45M " + 22V	10^3

of 5 & 2

1 mutate exp. to 22 protects against 22V! Because of very high multiplicity of 22, this may be adsorption - interference!

[redacted]

Titer of V4: 12×10^9, +++ $\times 10^7$ Confl. 10^5. All plaques clear!
(For more accurate titration, use 10^8.)

Qualitative tests:

	V:	1	2	3	4
LT2		S	S	S	S
927	λ+	R	R	R	R
666		odd plaques			
928	λ+	R	R	R	R
912		S	S	S	S
688	R	R	R	R	R

∴ can also be used to select mutants.

(over)

Some "smooth" streaks of 22V. + LT2.

Follow 4 through serial streaking:
gave self-nibbled streaks until isolated
as PLT-22, 22V-sensitive, non lysogenic.

On SW666 22V gave scattered plaques at 10^{-3}
(e.o.p. ca 10^{-5}). Plaques not very sharp, not lytic.

Restreak on SW666 to get 22B1V → retains lytic action on LT2,
gives turbid plaques on SW666.

However, attempt to reduce lysogenicity on SW666 gave 4/4 still sensitive

10^{-15} m. .2 ml ctg $10^3, 10^6, 10^8$ PLT22 + 10^9 LT2/ml (2 ml)
RT.

	PLT22				
A	10^8	count: 111×10^7	+V 3×10^7	288×10^5	
B	10^6	count: 120×10^7	+V: 0×10^7	22×10^5	(589×10^3) sic!
C	10^3	plaques: 23×10^1 (+ some mucoid).	with V: +++ $\times10^{1\frac{1}{2}}$		
D	0	count: 129×10^7,	with 22V: 6×10^5	1073×10^3	+++ $\times10^1$

Plate i 22V at various dilutions. also ⊕ titrates i C.
Stock PLT22 assumed 3×10^{10} ⊕ plaque titre and "protective titre".

Results. Phage added was 2.3×10^8 initial rather than 10^9.
Bacterial count was 1.2×10^9 containing 1.07×10^6 background resistant.

	Phage added	V22^R recovered.
A.	2.3×10^7	$+2.78\times10^5$
B	2.3×10^5	$10+12\times10^5$
C	230	—
D	0	6×10^5 10×10^5

$\therefore$ ~~$\frac{2}{100}$~~ $\frac{28}{23}$ = 1.2 resistant bacteria per plaque formed!

7 contaminated colonies appear at A ($\times10^{-7}$) dilutg. for all added virus!

E Test transduction of FA Y3 (22V4). .1 ml

SW V35	D(8)/8	Gal+/10	SW928: no swarms

all Gal+ stable. Test lysogenicity after purification: all lysogenic possibly exc. #1a (grows poorly!)
This is neither lysog. nor sensitive

Type of lysogenicity:

Kill resp. i $CHCl_3$, streak on LT-2

	type		
1	22	7	—
2	"	8	22
3	"	9	"
4	"	10	"
5	22		
6	—		

22 type is turbid plaque

6, 7 need ~~test~~ more in kinase assay i LT-2

✓ 6, 7 → turbid plaques.

FA43 × LA22.

1006E

10 Gal+ transductants. Restreaked and replicated to LT2.
All strains are lysogenic. #1 and 2 had possibly non-lysogenic components : E2 lysogenic

E1 appears to be non-lysogenic; resistant to 22, 22V.
H still reacts with anti IX-XII. May be an
"immune-1" type. Test for transductibility.

By growing E1 & LT2, turbid plaques were secured.
1006E1 may thus be another "weak lysogenic". Cf.
1009B4, 1009C1.

1006E1 plated alone gave numerous small colonies
D(a) + FA43 gave 7 large + " " .
+ FA22, 1/ml ca 300+ large colonies
1/6/53 435 + FA43 : 1, 3, 5
435 alone gave 6 !

Do SW435 now spont.-revertible ?
May be "intermediate" allele

Remember, small colonies may be living on added broth carryover.
Check these colonies (5 each)

			Gal –
1	SW435 sp.	++ ++ ++ ++ ++	–
2	1006E1 sp.	++ + + ++ ++	+
3	1006E1 FA43	+++ +++ ++ ++ ++	+
4	" FA22	+++ +++ ++ ++ +++	+
5	SW435 FA43	++ ++ + ++ ++	–

+++ strong growth in 24h.
++ " " 48h., mid low temp. (temperature ?) (over)
+ moderate " "

Apparent recessions may be weaker (correspond to intermediate ?)
SW435 now may be unreliable. Check requirements: recolate if nec. Compare
240, 279

Cf. supernates of

1006E1, SW435

= 1006E1A 1006E1C

X-ray of 22B

1007

12/23/52.

X-ray courtesy A Novick / U of Chicago

SW686 hr A no ☢ B 100,000 r C 200,000 r O: original stock

(pur. titr. 3×10^9)

		$\varrho \times 10^7$	Repeat		Calt / 666 .1 ml	Cassay on layg plates $\times 10^7$
Uncig.	A	92	53, 61	57	36	61, 70
sic!	B	26	24, 31	28	48	~~48~~
	C	117	3, 7	4	12	(7.3; 6, 2) 6.8 (73×10^{-6}....)
						original stock: 64

Repeat anyhow shows v. little effect of x-ray!

~~approximately 1 decade per 10 r.~~

∴ ϱ is attenuated ca 1 decade / 200 000 r^2

FA is diminished (?) from 26-12

In view of tremendous doses required, this avenue does not appear to be promising

C sterilized out: all stable salt.

Incidence of lysogenicity: 10/12

S. paratyphi A x—

1008

ca 12/20/53

SW948-949.

+ FA 9, 10, 12 gave no T or S except

1 extensive track 12 – x 948
a few swarms? 10 – x 948

FA10 → x948 2b, 2a FA22 – x948 2a.

SW948 (a) grows smoothly in broth but gives very rough colonies on NSA. Nevertheless, these react strongly with anti-I-II; IX XII

Check absorption of PLT-22. of 925

see 1045

Where are results of 22 – x and A-0?

Dec. 24, 1952

11:35PM. LT-2 Total ($\times 10^7$) PLT22 dil to "10^9". Contaminated.

A	B: count	117, 134
B	B/ml + φ/ml	78, 78 (sic)
C	B/ml + φ/ml	85, 88

D plaque assay ("10" phage) (Gratia plates: sal susp LT2: $\times 10^7$: 67, 73. ; fresh cell 130

dil in terms of original Bouill.

conditions of cells may affect the quantitative recovery of φ (as thioglycollate killed cells or other inhibitors

No effect of multiplicity or survival. The actual ratio φ/B was

~~13/78~~ in B and 130/

130/125 in B and 130/82 in C but amount actually adsorbed was not established.

Detail on B, C:

$r \doteq 10$ C$_1$ 85 plating 4 obviously contaminated. Many additional are vaguely mottled. Festuals these. Replicate to 22V.
central plaque general.

C$_2$ 88 Replicate to ~~LT-2~~ LT-2 4 contam.

B ($r \doteq 1$) Many colonies have a central cloud, difficult to tell whether definite plaque or not. Obvious plaques in 17/18; almost all others have a central plaque.

recover

Small central plaque are multiply infected?
Large plaque & lysed are single??

100901 Total 78 Test lysogenicity by replication

B# lysogenic

all non lys. were more or less lysed by 22V.

21 non-lysogenic
57 lys. of these, 17 showed obvious plaquing and are therefore contaminated.

On replication, 8 additional were ~~off-plaque~~ sector-plaqued

Streak out remaining lysogenic for purity (15 can be picked)

25 contam. or colony 27 not
21 non-lysogenic

15 picked, 12 not adequately tested on replica or not pickable.

$\frac{21}{78}$ non lysogenic = .269 = $e^{-1.3}$

actual ratio B:C = $\frac{130}{125}$ = 1.04

C: ~~B:C~~ C:B ca 10.

C2: 7/88 non lysogenic = .0795 = $e^{-2.5}$

Are these non-lysogenized? Test for sensitivity (all were 22V's)

also test "non-contaminated" lysogenics.

of remaining 81, only 4 were obviously contaminated

A: non-lysogenic on PLT22

B streak out "non-contaminated" lysogenics

In preliminary test of 21, 8 clones were picked (7 resist 22V)

All showed a few lysed colonies on streak. Conclude that all are actually contaminated.

UV'd FA22, protection vs. 22V ?

100 9

12/25-26

A dilute stock FA22 ca 1:30

B UV A 20 minutes (10ml) at 50 cm.

Add .1ml to .1ml LT2 (10 hour broth)
Plate out ± 22V. at 10^{-5}, [redacted]

O: LT2 & FA22 +22V, 10^{-5}: 5 survivors.

(A) ~~G~~. LT2 & FA22 ~ 10^3 plaques on LT2; ~ 10^3 survivors ± 22V at 10^{-5}

(B) H. plaques: 0×10^5, 0×10^3, 0×10^1

survivors ± 22V: 0×10^7 8×10^5. (check these for lysogenicity).

Note extreme killing (does dilution of the broth increase viricidal action?)

UV'd PLT22 seems to have lost its protective function, but the dose may have been excessive. Check transducing ability.

C (J) as above A, FA22 UV 10 min. 11 min rough No plaques at 1, 3, 5, 7.

K D. FA22 UV 0 1 min rough → rough. 10^{-6} 58×10^6 recovery ok!

E no FA22 16 min rough survr.

(F) 22V UV 10 min. assay. (titers of 1/30 diluted)

at 10^{-5}, C, D, E ~ 10^2-10^3 survivors with V. Expt n.g. owing to high survivors in the control. & D. indicating that this dose also removed protective power.

Diluted phage is killed by UV much faster than broth, as one should expect.

K: 10 plaqued colonies. See L-M for more thorough expt.

Any non-plaqued lysogenic? 2 lysogenic ones not obviously self-plaqued in original streaks these.

1009
B-B
(B)

B-B 8 "non-contam." colonies streaked out. 6/8 showed 1-5% plaqued colonies and are therefore contaminated. 2/8 showed no overt plaquing. Replicate for further test.

1 - colonies evidently not lysogenic. Restreak, first rub whole area, also an isol colony

2. " " " "

✓ sensitive! all sensitive. Initial account as lysogenic - non-contam was incorrect

Conclude that most or all colonies are initially "contaminated".

C-B 8 non-cont. colonies streaked out. 3 overtly clear.

Replicate

crude test, apparently pure lysogenic (might have been up to 2% of the 3 "clear" picks possible sens. for unknown. results.)

2 single colonies not sens. No self. lysis noted in controls.

Thus, multiple infection may give some uncontaminated lysogenic clones

B-A 15 tests 10/15 single LP22^S 5 are not 22^S (try 22V?)

1 self-plaqued. → all lysogenic (reduced?)

C-A 8 tests: 7 22^S 1 22^R → turns out lysogenic (reduced).

∴ There is too high an incidence of non-lysogenized once infected cells → pure sensitive clones? (over)

Save B4 and C1 as 1009BA and 1009CA

1009 BA, CA · seem pure on stretch.

Cf. lysogenicity i a typical "lysogenized colony

Transductions – lysogenizations – protection vs. 22V

1009 LT

also UV'd FA22

1/1/53

L. Added 10^8 FA22 to 10^9 LT-2. Absorb 10 min. Plate out to count incidence of lysogenization. (assume stock = 3×10^{10}/PLT22)
Also plate [redacted] & 22V for purity of these solutions.

M. No FA.

N. as L, UV 500 seconds.

L. 1ml SW414 + .2 ml FA22 (1/30) 9:45 –

M " + 1 ml "

N " + 1 ml FA22 (1/30) UV 500 sec.

A: Plate .2 ml on D(0) + M or H.

B. Plate 10^{-5} ml with 22V, EMB...

C. Plate .2 ml + 22V on D(0) + M or H

B): L ca ~~400~~ 878
M ca ~~800~~ (note 1:1 dilution)
N ca 7. Plate .1 ml directly: ca 100 plaques.
O ca 15

Stocks all right; UV'd phage PLT22 does not protect.

The design of this experiment is faulty for using 22V/LT2 rather than 22V/LT-14 in the last step.

(over)

medium useful?

0: (no FA)

		D(Hist)	D(Meth)	D(O)
	0	0	2	—
A	L	ca 50 v. small	ca 300	
	M	2	ca 300	
C	L	ca 10 v. small	ca 100	
	M			

O + 22V — 0 (pinpoints background) — 4

Important comparison:

LA - MA - LC

Note MA ≯ LA although 5× as much FA was used.
absolute counts seem rather high also, paramount of FA used!
Transduction by 22V may be ignored.

Survival of transductions = $\frac{LA}{LC} = \frac{248}{113}$

Survival of populations: $\frac{LB}{} = \frac{\cancel{400 \times 10^5}}{\cancel{10^9}} = \cancel{\frac{4}{100} = .04}$ $\frac{878 \times 10^5}{\text{"}10^9\text{"}}$

Expected surv = $^0/_B = \frac{10^8}{4 \times 10^7} = $ ca 2.5

New LT-2 mutants
UV FA22

ca 12/31

SW414: 9 sec UV (ca 400 cfu/plate).

Cal 13 plates
Xyl 5 "
Mal 10 "

1 clear Cal- (SW950) and Mal- (SW951)

SW950 also had some "fuzzy" colonies as well as clear ~~pluses~~ minuses: these → pure +.

∴ SW950 = SW414 (LT-2) Cal- .

SW951 is slow +, not suitable as selective marker although unequivocally scoreable

Response of SW950:

A Spont Rev: 1 + colony in one spread plate

B + FA22 .1 ml >10^2

C + FA22 (1:30) sec 1009 LM 23 papillae

D + PA22 UV (1009 N) 9 papillae + 59 plaques.

Thus FA22 behaves like PA10, 12 in response to UV. UV'd phage transduces, (thus hypersensitive). though it does not infect or protect

All Cal+ stable.

A: Lp^s

B: 20/20 Lp$^+$

D: 9/9 Lp^s

It can be inferred that FA22 can also be UVd so as to separate phage from transduction.

SW950 / EMS Cal. in a 15 plates, 1 sectorial → SW952.
This is slow +, like 951, and may be suitable only as an unselected marker.

Adsorption

1/14/53

A SW950
B 694
C
O – 948.

add (3x) 10^7 PLT22 (in 0.1 ml) to 1 ml heat-killed broth (10^9)

9:00 – 9:20. Plate 10^{-1}, 10^{-3}, 10^{-5} in SW950.

A: ca 20×10^5 discrete at 10^3, barely confluent at 10^1!

B: ca 100×10^5 at 10^1, ca 100 small sharp plaques.

C: ca $60 \cdot 80 \times 10^5$ (smeared) not discrete at 10^3

O: ca 100×10^5

694 lysogenic for LT-2?

Unfortunately, plates smeared.

Consistent with adsorpt: 950 > 948 > 694 > O. Rates seem unaccountably low.

Has antigen been destroyed? Perhaps should use live cells for adsorption, then kill. Each killed resp. aggl. (slide) in XII IX saline.

Adsorption of PLT-22 1011

2/2/53

overnight broth cult. Boil 10 mins.

add ca [redacted] 10^{4} FA22 in .1ml to 1 ml 10^{8} killed bacteria.

Room temp 10 minutes. Add 1ml 10^{9} ~~[redacted]~~ cw 414. ~~Assay (.1ml, .01ml)~~

Assay .1ml

A	LT2	156
B	694	344
C	948	81
O	—	270

Expt. n.g. Use higher densities.

FE 1/4/53.

SW 952

37° 4:40 – 5:10 Nominal 10^{10} B+ 10^{9} PLT22./ml.

A. Assay initial Bact. 10^{10}

B. Assay infected Bact. 10^{10}

C. Assay infective centers B. 10^{9}

D. Assay FA 44 surv. A 10^{7} – 10^{8}

E Assay FA 44 surv. B 10^{9}

✓ F Assay Cal + A .1 ml

✓ G Assay Cal + B .1 ml .05 ml .01 ml

✓ H Assay Cal + B + FA 44. .1 ml .... (used 46 hyperimmune on Cal plates)

✓ FF – HH same as D (meth)

A 43, 38 x 10^{8}, – 40 x 10^{8}

B 46, 38 x 10^{8} = 42 x 10^{8}

C 38 x 10^{7} 8 x 10^{7} poor plate (dimorphism of plaques: spreader contd.?)

D 114 x 10^{5} 4 x 10^{7} >10^{3} x 10^{3}

E 14 x 10^{7} (FA 44), 13 (+45 [redacted]) x 10^{7}

Actual initial conditions: Bacteria = 4×10^{9} PLT22 = 3×10^{8}

But 22V survivors: $\dot{s}$ phage = 1×10^{7}

$\dot{c}$ phage = 1.4×10^{7}

i.e., protection was not achieved. Note conditions: absorption in dense washed resuspension! (Adsorption?) Platings of B showed 1? contaminated colony in two plates (input ca 10% = 8). Test by replica plating.

Also note: plaques in C are of two types, 25 clear and 13 turbid.

(over)

check plaque type on LT2

22V1-4 : all lytic, similar sizes

1012B suspension : all turbid

Storks PLT-22 : turbid

2-clear plaques : lytic

2 turbid plaques : turbid

Especially since of 1012B, clear plaques must have been contaminations (myopicity, flannel sprouter?)

Replica tests of B: 1 lysogenic colony on two plates.

F		4		+(FA43)	2	
G	9,11	~~FF~~ (.1)		.02	1	.05 1
H		6,6 (.1)		5 (.05)		5 .02

Overall result is almost negligible transduction or injection c PLT-22.

(D-meth)

FF	.1	4	+ FA43	~~0~~		
GG	~~ca~~	11 156,	.05 56		.02 ml: 34	
HH	the same	160			.02 ml: 40	

v. effective transduction. This titer seems very high!

Apparently phage was insufficient. All transductants were presumed, but survivorship is obscured. Also note Cal+ transductants deficient. Inoculum may have been too heavy.

SUMMARY. ~~Also~~

Bacteria 4×10^9 PLT22 3×10^8 22VR 10×10^7

22VR (ϕ phage) = $4 \times 10^7 / 4 \times 10^9 = 1\%$ (cf.

	Cal+	Fla+
SW66 + FA46	− 1	—
928 + "	− 1	—

Host adaptation

1/8/53 X Y

of ., on SW666; [redacted]950:

FA 21, 22, 41A, B, C. FA41 = FA21 plaque resolution on LT-2.

~~2244~~ 43, 46

		X	Cal X	Y	Cal Y
FA22	A	~~50~~ $\times 10^1$	7	~~$300^{++} \times 10^9$~~	10^2
FA21	B	100×10^7	0	~~50×10^5~~	1
41A	C	100×10^3	0	$> 100 \times 10^7$	60
41B	D	~~50×10^3~~		100×10^7	50
41C	E	20×10^3	0	100×10^7	
43	F	10×10^1 (turbid)	12	500×10^7	lysed 10^{-7}: 3
46	G	100×10^7 (turbid)	0	300×10^5 (clear)	0 lysed

log ratio, essay on Y / X.

	adapted to	
A	Y	7
B	X	- 1+
C	Y (X)	4
D	Y (X)	4+
E	Y (X)	4+
F	Y	7
G	X	- 1+

The adaptation of PLT22 to SW666 is therefore reversible, and is presumably a host-induced adaptation.

It also affects 22V, which gives ~~turbid~~ turbid plaques on SW666 when adapted but does not induce lysogenicity.

Note that FA22 has a differential of 7 while the readapted phages FA41 have a differential of 4. This suggests a dual effect, part reversible, part not (host-adaptation; mutation). This may also account for previous impression that host adaptation was not reversible.

Purify, test lysogenicity → pure Cal+: all show same lytic interaction with SW950! (suggesting that 950 is lytic for 666!).

Transduction by lytic variant

1014

1/3/53

FA46 (FA45/SW618) → X 666 Probable lysis. No Tor S.

A. 928: 4 (long) tracks in 3 plates; no swarms.

Isolate 3 of the tracks. Also pick empty nearby agar. Test on LT-2 #2 and 3 or. show typical PLT22 plaques. #1 did not.

1/9 Retest isolated colonies: all lysogenic (PLT22 type)

see 1017,

FA43 = 22V/LT2 FA44 = 43/SW950 FA45 = 43/666 FA46 = 45/618.

1017: FA43 × 953 → H+

And But see FA44 increase of H+ from 22 × 950.

1006 FA43 × 435 → Tr+ Gal+ but low yields.

~~#~~

see also 1017

Persistence of H_1^*

1/8/53 FA 18 × SW 666 See 971018.

4 are i, $4p^s$. a, 4, c, d.

Prepare FA48 = FA22/a – d. save cultures under these numbers.

1/19/53. Plating ca 300 PLT2 + SW954 ± FA53

+FA53 ca 300 plaques, all clean.

100N, 1/ml → confluent lysis on part of plate; clear plaques on remainder
+ FA53 2×10^{-7} → 0. ∴ same ~~per~~ inhibitive particles are working.

Prepare S3A = FA53/LT2. Plaques are very small, scarcely discernible. Titer 10 or 10^4? $\times 10^6$.

D) 1-2 resistants ⇒ SW954/FA53 + PLT22

954 + FA53 seems to show some spotty mucoid lysis. Restreak to separate possible components.

E) SW951, 956 ~ not lysogenic / 950.
Some very vague interaction of 950 → 948, 956?

F) 954 ~~+~~ Lp+ (PLT22) showed no lytic response to FA53A or 53A+ PLT22. (v.sl. inhibition of growth or spreading noted).

& Grow FA53A (53/LT2'). Titre (SW950) 64×10^8

Felix' Salmonella O phage

January 15, 1953.

Ampoules received (duplicate) 1/14/53 as #1, 2, 3 respectively. (described by Lilly and Spicer; [redacted] conversation as "cosmopolitan" Salmonella phages. Label FA 51-3 resp.

a) Test on mixed serotypes. All behaved alike except as indicated:

SW950	S	all lysed clear (exc. occ. rough ?)
666		
948		SW948 had background regrowth to all 3.
927		
927R1		SW688 and S. coli 3 showed no response to any.
H901	S	SW900 had considerable background for [redacted] #2.
SW688	R	
550	S	927Rough1 is morphologically very rough (ragged broth).
S. florida	S	
coli 3	R	
London	S	

b) Plate. Incl #3 as rec'd i SW666, 950. SW666, [redacted] 550; i #3 in mottle

no papillae. no swarms

c. Select resistants in streaks of both mixtures. Clones noted:

1. LT-2/51	Retests:	53 R	FA22, 44 S	!
2. 948/52		53 R	" R	
3 950/52.		53 S	" S	

c1 was noted to show full lysis where streaks from /53 adjoined /22 / Retest synergism of mixture of #A22, 53. Note also resistance to 53, resistant to FA 22!

Repeat ✓ C1 is [redacted] lysed by FA22 + FA53, not repeatedly!

a) obtain C1 lysogenic! C1=SW954

C1 and C2 remain serologically smooth (IV, XII); (I [redacted] XII resp.)

C2 is not susc. to PLT22 + FA 53.

FA22 – 950 relationships.

1017

1/13/52

A. Titrate FA22 (stock) on SW950, SW953 on EMB Gal, D(H), D(M)

Titrations recorded for further studies.

1009LB showed FA22 to have activity of inv. $\frac{1}{6} \times \frac{1}{30} \times \frac{1}{15} \times 500 \pm$

~~10,000/ml~~. ca 100,000/ml.

(also 22V. and WV 22 and check transductants for Gal, M–.

1012 GG showed $1/\frac{1}{30} \times \frac{1}{10} \times 15 = 50{,}000$/ml.

If ca. 3000 previously assayed in SW 435

	FA22	D(meth)	cells have to be washed for D(Hist)
SW 950	.001 ml	30	(count may be conc. higher – plate somewhat smeared). ≐ 30,000
	.005, .01, .002	>>100	
	0	6, 8	
	FA47.1ml	>30 smeared	sic!

SW953	.005	3		
	FA43.1	~~ca 100~~	226.	seems real transduc! for leftie variant. 8 tested, app. Lp+ normal.
	FA44	2		
	FA47	9		
	~~1009N 1ml~~			
	EMB Galactose			

SW950	FA22		
	.1, .05	>>100	
	.02	454	ca 7500/ml.
	0	5, 2 (small, delayed)	
	1009N .1 ml	8+3 delayed	25 plaques
		10+3 "	21 plaques

Thus H+/Gal+ = ca. 10:1 for SW950.

SW953 has relatively poor response

(over)

D/meth) plated:

SW950 —	7
" + .1ml broth	8
+ FA47	ca 20 (sm.)
+ FA47B	10
+ FA52.	8

(presumably residual FA22)

Protection - transduction

January 16, 1953

A. B = SW950, 4 hour culture.

B. B 2ml + FA22 ("10^{-1}") .2 ml

C B 3ml + " .1ml (+ [redacted]) } [redacted] 8 35 PM - 8 55 - 9 10 R.T.

As assays involved dilution of FA22 1:300 and 1:3000, Bal+ is only practicable for A, B.

---

1. A, B, C at 10^{-7} for survival
2. A, B, C at 10^{-5}, 10^{-6}, 10^{-7} with FA44, 10^{-1}.
3. B, C at 10^{-6}, 10^{-7} for plaques on SW950.
4. A BC .1ml D(meth)
5. A BC .1ml D(meth) + FA44
6. A B .1ml EMBCal
7. A B .1ml EMBCal + FA44.

~~(8) A+ 1ml [redacted]~~

A)
1. 106,130 x 10^7
2. Ca 1000 x 10^3, 2 x 10^5
3.
4. 8, 7
5. 2
6. 0, 0, 1
7. 1, (lysed)

B) 93,100 x 10^7
78,87 x 10^7
1425 x 10^6, 1000 ± x 10^3 $10^3 \times 10^6$
$> 10^2$ — est ca. 3x difference
$> 10^2$
19, 24
56, 85

C) 107, 88 x 10^7
3,6 x 10^6. 0 x 10^7 same; 1000 x 10^6
phage ridden! 4, 4
2, 2
10^{-1} plaque ridden. 2, 1
0, 1 lysed

8) 1009N + 1A: [redacted], ca 10 plaques. 694 H+ colonies: test 8 for Lp+; all are Lp^s unfit transductants by inactive phage.
same in background.

Note excessive phage in 3B, 3C — Inadequate in 6C!

Summary: initial bacteria: 1.2 x 10^9 + PLT22 B) .97 x 10^9 C) 1.03 x 10^9 (possibly same will host uncertain)

FA44 SURV. 2.1 x 10^5 = ca. 10^{-4} = .83 x 10^9 5 x 10^6 = 85% = ½%!

Add PLT22, calculated 10^9 ⟶ .3 x 10^9

Why discrepancy between B and C?

C was allowed to progress only slightly longer, but FA44R is 50X too low.

(see over)

Accessory mcarp. phage

1018

A = .02 ml FA22 EMBCal ; .002 ml D(meth)
B = .1 ml FA47B
C = .1 ml FA43
D = SW950 .1
E = SW955 .1

		Cal		D(meth)
1 - 1	D'	.	1	.
2 - 2	E'	.	4	.
3 - 3	D'+A'	..	26,20	..
4 - 4	E'+A'	..	0, 6	..
5 - 5	D'+B'	•	4,	•
6 - 6	D'+B'+A'	..	13,27	..
7 - 7	E'+B'	.	3	•
8 - 8	E'+B'+A'	..	10,7	..
9 - 9	E+C	..		..

4. 3/6 no accessory.
4/8 ? lower yield.

Cetrimedine

1/21/53 Cetyl Trimethyl Ammonium Bromide (Cetrimedine). rec'd from D. Crum (Baker Lot # 1.1446 — opened.) Acc'd'g Bradley + Boyd. inhibits phage adsorption.

Mix 10^4 PLT22 + 10^9 [redacted] SW950.

Add .1 ml successive dilutions of CTMA, plate .1 ml.

0.				
1.	.1 ml 5%	= 5/1000	ca 10^3 bacterial survivors	
[redacted]	x 1/5	= 1/1000	" 10^4; occ. plaques	
[redacted]	1/5	= 1/5000	1/25	perhaps 50% reduct. count;
4	x 1/5	= 1/25,000	1/125	full count
5	x 1/2	= 1/50,000		
6	x 1/2	= 1/100,000		
7	x 1/10	= $1/10^6$		

Not usable for limiting cross-infection.

Diploid Hfr crosses
further commentary 6/10/53

952. (Hfr lac$^-$ x Het P$^+$), Abandon → H316 Mal-lacV TL− one960
W1940 W1590 F status? (Prod. Elimin.)

953 H312 x Hfr → MalV lacV SV TLV M+ No pure Mal+
F$^+$ H267 Mal$^-$ H318, 319. (self eliminating?)
(2n F$^+$ x Hfr) Held

F status? Elimin 1/33 stands?

955 H310 x1895 1/2 MalV. H313.
0/24 (Malt)
But appears to have segregated already in previous expts.

H310 then noted to give very high yields xP−
H310 segregants: 2 each lac−, lac+ are F−. Test more extensive pools: → Then 10+, 5− all F−.

956 H311 (Het 2n) x Hfr. No MalV. Reversion ~~not tested~~ not ~~reported~~ Reversion test.
23 lacV tested: all Mal− ! (Very few Mal+, but some found).

Abandon all these,

Continue H310 x
Hfr lac$_1$ x lac$_4$ .... (1940 x 1956 / EMS lac ....)

958. H245 x W1922. Sme Mal/lacV
Hfr Het 2n F+ Hfr.
Hbandera

Hfr F⁻
964 H310 x W1607 nEMSlac. 6/24 lac+
Mal⁻ SR Mal⁺

→ 3 lacV. 2 Mal⁺ 1 Mal⁻ (H322)

need hemizygosity test! (note H310 is Mal =).

superseded by 1057

July 4. 1952. Summary of Hfr × diploid crosses etc.

952. Hfr Lac+ − × Het Lac+ − —EMS Lac→ occasional Lac⁻ (balanced)
but mostly lac+ recombinants. ↓ H316 = Mal− Lac⁻ TL−
should be crossed × 154 × 161

953 (H267 s^R Mal−) = H312 prot. × W1895 on EMS Mal.
not ready yet → H318, 319 7/1 ~~should be zygotes~~
✓ Mal+ are TLB, −. Cross . Mal⁻

(mostly Lac+, Mal+).

955 H310 × W1895 (1895 × 1177 random. TL− Mal− Lac⁻) EMS Mal
In first trial gave 1/4 Mal⁻ H313 Lac+ Mtlr S^r
second: 0/24. But H310 itself is Hfr! [Mal+ Lac⁻?]

8/1. Recheck Hfr × F− → F− (haploids) only.

956. H311 × W1922 (Het diploid lac− Mal− Mtl⁻ prot). EMS Lac.
prot
gave numerous Lac+, 6/6 dip were lac⁻ Mtlr Mal− S^s
Rare Mal+ 5/5 Mal+ Lac⁻

Further tests needed, but presumably may mean that elimination is not bypassed in n × 2n (still n for Mal).

H320
A: Mal − Check for heterozygosity
B Cross zygotes

958 H245 × W1922

TL LacU Mal- EMS Mal. underway

Mal^{V} isolated. Test segregants for mutation, Hfr.

H321

960 TL H316 × BM W1895 EMS Mal. Check LacU Mal- for
Testel only Mal! homozygosity.

959 H295 sec. Centrale

To be done

a) Further tests on 955: for Lac+ Mal-pne (is Mal ever eliminated?). H310 itself may be hemizygous?

b) Segregates of H313 → recovery of Hfr?

c) Transmission of Hfr to Mal+ progeny?

d) Transmission of Hfr in H316 × Hfr? etc.

959: H295 recombinants: H317 Lp^{+}/s. $Lp^{2}{}^{R}$ segregating? Obtain Gal+ recombinants for coupling/repulsion.

954: Lac+ S^{R} recomb from F+ × F−. Check mesc markers to compare pattern with 1895 × 1956

957. What peculiarities of λ/1827 can be demonstrated? On gross test, λ^{4} diff not recog.

phase variation of Bumgendorf

2/22/53

Bumgendorf. 6145-52 = A2. single colony picked and matched = A1. = SW961

List of serums:
k1 Bumgendorf
2 minnesota 240
3 berlin
4 new mexico 10
5 para A 228 new
6 morehead
7 para A 228 old

2/28. Inoculate A into serum tubes:
k1, k6.
after 48 hours, k1 showed rough blebs which spread very slowly; 2/28: spread through.
k6 grew out and filled the tube = A/k6. Repeats + test antigens.
On slides, unspecific A/k6 = B1 reacts c 1,5(k1) but not 1,5(k6) or 1,2 reactions are quite weak.
(over)

2/22 Inoculate A1 on plates of k1, k6 again.
A23: bulbs on k6, not on k1 — slow overspread. (surface spreading = E
P23 pick → D. spread may obscure blebs. matches plates less satisfactory than tubes for prolonged incubations).
A24: numerous B+S on all k6 plates
1 small rough bulb on k1. Reinoculate to try to pick later.
C = choleraesuis c: — #153

D = A1/k6 plates (4 swarms / 3 small plates).
4 mass cultures all react c: + k1: ± k6 — in slide tests. ~~Test~~ use D3 as strongest reaction and test single colonies.
Each of 4 colonies react c +, k1 ±, k6 - in slide (= D1)
Prepare broth culture for titration. Broth D1 in k1: k6: E:
grows promptly through k6 is slightly inhibited in k1, strongly inhibited in c but definite diffuse spread. 2/28 still not spread.
E: one plate showed two rough blebs. Pick to Pennessay. (May have undergone two steps of selection)
after 4 days

SW
D1 = 958

		c	k1	q6
B1 (cryptic.)	at 1:500	+	—	—
	A1	—	+f	+
B1.	1:100	++	+	—
	(overnight: same)			
	supernates fairly clear.			
	1:500 overnight	++	+	—

D: Mor in k1, k6 SS. transferred k6 overnight; k1 in 48 hours = D2

2/26 D3 Mor D2 in k1

Titrate D1, living cf. heated 58° 1 hour & formalized.

living	c: 1000 +++		k1:	50 +++	100 ++	200 +	500 −	
heated	c: 1000 ++		k1:	50 −	100 −			

Repeat

	living	k1 : 100	++	k6	:50	−		
	form	: 100	÷		:50	−		
	heated	: 100	−		:50	−		
D2	living	k1 : 50	÷	k6	:50	−	c: 1000 +	(poor suspn.)
E1	"	k1 : 100	+	k6	:100	−		++
E2	"	ditto						

~~Retest Kunzendorf for c: 1000 D2....~~

carfunic notein of kunzendorf = 1,5, x... : c, x.., with x-component absent in other 1,5 sera. (cf. berlin serum). Heat lability makes somatic antigen unlikely, but reactivity of living cells is much better than formalyzed. Same type is secured by deletion in kunzendorf serum.

Titrate berlin serum : D1 living

1:50	100	200	500	1000
+++	++	++	+	÷

∴ ~~beheim~~ berlin is even more satisfactory some (titer of 1:500)

10 20

2/23-4 B1 (not pur.) into tubes of k1, k6 SS.
A1

overnight:

	k1	k6
A1	—	2 v. small balls. +++ near end of tube
B1	slow diffuse spread	

2/25. Titrate D1, A, C: (1 hour 37°).

A. C: 100 — 500 — <100

k1 : 500 +++ 1.000 +++ 2. +++ 5. ++ 10. + 20. ± 1: 10,000

k6 : 500 ++ 1. +++ 2. +++ 5. ++ 10. ++ 20. ++ >20,000

C: c 1.000 +++ 10. ++ 100.000 — <100,000

k1 100 — k6 100 — <100

D c 1.000 ++ 2. ++ 10. + 10,000

k1 50 — 100 — 200 — 500 — 1000 — 2. — <50

k6 50 — 100 — 200 — 500 — 1. — 2. — ! <50

of B1 at 1:100, 1:500! 4 hours: ib. (±k1 1:50, 1:100)

Ax to c++ k1±. still shows in shide aggl. from colonies from streak of D1. Cf. heat killed cells (somatic O component?)

Kenyondorf - 958

1020

further titrations

2/27 4 live cells and heated in boiling water 10 mins.

	c 1:1000	berlin 1:500	berlin 1:100
SW958 liv.	++	+	++
heat.	−	−	−
cholerasuis 153 liv.	++	−	++
liv. D2	++	−	÷
liv. E1 purified	++	+	++
liv. E2	++	+	++

∴ factor is also present in 153, presumably selected by a non kenyondorf serum. It has been substantially eliminated in D2. The factor is presumably absent from diphasic cholerae suis which migrates readily in kenyondorf serum. It is heat-labile (presumably H.:.), but also formalin-labile!

Test serums at 1:50 with D1 living

berlin 1,5 (k3) and ⑤ (berlin absorbed c 157) ++++

all others negative: k2, k4, k6, k7, 1,2; kw, enx, d., 1,2,3 (colindale)

To select ≤ ~~4~~ or 4 phases from c: 1,5, presumably should use 1,2 serums. "phase stability" of javiana may be due to similar cross reactions of ~~kw~~ d – 1,5.

Effect of formalin. (Add .5% formalin to D1 cells)

	c 1:1000	berlin 1:100
liv.	+++	+++
form.	+++	−

	c 1:100	berlin 1:100	TVi 1:50	c 1:50.
SW 958 1/V.	+	+++	—	
SW 961 1/V.	—	++++	—	—
D3 (not pur.) (of Glidden ref. swis 153)	+++	—	—	

c' factor is evidently absent from Glendale c.

Further questions:

① Does this explain all anomalies (cf. 45-47).

② Recession of c

a) 958 appears to be stable in c (Glendale) SS. cf. D3.

b) does Glendale c inhibit migration of [redacted] 961 : tested 2/18 no.

③ Is c' present in Edwards' c serum? (not previously detected with 1,5,c'... owing to formalin-lability.) If so, c' reagent possibly best prepared by absorbing cc' with D3 ~~with heated cc' cells~~. Absorption of berlin serum with, e.g., para A 1,5 would be less safe.

Misc. tests:

3/1 E.153 is somewhat restrained in berlin serum. (verify c')

SW961 is not " " c (Glendale).

Titrate in c, berlin:

	c 1:1000	1,5,c' berlin 1:100
(mono. c-) 902	++	—
D3 pur a. 958 c	+++	—
SW 958 c (b.)	+++	—
958 2c (slide agg.)	—	++
961	++	

3/4/53. Stability of c phages:

F) Broc c: SS 3/4/53.

		3/7:	3/10:	3/13	3/15
1 SW958		no			
2 958C	motility whatever.	idem	idem	idem	no motility (well-purified stability)
3 #153					theirs.

Other serums: SW961 in SS:

G.

1. 1,2,3 bleeds — 24-48 hours screamed through
2. k5, k7 (para A 278) - 2-3 days " " (k5 slightly slower)
3. k3 — substantially immotile in 4 days: rough beads.

"1,2,3" serum probably pufunal (try Edwards 1,2. of Lw!)

However, k5 and 47 are "effective serums", may still have some residual components (agg. titer < 1:50)

4. 961 / 1,2E (157 serum): screams about equally prompt in k6, 12 Edwards, 1, 2, 3.

Stability of O-901

2/24/53.

Fresh stocks of 0901 received from A. Felix. Also test SW 542, an absent, and SW 586 resuscitated from lyophil. (0901 from Kauffmann and Edwards respectively).
In tubes, 0901 Felix 1-2 remained stable 4 days. On plates, (1 each) #1 stable; #2 gave 1 swarm 48h., 2d 72h.
Requisify for further test.

A: 0901-11 → both (d).
0 sw. 1st. 2d plate: 1 swarm: (d)

C SW 542 0 swarms 1st plate; 2d plate: 1 swarm: (d)

D SW 546 1 swarm : (d)

∴ All cultures are about equally stable (ca 1 swarm / 2 plates)

E 0901 #1 + FA 22: 8 swarms after 24 hours (control 0) } all d
v. numerous trails
48h. 1

F + FA 9 (SW 663) 3 swarms several trails
+ FA 12 (SW 628) 4 swarms several trails

0901 + Flg. −

motility /f

1022

10/23 ~~Dor 5 tubes each SS~~ A B ~~W6, W1678~~.
~~overnight: notably not diffuse but from coalescent swarms of each.~~

C - D single colonies as above.
W6 W1678
C1-3 "smooth" 4-6 "rough".

10/24 overnight:

C	1	spreading is initially rough and patchy; later diffuse from loci of original origins
	2	
	3	
	4	"
	5	
	6	
D	1	"
	2	
	3	"
	4	

10/27 Reinoculate All marked bottom except D4

Second passage: all marked bottom, ca 24h.

A. 3/2/53 Streak out on N.A. Purified isolates to H.L.B. for compatibility test

	×1876	1956 ①	② 1876	1956	
C	1	+	+	+	±
	2		—	+	—
	3		+	+++	++
	4		—	—	—
	5		—	+	—
	6		—	+	—
D	1		+	—	±
	2		+	+	—
	3		—	—	—
	4		+	+	—
C 6-0		↓	+	+	—
D 4-0		—	+	+	+

① Grown in both together 24/h plated on EMB Loc.
② Grown separately D(0)
③ by JL on D(0) A × 1177 B × 1817

over.

	xA W1177	xB W1876	
c2	—	+++	
c3	++++	++	D1-0 +
c5	—	+++	
D2	+	1	
D3	—	4	1607 —
c6-0 (58-161)	+	+(+)	D1-0 +
D1-0 (W1678)	++	1	[redacted] ++
1607	—	++	

W2207
W2206
W2208

c60 1 c3 + W2209

D4 ++ nd. ± still F+

~~c3 is Hugh fey.~~

D3 seems to be F⁻ but also sterile.

D2 is poorly futile, not F⁻

c2, c5 seem F⁻

tip D4 now.

1177 x 2209	—
1876 x 2209	20
1177 x 2209 (1876)	40
1817 x 2209 (1876)	~~10~~ ~~20~~ 3
1177(2209) x 1607	—

Defibulation and F test:

2209: after grows c 1876 becomes futile c 1177 and 1607 (but also futilates W1177? ?) Recheck this test after purification.

needs to be checked.

2206 futilates 1876

2207-2208 are futilated by W1876.

F/ mot. recap. 1022

4/6/53. 6 cultures 58-161, 3 and 1, resp. 4 of W1678 sent through motility tubes (2 pass.) were altered.

58-161: W2207, 2208 : F^-, ~~non-infective~~, infectable
W2206 F^+ nearly Hfr. Infective. Not studied for mixture. (see TCN) (found no SR+) see also 1113

W1678 W2209 F^-, non-infective

3/ Prepare purified (5YA) from purified z_6 phage of 3 ggs.

3/13/53 P: 5YA – X LT2 ^{2} <24h → d: 1,2

~~(R) (S) (T) 5YA – X SW999~~

R 891 x–
(S) 959 x– | 5YA. swarms overnight. → d
T 960 x– → d

Dose these bottles, unpurified, into d+1,2 for possible z_6 phages.
3/18. No alt. phages.

Repeat 3/8/53 using restolyzed 891, 959., in d-1,2 serum.

3/19/53. Swarm in S (in d 1,2 tube). Other R, S (2 d: 1,2 each): no swarm overnight ~~~~ T.O. (T.O.) T.O. 3/28.

3/20/53. 1 235 swarmed. 3/21. z_6: – purified, no swarm in z_6 in 24h. Send to Edwards as SW999.

SW999 / z_6 = 999B: wants z_6 ++ 1,2 ++ 1,5 ++
2 – 5 ++.

∴ new phage is z_6, 1, 5 ... or mixture. Restricts

	z_6	1,2	1,5	2	5
cf. SW999	+++	–	– +	–	–
999B (not pur).	++	++	+++	–	+++
SW959	–	+++	÷	+++	–

↓ 5/5 single colonies behave same way. SW999 apparently wants fairly specifically a z_6 Still, worthwhile in 1,5 SS for further selection

SW999 and 999/1,5 serum still react a bit in 1,5 serum at 1:1000 but are not inhibited by it.

Misc: transductions

A. SW938 (1,2 : enx) ← FA40 (sendai a: 1,5) [2; enx serum SS]

B. " ← FA3 (attendorf c+1,7) ["]

C. SW676 (z_{33}) ← FA22 (i: 1,2). [z_{33} SS]

D. SW933 (i: enx) ← FA40 [i; enx]
dense bulb but no swarm. 2/28 swarmed through.

E. SW676 (z_{33}) ← LA-2[I] (i: 1,2) [i; 1,2]. 2/6: Moderate spread of inoculum in control (inadequate ?)
FA49
Terminate: 3/1 no buds, continued slow spread: 3/3 all are i; exp. not adequate

2/26 F. abony 1+2 ← FA55
G. " ← " 56
... H. " ← " 57
(−1,2) } 3/3 No buds in exp. or control. later slow spread of v. dense bulbs.
[b, enx serum SS] all still b recover

2/28 24-48/1. A: contols and test show bulbocities but no swarms. } swarmed through see note *
B. " " " .
C. control: no spread 3 tests: all swarmed + through.
c1 : i test s.c. in i SS: no buds.
c2 : i " " " + slow spread
c3 (incomplete swarm) : still z_{33}. spread (i probably weak)

D. after mobilization, a:

J FA54 (76) ← SW891 (−1,2). Swarm through in 24/hour. d
K " 959 " Contols unmodified. d
L " 960 " (959 shows v. slow diffusion) d See 1023 J/K/L

2/28. M. FA50 (SW946) ← mania (6500-51, a: 1,5) [a/5] suggests all of these are $H_{1,2}$ like #153!
3/2. Slow spread in expt., not control. → all wait (see over) 1,2 + ② + 1,5 − ⑤ − (glide)

3/2 N 15 (abony b: enx) ← pairing (732-49) (lev: 1,5) [lev: 1,2] :
see 1028

* Note (2) - serum seems to restrain attendorf (1,7) (+c serum) but not sendai (1,5) (+a serum)

A - failed ? Needs multiple injections

B - " . Probably ⑤ has anti 1,7

C - i: 1,2 × z_{33} → i: — best evidence so far that z_{33} is a phase 1 homologue.
save one as 1023c1

D (parallel to A).
"i: enx" × a: 1,5 → B: a: enx. SW 975 (of 925 = D, 9, enx)

FA 40 (nominally phase 2) maybe ~~not~~ mixed?
~~check phase 2:~~

F&H: (—: 1,2) × abony 6: enx 3/7 : no consistent spread in any expt'l. or control. Activity of PA? 3/10 Isolate spreading dense bulbs.

23F Rep. - 3/31 still b.
G Inocg. →
H "

SW 973 (3 cultures)

M. 1,2: — × a: 1,5 → 1,2: 1,5 S.c.i all passed through

⑦ serum. → ⑤ ++ (slide)
⑦ -

M1 in 1,2,3 serum gave bulbous/fans restricted growth. 3/7: no exam.

5/6 in ⑦ : immobilized

M (control) eventually swarmed through:
⑦ — maybe 1,10 or 1,11?
⑤ —
1,5 ++ single colonies again
1,2 ++ reacted i ⑤ as did older broth.

~~SW974~~

∴ control gave no change

z_6 – X : — : 1, 2 —

1023
JKL

SW959 eventually gave spread through tube; 891 and 960 remained immobile. → maggs. b, i, 1,2. pv± Rebuilt ✓ inaggs. Put through SS.

3/1 JK,L, ~~M~~ reacted (weakly) in d. Plant single colonies in d ~~agar~~ serum:

3/2. J Inhibitor bulb, i subarg. spread = J'
K " " s ? " " = L'
L. " " i " " = L'

zega/z_6 uninhibited spread.

through d: 1,2 → ② + ⑤ –

1,2 ↓

② +++
⑤ ++ !

Single colonies of both J and L / ~~and~~ react both in ② and ⑤ note

L, originally behaves similarly.

This would suggest

d: z_6 (phase not necessarily pure) → X: 1,2 → d: 1,2 ✓!

Test J', L' in 1,2 ~~SS~~. → ~~—~~ d. ∴ J, L are d: 1,2

J", L"

→ single colonies: J: ② ~~,~~ +++ ⑤ –
L: ② +++ ⑤ +++
SW 960: +++ +?
} z_6; lw —

② 1:2000 ⑤ 1:1000

In tubes

J'	++	—
L'	++	—
960	++	—
891	++	—

∴ ⑤ cross-reaction appears on slides (at higher conc.)

K finally gave out in d serum : maggluttinable.
Select in SS: → inagg. (j ?) K itself lost. save K' (inagg.)

J" = SW 974
K" = SW 977 v. wk. reactions even after passage twice in SS d?
L" = SW 978

B phases ~~I~~ 891
959
960 →) see 1031.

Hold off further work until → X – ; ent str. is understood.

1023 FGH repeats

3/29: F —

GH small buds / not progressive)
(56,57 → x above)

G: Z33:
maybe 22s. Test single clones +
verify Z33: ent

Miscellaneous ♀ and stability tests 22/

2/26/53 et seq.

Test misguided strokes.

		12	22
	SW676	++	—
	SW546	—	++

A.

	938	++	+
	altendorf	—	++
	0901-1	±	+
SW959	Hines 1,2	++	+
SW 960	5394-51 M2	+	—
deuben	SW843	—	—
kudemm	SW730	—	—
bispebjerg (type ±)	SW725	—	±
	SW714	++	++
J. Taylor	ebony 133-52	—	±

i + 12 ++
b ++ enx ++

migrated readily in 1,2 serum to give i - rabbit culture

not morphopic but might be somewhat stable

B

	ebony 177-53	—	—
	ball 16-50	—	—
avian 9DH	l.l. 783-52	+	++
-1,2	SW891	++	++
	jaso. 732-49	++	++
	denv 135-52	—	—
	schwitzi V726 52	—	—
avian 5DH4	l.l. 4102-52	—	+
	Jega 317	—	++
	shoulditch 208	—	—

a ++ enx ++
lev: - 1,5: +++ z_6: +++

cf. stocks.

a — enx — after neutralizing ± ±
d ÷ z_6 ++

isolate 2 phases d agglutinates poorly.

C →

	Stanly 5099/50	—	—
	mmini 16570/51	++	—
	ball 268	—	—
→	l.l. 874	++	++
	848 salm.	—	—
	i:- 890A	+	++
	mmini 1885-52	++	—
	" 3840-52	—	—

d ++ 12 ++
a ++ 15 ++

a ++ enx +++

a + 15 ++

mix x poorly on slides

ebony (try 2ga!)

SW874 stock: slide 4a : 5 maggl! agg. on colonies + ca 2-3 hrs!

passage from single 9, enx
874: colonies through 2 SSagar gave cultures each agg. nearly both i 9, enx ... too unstable for present purpose

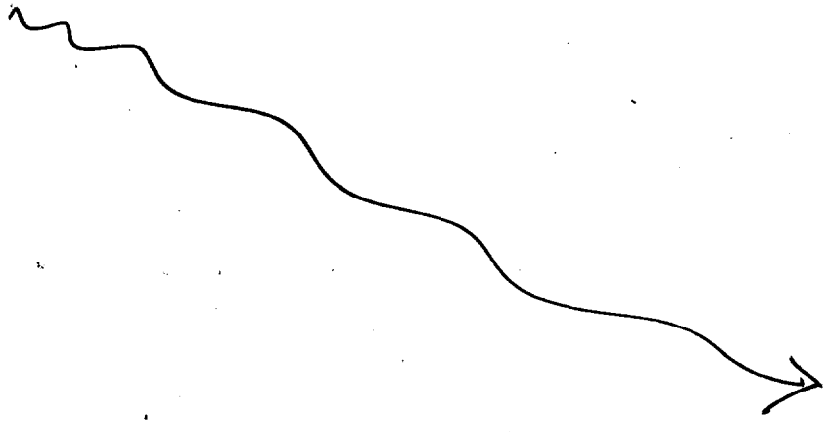
(over for page B)

Test 4 type para B strains for phage purity.

B. bifidum from stools.

a) slide agg.

	6	12
~~Jersey~~		
Dundee	++	+
1	++	++
2	++	++
Taunton	++	++
Bucles	++	−
Jersey	++	−
3B	++	+++
BAOR	++	+++
3aI	++	++
3a	++	+++



Repeat

			12	22	
	brandenburg	24	—	—	
—:enx	ab. equi	26	+	++	
	ab. obris	28	—	—	
	" "	29	—	—	
	dublin	65	+	+	
D -	d.e.sal.	72	—	—	✓
-	para A	2	—	—	
-	"	229	? -	? -	✓ numerous faint, tiny plaques!
	urien	281	—	—	
	wagumi	290	—	—	

S. bredeney strains from Edwards (non-~~XXVII~~)

		PLT 22
1	3807-52	R shaded
2	4102-52	S num. plaques. lv: 1,7 ~~Sw986~~
E 3	4641 52	shaded
4	5435 52	"
5	5437 52	"
6	6594 52	"
7	6612 52	"
8	303 53	"
9	517 53	"

4102 proved to react D:a ! Of previous page: this culture is recorded as ~~lossa~~ livida.

	Tube agglutination:	b	1,2
F 1	Beede	+++	—
2	Jessey	+++	—

b: —

Inoc b serum to isolate 1, 2 phase for subsequent transduction to b:enx : no agglutination — these are monophasic:

L

Notes on cultures from Chamblee

3/1/53

(Brought back 2/15+/53)

SW961 = S. choleraesuis Kunzendorf recently isolated, 6145-52, purified and motilized. = —:1,5,(c'?),...

SW958 = SW961 selected at GW / immunized serum. = c,c':—

958C = 958 / Kunzendorf serum = c:—

Non motiles: 1520- to 1526-51 (see SW552-553) presumably all nonmotile dublins from out kwale via Guatemala. See letter to Munoz ca 2/53. 1523, 1526 should not be found. Other NM not selected in accessions. Relationship of 3010-49 to 3012-49 not questioned.

? Kauffmann's D nm: is recorded as highly aberrant

= Stocker 234?

Monophasics: —1,2 Stocker 191: history ? listed as typhimurium

SW960 = 5594-51 (Kauffmann...) Isolated in Berlin. Phage types as para B but is —1,2. (Bekeken ontbreekt?)

SW 959 = Hinsies VA H -? no other label. listed as —1,2

3550-51: 2 cultures found, marked monophasic 1,2 and b resp. Worksheet names 3550-51 as b:—, 3551-51 as b:1,2. [3550-51 is probably a fairly stable b that later did give a 1,2 phase. This should be verified].

SW 891, acc. to PRE letter, is e,n,x:1,2,3 (Theill-Esmell) ∴ TM.

z_{33} homologies

3/4/53 occurrence: (see also sw. in)

991H1A = SW937/b → z_{33} ++.

ef 3eq

K1A
K3

See 979-J and K. J14, K15-16 kept as maggiotwinkle.

Now tested as possible z_{33} variants. — not z_{33}

After mutilization: +	(through z_{33})	& / through enx
J14 enx, i	i enx	
K15 enx	enx: z_{33}? v. weak	enx ! z_{33}?
K16 enx	enx	~~maggi~~ z_{33} ↓ enx

∴ K16 = z_{33}:enx & typical variability = SW981

K15/K also wants z_{33}: 1,2 at 1:500/-1:1000!

but strong spont aggl: maybe Rubenoid! rough. T.O.

3/13 Note 1023. SW676 — x.. failed. Repeat 3/15.

FA49 — x SW666 swarm. 16 slow buds only. eventually grew out discard, as basic improvement still i.

→ x LT-2 " " "

→ x abony no mot. at all. T.O 10/21

ca 4/15. ~~Sw~~ SW1036 plated from SW703/b+1,2 in tube (1/3, after mutilation) by motility selection of ph1. z_{33}: 1,2. The ph2 appears

L SW1006 abony (mot.)/b, enx plates — indestructswarm. z_{33}: enx (z_{33} ruined/enx. Mutilation → enx.

Cross-x i b & 12 presumably parent + presumably outgrowth.

Test 676 in b, i: 12 seq markedly inhibiting in b. Not especially in i: 1, 2, 3.

3/2/53

P2

SW726 = Edwards 25. Secr. to PLT22 } A4: no swarms.

A. Broz enx S.S. } all negative

B " a-enx " } 3/7/53. T.O.

C " " " + FA 54 (Zga 31) } enx sufficient to block.

3/7 D 726 × FA 18 [enx serum] 3/10: no spread! Isolate rough buds 3/15

E " " FA 22 [enx serum] 3/15 finally grew through. → a a (ozzy)

3/6. F 726 (FA 58) × SW666 : +++ Isolate ± 6 ss. tests.

−b +++

+b no swarms after 36h. — swarm: appearing → a. (check ax, c enx!)

A8 P8 H10 SW985

3/7 G 58 × LT-2' [i, 1, 2] — — — Isolate incomplete SW 3/15 steif a

H 58 × LT-2² ++ +++ → enx : —

3/9–10. SW985 migrated promptly through enx; was immob. in a repeat : up 985

Velbo appeared → pontyagyl, but v. weak b, z33 + .

58 H1 remained immobile in enx SS! (possibility of contamination? excluded later)

Why is 726 un-transducible? Note stability of H1.

3/10. Broz 726, H1 in eh SS: 3/11 no swarm! 3/13 steif immobile

3/15 " T.O.

Possibility that H1 is ab. equi contam? Try Pharmacia ferm:

LT-2 : AG + (24h.)

H-1: AG +

726 : AG ± and sparser growth. Not decisive diffusers. Should be repeated. (Try 950' - 2½)

note "985" itself agglutinates weakly w/ b, z33. Recheck purity 985 represents wide immobile w/ a

,026 D, E

in a serum, inhibited 6th, buds from surface.

↓

enx

Probably a spontaneous enx : a.

Check stability of these enx phases. (grow possibly on nutrient agar)

3/21.

3/13. —x 726 have failed. x— typhi murium gave peculiar result (enx: —)
—x SW666/b gave "a": —, apparently cross-reacting i b or 333.
(985 might be mixed). [From past experience, enx does not hinder 1,2— str.] J.

M FA58 —x SW891 ∠ 24h. → enx: — after 48 hours:
N 959 " → enx: — still enx
O 960 slow but only, 48h → 3/16 a, (enx?) Purify and retest

P2 58 —x SW950 (heavy FA). → enx: — OK, Cal —. SW986 (22R)
P3 " —x LT2 → enx:
P 58 —x SW703 II. → enx: 3/19

(not. l.)
S SW726 x— FA18 (LT2 II) } very limited if any spread. S2 recover
T 40 (sendai II) } shows some rough blebs. → eventually 1,5: — SW998
U 24 (703 II) } eventually gave SW1001 1,2: 3/24 Rough! Try to recover smoother isolate through
V W X 55-57 (—1,2) } U (55—x) SW1000 rexamined 3/19: 1,2 : — not again

R SW985 (58—x SW666, a) /a gave a "b, 333+". S.C.-1, motility, gave same response. Also, 985, unspecified, gave similar "weak b"., but did not produce a serum though a again. Probably mixed, impurity.

Thus enx of abortus equi is intrinsically monophasic, even when transduced to another strain. ∴ its homologies are not directly deducible.

O. 9 single colonies all a+++. 4/4 tested weak enx? Broc single colony and mass in a, enx serums. single colony and mass migrated through a, and O-1 not at all hindered in enx:

O /a → 1,2 ~~(enx? Probably)~~

O-1 /a → 1,2

Note SW726 itself was poorly motile in first transfer in motility agar.

52 → a. 22^R

↓

enz readily. ~~Test phase stability 4/5/53~~

rather rough T.O.

need test to discriminate spont vs. transduc. origin of these a: enz types. Prefer smoother ab-equiv titrations

S. aboatus equi

1026c

3/20/53. Repeat S, V, W, X (726 mut ← FA18·22; 55, 56, 57 - resp.) but no swarms appear. Title carried 726/enx.

3/27 FA18 (~~FA~~ TM12[2]) × 726 gave a. = 102652 (of D/E)

3/29 others still immobile. Seal off whatever swarms

D-E. Note enx → a → enx. Test diphasicity. ✓

D/enx gives scattered buds overnight, but these remain rather rough. and move very slowly.
E/enx goes fairly promptly. → a.

∴ 1026E is now a: enx diphasic Was this a transduction of a modifier or simple selection of the same?

1026D moved very erratically and slowly through enx, but these buds are a. Probably rather too rough

Are these a: enx now a spontaneous enx: a or a transduction of a variability modifier?

3/29. 26V SW100 appear to be monophasic on 123

H1 G2 26M S3 eb. enx immobile → ~~still~~ in enx 3/31 enx +++ i + (quite slower)

v. rough.

Smoother cultures of ab. equi would be essential for further studies. (Write Morag)

3/30

18-x726
726M'
2603 / cux
26P
726'

still cux

26H1

4/3/53 ab equi (−: enx) → × TM (i: 1,2) gave (+−: enx). SW986.

① attempts to obtain i phase by selection have failed

② Try to substitute a scibbli and distinguishable diphasic H_2 allelo.
FAYO (sundai^2) → SW986 1 attempt → a: enx
This cannot be interpreted as sundai itself is a: 1,5

③ 1026G2 / enx 1 passage gave enx +++ i ++ (i shown but fully developed). This pattern also shown by single colonies.
Possibility of i enx: enx Compare unselected culture.
also eb+++.

Compare sequences of (same patterned)

4/12/53.

		a	i	enx
1	SW986 (stab stab)	−	++	+++
2	SW986 stab	+	−	++
3	SW986B (/enx)	−	+	++
4	SW986B2 (/enx/enx)	−	++	−
5	(fresh SW986-1 / enx)	−	+++	−

Thus SW986 goes through sequence:

enx (a) → enx i → i,

but patterns of a reaction are somewhat obscure. Restreak each culture for retesting.

4/s.c. i. each from (tested as a, i, enx) stab stabs.

	1	2	3	4	1026G3 1026H1
1	enx++ i+ a−	do.	do.	do.	enx++ i− enx++ i− a−
2	a++ enx+	do.	a+++ enx±	a++ enx±	
3	i++ enx+++	→			
4	i+++ enx− a−	→			

SW986.

1026 e.

Recap. 4/13/53 From FA58(aborticus-equi) → SW950 following cultures were thus obtained, verified by single colony tests:

		s.c. Vx	alt. phase
2	SW986 (slant) Gal-or ±	: a, enx	
1	SW986 (stab) Gal-	: i, enx	
3	SW986B = 986(e1 or e2?)/enx	: i, enx	i
4	SW986C = 986e3/enx (thrice 2 passages)	: i	—

Save/each of these mesolations for further study. Also note.

5 = 1026e1/enx i.

From similar experiments, 1026G3 and 1026H1 had been isolated (both → TM2) These now react as pure enx, as SW986 was originally reported. (It could have been overlooked as a stray i)

Gal- character, even of #2 (which is the most puzzling) seems to rule out any possibility of confusion, e.g., a 1026G2 (= FA58 → SW726). Recheck gal character of SW726. Actually, 1026e2 shows some fermentation of EMB Gal! Disregarding 2, there may have been an increase in i-reaction since SW986 was first isolated. Cf. G3 and H1. Since above rx all come from single colony isolations, 3 and 4 are definitely different, presumably not mixtures or notability.

-2 is weak Gal+ as is 2652 and SW726.

4/14. Try SW986e1 in i., 986e4 in i; G3 and H1 again in enx.

			overnight	
e1	i	++	→ e1'	i- or ± enx + s.c. i → enx+++ i++ (delayed
e4	i	−	} no migr.	4/25 still many limited spread: i, no enx
G3	enx	−		" " " " : enx+++ i − (or i+ v. long delay)
H1	enx	dense bulb	48h: slow spread. →	4/25 : enx+ i −.

cf. 1039 SW950 is also anomalous (more or less i: i 1,2)

e1' indistinguishable from SW986

Thus enx → TM1 makes the latter monophasic vis-a-vis either i or enx.

Try → SW950 to restore diphasicity.

→ 950 shows the double reaction; → TM2 more typically —: enx

enx i : —
— : i

Cf. SW986 in i, enx seems vs. phases of SW924 or 941

Non Motiles 1029

3/2/53

hor SS tubes i + ii PLT22

A)

	FA 12	PA 22	Control motility		36h. YE+ FA22	H:
962	±	±	+	++	+	i: 1, 2
963	—	÷	—		+ ✓	i: 1, 2
4	—	÷	—		+	
5	—	—	— slow spiral	slow +	± irregular	i
6	++	++	—		+	b
7	++	++	—		+	gm+
8	++	++	—		+	gm+
9	++	++	—		+ ✓	gm+
970	++	++	—	—	—	
1	++	++	—		+	gm+
2	++	++	—	—	—	

970, 972 only non-motile unmotilityed. Grow PA22 / 970, 972

(Plan FA9 — × to obtain Fla,⁻ ?)

Single colonies of 962 were motile, agglutinable at first isolation; miss stock culture is actively motile!

3/6/53 ~~Also try i~~ FA 9 — × NM's

Repeat: see also 1029

B)

	A7 (12h.)	36h.	FA9	FA11
963	short T.	long tails		
964	—	—		
965	+ short	slow ✓		
966	++ T+S			
967	T. very pronounced	no SW. T no S.		
970	—	—		
971	—	—		
972	—	—		

schedule to 6PM

note: 9 — × 967 tails continue to elongate! (complementary allele of Fla,⁻ ?)

Try in gm serum.

(But note SW662 : 553 — × 646 → 12⁺)

3/11/53.

3/6/53 9-x967 gave a continuously extended track. Marks at 8³⁰A10, 10P10, 8P11.

3/10/53 ~~2-x 553~~ no T or S
2-x 553 S

Tubes: 9-x 553 1 Track.
~~2-x 553~~
22-x 553 T+S. /gm → no sw.

Later:
Sw: i

1027C1:
S.C. i: — 3/15
3/19 gm through still i.

C)
2-x 967 no T or S
967
9-x 967 [10-x?] ~~numerous T+S~~ Numerous T, no S " T (tube) (?)

Plates
D)
12-x 967 Numerous T+S.
10-x 967 numerous T; ~~swarm~~

tube 22-x 967 Sw /gm no sw.

later → swarm: 1027C2:
i, gm±; 1,2 — pv ++ -

Repeated later: 60, 60A
60 } x 967 gave 0.
60A-x666 gave sw.

60-x 553 Trailers! [used 967M for FA60? carryover of FA22? Repeat FA pup.]
60-x 967 " !
60-x 666 Sw +++ /b →
972-x 967 T+S
970-x 967 "
970-x 972 0
972-x 970 0

gm±?
Try gm or gm sectors.
SW993 Later
(gm) + pv ++ gm ± i
Repeat S.C. i in ~~FA~~ pv+++ gm± i

Could one show that these strains are double mutants?

Tester:
F/a:
1 543; 666
2 SL13
3 (also pa
4 544
5 545.
6 541
7 SL15
8 5-8 = 5?
9 549

	1 (pw.)	2	3	4	5	6	7	8	5	9	
control	—	—	—	—	—	—	—	—	—	—	✓
970-+	+	—	=	=	+	—	+	—		+	3-4-6-8-
972-x	+	—	=	=	+	±	+	=		+	3-4-5-8-
FA22-x	+	—	+	+	+	+	+	+		+	all +

Repeat 9-x967: gives numerous trailers, v. rare swarms
10-x967 " " " and swarms. Need direct comparison of efficiencies and b: gm ratio. See 1033.

3/2/53.

=SW979

Use 732-49 unless otherwise stated. PLT-22[S]; story-rx i 1,5 not lw when first examined (also Z_6, presumably cross-reaction).

A) Test stability in 1.. sera:

1,5 bulbis restricted inoc. all gave swarms out.

1,5 (kb) " " later swarmed out → lw+ (phase I)

1,2,3. diffusing moisture, definite swarms

1,2 3 and 1,5 (bulbis) did not swarm pre- as allowing fastest spread of swarm presumably. i sharpest inhibition.

N) see 1023 N However, control for N) gwvont in lw+ 1,2

3/2 abony[2] — x javiana [lw: 1,2] 3/3 2 exptls. swarming Control fixed.

(✓PLT22[S]) two colony types, both very[A,B] large probably partially cough) N1 enx: lw Lp^S = SW980

N2 enx lw Lp^R

eventually swarm 0 2 BW-0

aggl. faintly in lw, 1,5 same

B) FA59 — x SW666 (979[I]) SW984. i/s b serum ++, +++ growth. Recover (lw) — titrate① i lw, 1,5'... No rx dextrides i 1,5 of javiana

C) FA10 — x S[redacted] SW980 [lw; enx] for "stable" b: enx

1 980[1] 2 980[2] } numerous bbls but no entrances spread at first. very slow spread further.) both still lw.

3/13: abony is destructly retarded in lw: enx serum but does eventually migrate. SW942 did not agglutinate in lw to 1:100, but ++ in b. 1:1000. This might account for failure of C+. 546 is not delayed.

942:

D [redacted][1,2] x— FA22 164. → i: enx

E x—

F V. slow x— 23 (Para b 1:2) 3/18: b: (originally!) F1 - still lw.

G broth x—

H x—

(rock)

3/29. Seal off incomplete buds of 1028:

1	180' x— 14	all lev
2	' x— 5	
3	2 x— 5	
4	2 x— 14	
5	' x— 22	

S. pullorum - gallinarum

3/3/53

		XII	PLT-22
P 1	75		
2	76-53 (2)	2	
3	66-53 (2)	2	
4	2292-51 (3)	3	
5	9823-51 (XII 2)	2	
6	5462-52 (2 3)	2 3	
7	5464-52 (2)	2	
8	5839-52 (3)	3	
9	5840-52 (2, 3)	2 3	
10	6319-52 (XII 2 3)	2 3	
11	6689-(3)	3	
12	6694-52 (2, 3)	2 3	

3/4/53 + FA 22 on motility agar

EMB Mal — EMB Gal D(B)

all S. all negative 3/3. Store at 30° 24 hrs. still negative 3/10. throw out.

SW970 weak + 972 " "

all + +

all S. see over

G 1	74	
2	2923-49	
3	2927-51	
4	309-53	
5	3728-52	
6	3966-52	
7	3968-52	
8	4614-52	
9	5285-52	
10	5522-52	
11	5933-52	
12	6299-52	
13	6475-52	

P1, P4, G-1, G-12 selected for further study. PLT22 grown on each for FA. Twenty on SW666, EMB Gal; SS.

(Storkan found PLT22/gallinarum SS to have v. low e.o.p. on LT-2 ($< 3 \times 10^6 / 10^{10}$) or 53%.)

see 1043

B. Test P1 ... + PLT22 for ~~gallo~~ lysogenation

	→ ×666 not. A7	A8	→ ×666 EMB Gal
P1	−	−	0
P12	−	−	0
G1	−	−	1
G4	−	−	1

FA 22 +++ T+S /b:

±b: SW970 +++ → gm+ SW983 +++

±b: SW972 +++ T+S. → gm+ SW982 +++

Hold further for Mal + pullorum plan later work for intra-team transductions.

(over)

G8 × ~~FA~~ FA22 showed faint fuzzy

extension 3/10.

Remoi ± FA22 and reincubate.

ditto 972. no motility whatever to 3/19

(probably precipitates).

~~P1 × P1~~

P1 × FA22, 972, G4, G1 all 0 on EMB Gal.

FA10

~~P1 × P1~~

955 × P1 P12 control FA9 FA10 on EMB Gal

5 3 5 5 8

970 972 G4 G1

8 6 5 9

no likely effect.

P1, G1 × 967, 971 all negative except a single swarm (1 plate) in ~~and~~ 1/3 plates of P1 × 967

Both P1 × and G1 × 967 gave (gm) + 1 each.

P1, G1 both gave tracks + occ. swarms (d) on 0901. (presumably ±)

× H901 /d. Both gave mags. phases that later reacted slowly, i d

"Antibacterial i"

1030

See 1001. i FA12 – x 666, b swarms were delayed relative to i! could not show motility differences later

P6. Inoculate motility tubes i 666; ~~&~~

A7. Add 10^{-7} ml SW 680, 681... 11AM 10^{30} A8

i	A.	680 / 666	mm. 50, 53, 48, 38
	B	680 / i cells	64 = bottom = +
b	C	681 / 666	61+, 60+, 61+, 66+
	D	681 / —	66+

i! b! ..

Thus 680 was stored prior to 681. (Instrument motility diffs not determined: further controls needed. Diffs in inhibition proportionate to 666 large not readily discernible. Use B+D as motility cultures in further expts.

Remix → B, D Mainly 10:40AM 3/8 — 4PM:

B	23mm., 23	dye tube 17
D	29, 28	" " 22

∴ Intrinsic diffs in motility. 991C should be repeated to provide raw material comparable to 1021.

D still > B after motility selection.

3/19/53. Repeat 9992IC: Dilute FA12 → SW666. Ca 2-3 swarms per tube (5-10 × .01 ml samples of FA12 1/300). 6 early 2 later swarms. These were i: 2b 2i 0b respectively. Result previously stated may have been a coincidence! - See 1021 (m.)

1 b
2 b
3 i
4 i
5 i
6 i med
7 i late
8 b late

} early

} all 22^R Test 12.

Monophasics.

A. SW942 (N97 :b—) in b SS tubes = D3 see D
Edwards dug up some other N97, "1,2" presumably. Discard of possible doubt as to ancestry do not use these unless essential.

B. ① 3550-51 "b" } "monophasic" was [redacted] viable. → "b+ reactions, 1,2—.
② " " "12" }
Mor. b SS 3/13.

C. 546 in ② 12 [redacted] /46: } 1/2 immobile / immobile
C2: still 1,2.

cf. D. 546 liv. agglutinates nearly 0 in b (Edwards) 1:100 b+ not b (Colindale — absorbed?) [redacted] sera may be imperfect for phase solutions!

D. 942 in b 123 (Colindale) 12-E / 46: — / +++ / + def. retardation! (cf. Speicher's titre)
D 3+... sig. redo / b. 3/10/53
3/12: D6 → {3 3+ 1,2 +}
3/7

Note: in tube agglutination, 942 reacts i 1,2 E (#157-serum) to > 1:200, < 1:800
i Colindale 123. 1:200 +++ 1:500 ÷

E FA 54-x 666 ++. SS → ++ SS+b → d: (v. weak in slides) SW987
55-x ++ —
56-x — —
57-x ++ —.
} Repeated 3/19 i same result. Is FA56 reactive? Trypan SW967 Agglut. 1:500

F 959/1,2 maggl. at first, later after SS → 1,2 ++, ② ++. Repasses in 1,2,3)
959/3: reacts b, also i!??

G ~~B91~~/1,2 2 passes in 1,2,3 : maggl. (pr)

H = 960/1,2 2 passes in 1,2,3: → still 1,2

} Save and send to Edwards as B

~~55~~

12 1:100 : +++

942 : 1,5k3 : —

same lib + ~~pure~~ pure kb : —

b 1:1000 +++

∴ cross-reaction
& gpn 1,2 only.

In repetitions of E, FA56 was inactive (4 control?)
55,57 /b gave nothing (maybe useful as H_1^0).

56 → 967 also gave no swarms.

Try 55 → 967
57 →

both gpn + numerous swarms.

~~5557~~ → pus; slow outgrowth 4 times.

gpn → 57: still (gpn) +

Mycoplasmas: stability

3/7 SW942 in b SS

D.

1	duct from	3/13 →	z_{33} V.sharp	→ sci	z_{33}: T.O 3/29
2	stalk	3/13 →	z_{33} ✓	→ sci	
3					
4		3/17 →	z_{33}		
5					
6		3/12 →	z_{33}+1,2+.	Rep.: z_{33}+ 1,2?	Titrate (after S.C.I.)
7		3/19 →	z_{33}		
8	T.O others 3/29				

following what appears to be an initial stage, a dense well demarcated bud, diffuse spread sometimes later ensues as if in two steps.

C. SW546 in 1,2,3 SS. 5 single colonies 3/14.

3/24 1. Stiel 1,2 ± 2 ?

others T.O 3/29.

SW997 B: 3550-51 (= 1031B3) in b SS 3/13. 3/15 slow migration. Pullout after limited travel and reinoculate. = B1, after second pass, with z_{33}? mixed. Put through SS mag. = z_{33} (weak) 4p

3/15 Single colonies after motility agar: 2-4

B2 → Rough! B3-B4-(Rough) z_{33}.

FGH Conclude that 959, 960, 891 are substantially stable. Present FA pups inadequate to elicit non-b from SW966.

#031

JKL

J 891 } x— FA15 (along² enx) / 1,2 Eclos. ③
K 959 } 1,2,3 ①
L 960. } ② swarms. ②

Buds + swarms in 20 hours. Fastest progression in 1,2,3 (2 simult.) = ①

J1 b: slow → 1,2
K1 b: — at first → ≥33!
L1 b: (slow) —
Because of phase again.

b:1,2→b (single colonies wait b, 1,2++ ?) then ① ③ slowest.) ~~these crawl~~ through ~~later~~. then
b:1,2→b^c Test reversibility wait primarily 1,2 (over)

(try in b, 1,2!) of: zega & JKL, same pattern! As phase II intrinsically poorly motile? ~~[redacted]~~ Pass 891... in motility again! (31-50...):

A16 J-0 moderate +.
K-0 v. slow initially, gave fast bud.
L-0 moderate +

Note 959/3 finally gave (after 2 passes 1,2,3 swarm + 2 not again, + s.c.i.) a phase reacting, in slide aggl. b:++ ≥33:++ (i, 1,2 ±?) In tubes at 1:1000 b++ i ± 1,2 ± ≥33 +? Record as b, (i, ≥33, 1,2?)

SW992 After passage through b ~~[redacted]~~ 1,2 readily.

SW995	J2	b+++	enx?
	K3	b ...	enx +++
	L2	b+++	enx ±
	L3	b+++	enx ±

Assume that b: 1,2 and i: enx are both formed, but that the b phase usually predominates.

gives v. slow progression "Lie-agars (enx) bands"

L3 L2 eventually gave through: still b.

Select J2, L2, L3 swarms in b: 1,2 agar to isolate possible residual —: enx forms. [Similar sit. c d: ≥6]. No alternative phases appeared (already pure!) No further test except K3 enx: — 3/11

Hereafter, use motility J0-K0-L0 in further experiments. These are still pure —: 1,2.

① Try separating b from 33 colonies Record as b, (≥33?): 1,2

② ~~b, ≥33, b+≥33 selection.~~
↓ ↓
1,2

(See over for summary)

JI': 1,2 (primarily) doubtful b mutants
in several single colonies

Put in 1,2 serum for "
migrates in 2/4. → b

---

single colony swarmed directly through b, 1,2 but
not b+1,2

∴ JI' is now b:1,2 reversible.

Summary: 891 and 910 x— always have given so far
only b:1,2 becoming
diphasic.

SW959 x— always has given ① b:— (z33) might not be transductions
② —:eu x

Alt phases: SW959 → 959/3 which acts b(z33:1,2):1,2
not clear whether now diphasic.

These selections need to be repeated using motile of SW959.

3/29/53.

STATUS.

Wj. Hss:

1. 53-666-948.... (See 1008). AEC / L+Edwards.
   Combinations, tracks, etc. in progress.

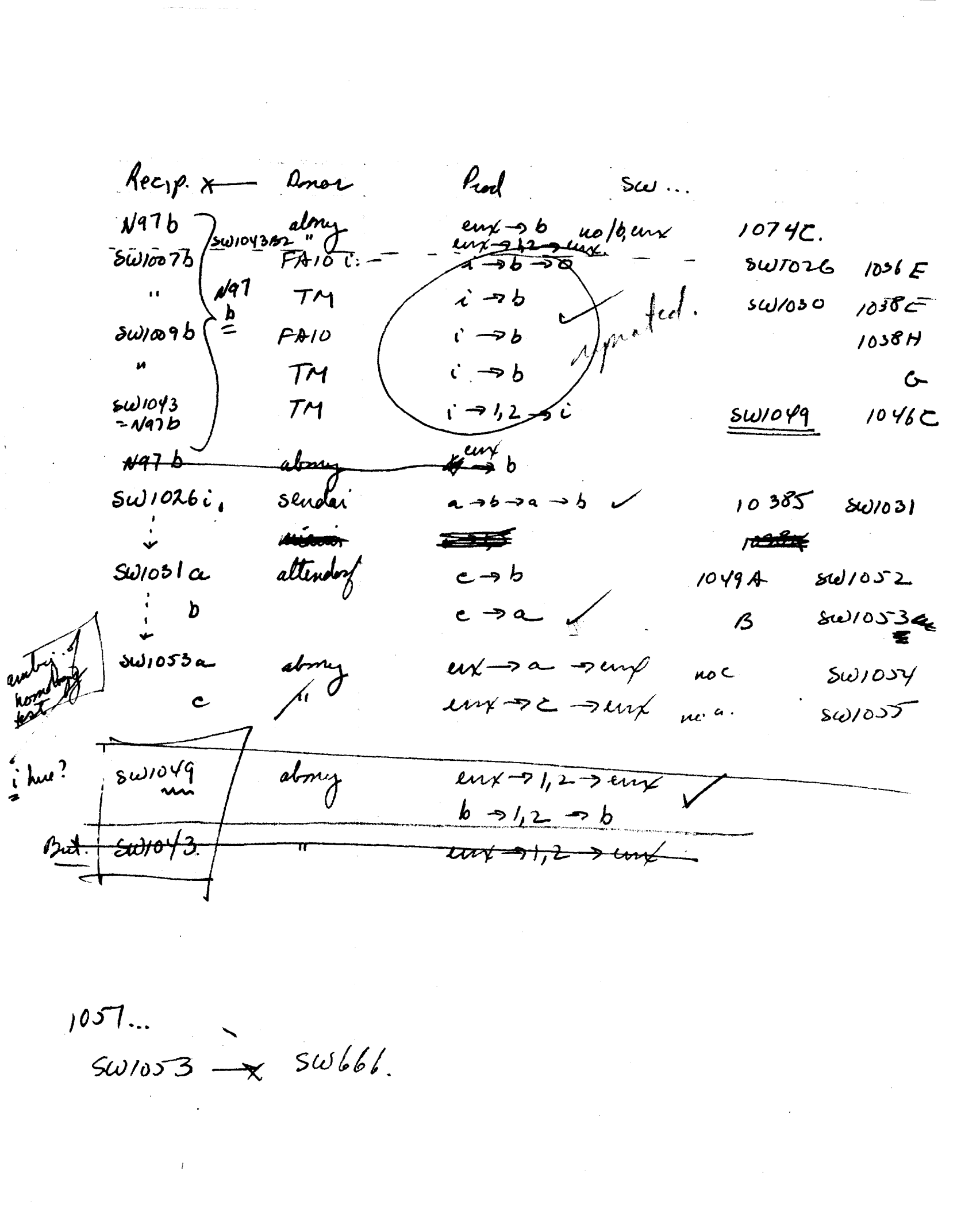
2. Monophenic 1,2's.
   a) no first phases clearly produced (Recheck b from 959 - of Edwards 1...)
   b) → other strains. Failed on abony, no explanation. Ref.
   c) → 666 to reveal first phases. No swarms 0901 - Felix '30
      → 967 " " " In progress (cx. Fig. x)
   959 → x aborticus equi gave 1,2 : — (sw1000) This is the only transduction from these monophenics. Possibly phage titres are low? Should be checked.

   a) → x 959-960-891... d: z6 gave d:1,2 in each case (977 may be d: - ?) 976-8
      (a) enx a:1,2 sw994
      b: enx b :12
   (959 seems to give stable types d:- , b: — and -:enx). Use 959 motile for further exp.
   (. also z6 :-)

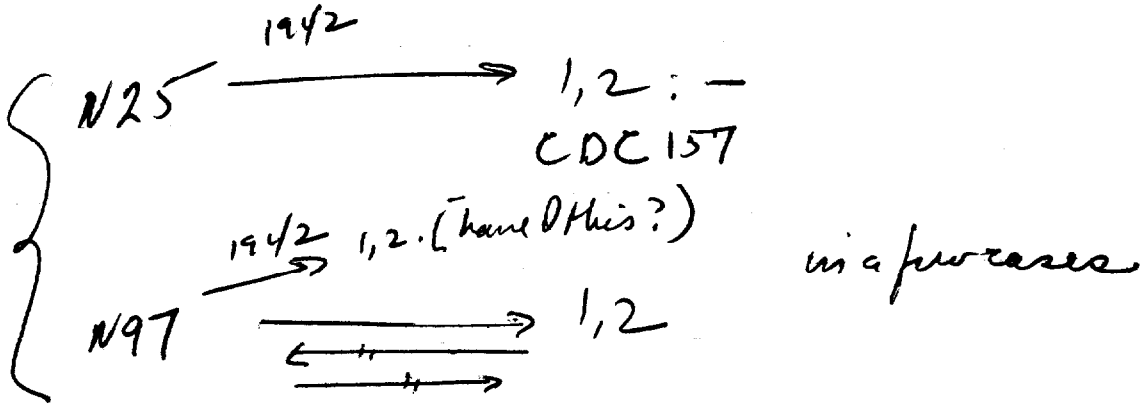
3. Aborticus equi. → x TM or para B gives —:enx! (sw986)
   → x ~~sw~~ 960... a:1,2 ...
   x ← 959 —:1,2
   x ← para B 2 —:1,2
   x ← TM (not yet sure in control) a : enx !

Need smoother culture for / utter work on Odiertig' monophenics and 2
Prepare FA 9, 10, 11, 12. substitute enx ...

			H_1	H_2	H_2	×	H_1	H_1	H_2		H_1 H_1 H_2
	N976 ×	abmy	b	1,2	–	{	b	–	emx		b (1,2) emx
										1074	(b) 1,2 emx
	TM		b	"	1,2	– {	i	–	1,2	SW 1026 1050	b i –
										SW 1049	i 1,2 –
1029: i obtained.	SW1026 ×	scudai	b	i	–		a	–	1,5	SW1031	a b –
	SW1031	altendorf	a	b	–		c	–	1,7	SW1052	c b
										SW1053	a c
	SW1053	abmy	a	c	–		b	–	emx		a (c) emx
											c (a) emx
	SW1049.	abmy	i	1,2	–		b	–	emx		b 1,2 –
											(b) 1,2 emx
1036D	N976'	× TM ✓					b → 1,2 → b				b 1,2 SW1027
	1007b	× mein ✓									b – 1,5 SW1028
	(1009 b2	abmy ✓									1,2 – emx
	SW1043 N976	lonne lmda a emx ✓									b emx
	SW1026 i	mienn									i 1,5
wh. 1046.	SW1031 a	SW1046									a 1,2
	b	"									b 1,2


Recip. x
Donor
Prod
Scw...
N97b
SW10073b
SW10093b
SW1043 = N97b
SW1043B2
N97 b =
abmy
FA10
TM
i → b
i → 1,2 → i
SW1049
1074C.
SW1026
SW1030
1036E
1038C
1038H
G
1046C
unmated.
N97b
SW1026i
SW1031a
SW1053a
SW1049
Bad. SW1043
sender
attendorf
c → b
c → a
1049A
B
SW1052
SW1053
SW1054
SW1055
1057...
SW1053 → SW666.

stated in 1946 that $b \rightarrow 1,2$ only once
1,2's are stable



"AMS" source of N97?
1036 - 1046.

N97 → 1,2 sc → 0
type 3b.
1046A1

1036B1
N97 → 1,2 → b → 5sc → 00000
mess
SW1009
SW1007

sc → b
SW1009b
5sc → b → 0
b → 0
b → 0
b → 0
b
1036G1

N97 → 1,2
mess
SW1043

8 s.c. → 1,2 → 0,0
→ 1,2 → b → 0
→ 1,2 → 0
→ 1,2
→ 1,2
→ 1,2
→ 1,2
→ 1,2
1046B1

again
→ 1,2 → 0
→ 1,2 → 0
1,2 → 0
→ 1,2
→ 1,2
→ 0

History of cultures.

COC-157 = S para B N25 — 1,2

Ky Bull 1939 Ky Ag Exp Sta Circ 50.

[Useful to obtain a Vi⁺ H⁻ strain of Ty 2.
Phase reversal in Toluin]

Sw 66 = 0248 = Jersey taft +.

11/53 – Phil offered other cultures from same outbreak.

received? Saved?

{ N25, N97 } from an outbreak in C.Z. c 1942
one typable (Cherry)

{ N25 → COC 157 tartrate pos.
N97

Same outbreak

1025.

4. z_{33}. SW981 is typ. z_{33} : enx (Try prioffate for *i:12)

SW96 → x i:1,2 failed. (inadequate ♂?)

5. Monophasics 1031-36. No occurrences of 1,2 phases in available material tested. Others underway. 3550-51 (SW997); 942 gave only z_{33}. 546 gave nothing new. [Try to get viable 3550-51 (1,2)].

6. S. javiana SW980 ✓ IX XII enx: l z_{28}

996 1028F2 inaggl. x—para B 1.

990 IX XII i: enx

980 — x 666 → l z_{28} : —

lw seems to inhibit b.

7 Nonmotiles. See 1027. Homology tests on SW970, 972 incomplete. H1 - F/a ~~too~~ Linkage of SW953-967-8-9 in process.

8. Pullorum-gallinarum → x 0901 → p_6^+. Other homologies not extensively tested.

9. Motility-F. 58-161 2/6 F^- 1 $P^{+++ H?}$ W1678 1/4 F^- hyfactile

10: Tracks: 3/27/53 On basis of Morse' findings on segregation of Gal_2 → Gal_4 → + → both Gal_4, Gal_2^- tracks may have other genotypes than receptor stem

Selection for H.f. transduc.

3/10/53

(cf. Morse' contemporary experiment):

FA22 × SW950 on EMB Gal to isolate transduc. phage. Isolate 32 papillae. After purification, grow mixed culture ± LT-2 and grow phage. Assay 1 drop of each phage ± SW950 / EMB Gal. 1–22 individual, 23-28, 29-32 as pools. Look for marked discrepancies as compared ± SW955 + LT2; PLT22+LT2 preparations.

	<50	50-100	>100
#'s	9	13	
	8	22	
	7 (2)	11	
	6		
	15	18	
	19		
	20		
	21		
	5		
	16		
PA22			
	11		
	14		
	1		
	0 (0)		
	29+		
	23-28		
	10		
	955		
	4		
	3		
	2		
	12		

	pop/.1	plaques/10^7	ratio
A1	~~88~~ 131	773	.169
A2	241	1251	.193
A3	93	576	.161
A4	296	1456	.204
FA22	~~144~~ 231	1448	.160

later platings ↗ too high for accurate count!

ratios in fairly good, constant accord; about 1.75 transductions per 10^7 ϕ. (Yesterday's determinations for this factor, which is fairly incomplete

Some of these are very crudely estimated. Save nos. 11, 22, 3, 7 for further assay as 1032 A(1-4). Assay ϕ, FA Gal+. Also pass papillae further test of same sort.

Note: In spite of ~~semi~~ bi-toxon $Zn Cl_2$ in t.f., the pups became obviously contaminated, presumably ± LT-2. This is apparent in terms of overnight papillae. A2-3-4 show this property. Initial readings, however, are probably OK, so repeat assays after vibrating and shaking ± chloroform in closed vials.

(over)

169
193
161
204
} transductions per 10⁹

160

Repick 4 papillae from A4 = B1-4 for recard pass
Assays, .1 ml → x sw 950

3/27/53.

B1	315
2	352
4	307
3	480

~~[redacted]~~ Pick 4 papillae for C.

11/7/53

C1	57
2	131
3	116
4	3 (probably nonbyog.)
FA 22	113

---

no effect obvious. Note variation in assay (little rare an indicator).

Isolate C2 and save.

SW684 is sole Gal v

K. SW684. ~~Gal+~~ colony (selected by MLM, must ~~be~~ Gal v) + culture Lwoffed. But in pest test lysate gave 0 Gal+! / SW666.

of 973B K1 K2.

L. Reisolate SW684 Gal v.

not recoverable 4/53.

S. dublin NM. F1a, ? 1033

cf. 1027

3/19/53 FA22 —× SW967 /gm. → after 48-72 hours swarms: again c'. Unless 2-step transduction is involved, which seems doubtful, SW553 also shows limited transduction.

Recapit: 1. SW967; 553 —× SW666 docogive F1a+ b and gm.

[Compare b:gm ratio with NM and motile SW967....]

2. SW666 —× SW967 gives (only?) gm

3. LT2 —× SW967 gives mostly gm · i selectively.

[Unselected ratio gm: i].

[b from 666 -× 967? Note rarity of any swarmers. cf. swarm: trailers 666, 666 F6]

4.	FA9 —× 967	5/5	gm	save 2	22s	1. SW1045
5.	12 —× 967	6/6	gm			
6.	10 —× 967	54/54	gm	save 2.	22s	
7.	22 —× 967	67/71	gm	2i	2 rough.	Hg 2i } save 6 22s

Renew some of these for sensitivity to PLT-22 for further tests.

Thus confirm occurrence of "limited" transductions.

Detection of suppressed phases:

			− gm	+ gm i swarm
8	FA55	(SW959)		
9	56	(SW960)	—× SW967 ± gm.	
10	57	(SW891)		

cf. 3/19: —× SW666 ± b. 55, 57 gave swarms i but not i b, cf. 1031 E

JAN 25 1955

FD58 = abortive eggs
supposed to be H.
Both to use SW1067
which may actually be H.

F1a, ... : Trackless segregation

3/29/53 (666) (609) (623)

A. FA9 –x SW967. B. 10 –x 967 C. 12 –x ~~###~~666.

D. FA60 (SW967) –x SW666.

3/30. A has almost no Tor's. (new prep. of FA9, may be lost later)

3/30. Repeat B, C, D 10 AM.

B. Most plates too heavy: isolate trackers (and purify former) from 1 plate

C, D. Too heavy

B. Pick 6. #6 shows a few motile cells – rep. Repurify all 6, but test 1-5 also directly ? FA28. (1-5) x – 58 → gm, not b. Also test each x – 60

6 x – 22 → gm not b

A. After 4 hours trackers appeared. Pick 17. Spot as SS ± FA22. ~~12 tested all gm~~ #3 ? ~~5 confirmed + #3~~ all → gm +

3/31. Repeat A 9:30 AM. Use 9 28 hr offgate –x SW967. 30 trackers picked 21-50

B 10A –x 967 33 picked #1-~~39~~.

C 12 –x 666 ± b, i serum

D 60 –x 666

E 60 –x 948

F 9 –x 948

G 10 –x 948

H 22 –x 948.

4/1 and 4/2

A. After 15 hours, trackers and swarms are completely inhibited by gm serum. Swarms are reduced in number but trackers are scarcely affected (number?) by b serum. i serum, trackers and swarms are very profuse. Pick trackers away from vicinity of swarms

C. Trackers are not very numerous compared to swarms. In b, i serums numerous swarms (somewhat reduced?); no trackers at all. b+i: a few inhibited swarms. No trackers that could be isolated.

B. Dilute plating. Pick isolated occasional trackers

What was 1033 expt? ① Look for crossovers ② Serum effects

JAN 2 4 1955

A: (SW666 → × SW967.) T × — FA22 all gm (17 tested)
all ×≠ FA60.

S.50: all gm

B (SW609 → × 967 / much heavier guild than A).
T × — SW726
or TM2 → 6 all gm.

~~No record of Ha_x dosperiis, but note to do it.~~

Repeated. ♀ serum test. ♀ gm serum, no Tor S from A.
no effect of ♂ serum. (cf 6 → ♀ !)
(or gm → b)

C. 12 → × 666. Note that T ≈ S. Dor inhibited T.

D. (60 → × 666) S 6b, 4 gm
T (4) × — TM2 → b.

G. Found that (948 × — FM0) T was more susceptible of transduction.
T: S ratio here is 120:10

D. Occ. trailers and swarms. Pick as possible. + FA22 4/trailers → b.
6b 4/gm.

E. No swarms, rare trailers E2 x— FA22
E1+22 several trailers, no sw.

F. No swarms or trailers (smeared)

G. Rare trailers G1 x— rare trails G2 x— +++ G2, G3 x— FA22

H. Rare trailers H1, H2 x— FA10 rare trailers.

A 1-17 tested x— FA60 (SW967) No swarms. All trailers are 22^S
(1 diff. plaqued)

♀ G2, 948 x— FA22 for diff. transduc, etc. 948, G2 both 22^R.,
G2 may carry some ♀/SW950 ?
(0. small plaques)

[A Test (original) 50 swarms all gm, no b.
% O. $6, 22^R : 4/4, 22^S$]

Set up FA22 2: SW948, G2 1. Shoc .02ml samples on nut agar.

18 hours: → 948 no T or S this time.

	T	S	
→ G2	28	5	G2 is apparently selected as more amenable to transduction
	47	3	
	45	2 + ?	
	120	10 (all a)	(X11 form variant ??)

Possibility that G2 has had a substitution of Fla_1^-? But derived from FA10 →.

O transductions.

Serum marked Typhimurium O 1:2", presumably absorbed on typhi, ~~but for~~ found to react ~~with~~ alcoholyzed LT-2, not H901. (Once marked by CCS "1x only · D", presumably incorrect.

In slide tests, stock cultures of Ellendorf, Zoga, Sandiego were not agglut. but aboriscanis (though already rather rough) was.

Distilled not ~~with~~ aboriscanis and sandiego.

Use 2 ml SS agar + .05, .1, .2 ml O serum. (presumably mostly 10)
3/24 None inhibited.

~~Exp't LT-2 into 2 ml~~

about x — FA60, FA61 and control in .2 ml serum / ca 4 ml SS.
=C control (persistent surface) swarmed nicely through overnight.
A, B expts had gone ca 2 cm in 24 hours. Cut off this and also remainder
A: 4/4 still O+ T O −
B: 3/3 rather rough, but B+ T −
O901 T+ B−
results inconclusively negative

3-20-53. Motilize abony 1 and 2. Prepare FA 14C and 15C resp. from single colonies.
Plating of lysate before heating showed: 14C 24 b : 1 enx colonies
15C 0 b : 20 enx
These FA should behave substantially pure.

Prepare suspensions of TM2 and SW950, phases i and 1,2 from single colonies.
Plate mixtures with FA on i:12 serum SS agar.

9P26

A. SW 950 (i) + TM2 (1,2) x— 14C [b:enx] .1 ml 1:1 culture mix + .2ml FA pipette spread.

B. i- 12+ x— 15C b:enx

C. SW 950 (12-) + TM2 (i+) x— 14C 10A27: All plates rather overspread (medium still too moist; insuff. antiserum?)

D. x— 15C

Pick whatever swarms as possible, and stationary growth (A_o, B_o...). Streak these out as well as inocula.

	gross slide agg.	Colonies on EMB Gal	Individual colonies		
AB inoc	i++ 12++	- = +	5+ all 12 5- all i	all ok/	
CD inoc	" "	- = +	all i " all 12		
Ao	i± 12++	ca 5+:1-	all 12 all i	ok	
Bo	+ ++	3+:1-	4i 1"i(12) "		
Co	++ ++	+=-	5 i:4i 1i(12) all i:12	?	
Do	++ ++	+=-	5 i(12) all i:12 !	?	

A30

Co and Do reacted very poorly directly from colonies and were therefore reinoculated into broth and then tested. It is still mysterious that they should show this diphasicity. Restreak and cf. C-D inoculum.

In first run, A and C gave discrete swarms; B and D were badly overspread, and must be regarded as pooled (and possibly biassed) swarms.

A: 1-5 all Gal- b
C: 1-3 Gal+ b

These are in agreement with result of 979JK, and may also show directive preference of recipient phase (homophasic)

B: 1 Pred. Gal- (5/5 b); few + (5/5 enx) Count 1 b- 1 enx+
2 almost pure Gal+ 5/5 enx " "
3 pred,. Gal + (5/5 enx);few - (5/5 b) " "
4 pred. Gal+ (2/5 enx 3/5 12*) - 5/5 b " "
3 b- 4 enx+

D: 1-3 All virtually pure Gal+ b. 3 b+ .....

P28 Rerun B,D using smaller inocula (same suspensions). Still overspread, but mod. well isolated swarms.

B: 2 Gal- b : 2 Gal- enx [sic]

D: 11 Gal+ b : 2 Gal+ enx : 1 Gal- enx

Total	homoph b	heteroph b	homo enx	heteroph enx
A	* 5	—	—	—
B	* 5		* 4	2
C	* 3	-	* —	—
D	14	.	* 1	2

4/8/53

See → for summary.

FA15C × TM2 ¹ 1 swarm: enx

² 7 swarms: enx

(old suspension) v. dilute FA. well-isolated swarms

(This fits previous data much better.)

4/10 .. FA15C mixture 5 swarms: enx

dil. → AB gal: 3+ : 2i

35B' (sucrose) (hemoph) (hetrophil)

Ca = gal+ : −

5+ = 1,2

5− = i

(of 4:2 previously!)

35D' comparable to above. discrete swarms only.

4/13. + plate made heavily inoculated → pool

mix CD streaked out. predom Cal+ 5+ {1 i, 1,2 +o
{4 i

5− {5 i 1,2

of AB inoculum! There are much more motile

15C × CD . Discrete swarms:

75b all Cal+

9 enx 3 Cal−

6 Cal+

These mixtures are peculiar. save as 10 35 C, D, CD.

D: pool, streaked out ca 10 Cal+ : 1 Cal− . pool makes mats b.

Pick 10− enx

10+ b

C-D show the major descrepancy. Possible sources of error:

1. Impurity of FA. (in spite of preliminary control!) Repeat with other pups.
2. Intrinsic motility difference favoring Gal+ cells. But cf. A. or i
3. Differential effect of the serum preparation, favoring G: — over ent.
4. Cf. peculiarity of phase of Co-Do!
5. Contamination of FA15 with abony. (Test some D for phase 2) but B is also descrepant. Not likely.

---

2) 21/2/53.

Mor AB mor CD mor / not agar

At margin, steahont: AB pure + (1,2
CD ~~pure~~ 20: i + 1,2)

Try To i Through motility agar: 2 passages: reacted 1,2++o i —
∴ phase 2 is more motile.

Materials of s.c.i. from A-B-C-D mor,

950(1,2) showed mixed contam. in both from each of two s.c., no others.

E. 4/8/53. Test quality of FA15C, at dilutions to permit discrete swarms.

→ × TM2' 1 swarm enx Same susp. as 1035.

→ × TM2^2 7 " enx.

B' 4/11 FA15C → × AB. 5 swarms: all enx, 3 Gal+
2 Gal-

Control AB 5 Gal+ : 1, 2
5 Gal- : i

D' 4/13. Same suspensions, diluted FA.

FA15C → × C (SW950^2 + TM')

Control: pseudo Gal+. [5+ : 4i 1 i, 1, 2.
5- : 5 i+1, 2

Discrete swarms: 25 b all Gal+ cf. 3/28 D - quite homogeneous.
9 enx 3 Gal-
6 Gal+

pooled swarms, ca 10 Gal+ : 1 Gal-

10 Gal- : 10 enx

10 Gal+ : 10 b.

Thus if we regarded Gal- only (= 1, 2, in this case,) all would be homophenic, but 25 : 6 of the Gal+ (TM' !) are b. Swarms may have been too crowded still. TM has not, as a rule, given any difficulty in scoring i vs. 1, 2 but should be examined further.

Cumul. totals (discrete swarms only): • homophenic

		Gal+ b	Gal+ enx	Gal- b	Gal- enx
A, B	i- plus 1, 2 +			5 • 2	2
C, D	i+ plus 1, 2 -	3 • 36	8		4 •
E	1, 2 +		7		

See 1039 for further analysis of TM; SW950.

3/26/53.

see 1031

A. "7-119" from Cheny 2/53. "Peters Serum Co" Shipp Nov. 1942
S. paratyphi B & type 3b. Monoph. nonsp. tactiate +.
= SW 1006.

SS = sarcolid In my hands — rather rough. Rapid smoothest colony for stock.
1+ = agglutinable. Does not swarm through SS agar either 37° or 30°.
3/28 Microscopically: occasional cells (ca 10^{-4}) show definite motility, others stationary. Some diffusion in SS, but no progressive swarms or blebs. Cells about stationary under microscope. (nutr. req. for motility?) — Chalk with Cheny. On SS plates, numerous blebs appear, enlarging to swarms which are radially inhibited near margin — probably accounting for failure in SS tubes which are more restricted. [Antarctobacter system?] 2 swarms: 1, 2 ++, b ±. A smooth-looking colony (in SS subtilis) was actively motile. Note: self-plugged!

B N97 "A.M.S. Source unknown type 3b Monoph. sp. tactiate +"
agglutinable in b. ~~Treatment for single colonies & mass. b serum.~~
Stock: grew through b in 48 hours (after def. inhibition) = 36B1 = 1, 2 — = SW1009
Herrothers 3/29. N97 orig lost. Put mass 36B1 through
SW1007 (recovered from mass 1036B1) = b. for reversal 1, 2, 3 N97, if any.
Treatment and test single colonies in b serum.
4/5. 1036B1, s.c., also gave b after prolonged incub in 1, 2, 3
∴ N97 is reversible b: 1, 2. (and just as stable as parent N97)

stock tubes inadvertently discarded

C. "N97 (3) 1" java nsp. Grew very slowly in Pressey or nutrient. Test swarm through sarcolid — 1, 2.

NOTE.

SW1007 (= N97 or ?), and most other b phases react b++ z_{33} – .(including SW1027, 942,)

SW1009 = N97 ph.2 = 1,2, not z_{33}.

SW1009 → SW1009b (ph.1?) but this is bz_{33}.

In 1036 G, the b phases are all from a single ~~colo~~ selection of SW1009 1,1,2,3 and are evidently all bz_{33}.

For comparisons, F1–F5 should be compared. F1b is recorded as being b++ z_{33}–

SW 1009b (G1) does not revert to 1,2,3... but slowly gives z_{33}.
Other b's from SW1009 should be checked.

SW1026 is stated as FA12 (SW623) × SW1007 (and not × 1009b)
It was isolated in i phase, readily → b, but the b phase (4 colonies of 1 isolate) gives only z_{33}. The b phase has little or no z_{33}.

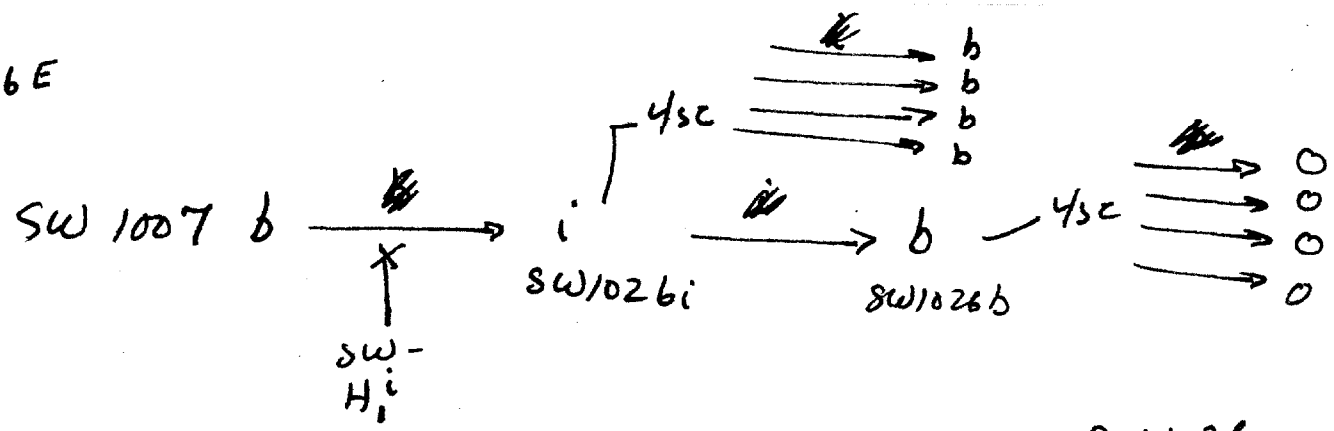
1036H

① SW945 | $\frac{1}{1}^{2\,3}$ inagglut though actively motile

② 1000-C1 | 1,2,3 often not → slow spread 1,2 ++ 1,5 – + all b phases
original ribbon spot.

1,5 Revert; 1,2 both ++ for main ph 2

1036E



∴ SW1026 is b:i

1036D. ✓ S. abony b:enx → 1,2 → enx; 1,2 → enx

SW1009 (1,2)

1038B S. miami a:1,5 → SW1007 b → b → 1,5

D TM i:1,2 → 1036G-1 b → b → 1,2 → b

1038E SW1007 b → TM → i → SW1030 b

SW1009b b → TM → i → b C, H.

ditto FA10

TRANSDUCTIONS WITH (N25 and) N97 derivs. as Recipients.

(A)

Recip.	Phenotype (Inferred Genotype)	Donor (Genotype)	Phenotype	Prod.	Inf. Genotype	Label
1036E	SW1007	b	$H_1^b H_1^{1,2}$	FA10	H_1^i	i → b → 0	$H_1^b H_1^i$	SW102
				TM		i → b		SW103
1038 D	E ✓ SW1007							
1038 H	✓ SW1009	b	$H_1^b H_1^{1,2}$	FA10	H_1^i	i → b	$H_1^b H_1^i$	1038H
1038 G	✓ "	b	"	TM	$H_1^i H_2^{1,2}$	i → b	$H_1^b H_1^i$	1038G
1046C	✓ SW1043 N97	b	"	TM	"	i → 1,2 → i	$H_1^i H_1^{1,2}$	1046C SW1049
1038 J	✓ SW1026 i	i : b	$H_1^i H_1^b$	sendai	$H_1^a H_2^{1,5}$	a → b → a → b	$H_1^a H_1^b$	SW103
1046 DE	✓ SW1031	a : b	$H_1^a H_2^b$	Saltzdorf	$H_1^c H_2^{1,5}$	c → b (1)	$H_1^c H_1^b$	SW105
						c → a (2)	$H_1^c H_1^a$	SW105
1051 GH	SW1053a	a : (c)	$H_1^a H_1^c$	S. abony	$H_1^b H_2^{env}$ b : env	env → c → env	$H_1^c (H_1^a) H_2^{env}$	SW105
SW1052	SW1053c	(c) a	"		"	" env → a → env		SW105
496				S. abony	"	5 mod → i? env → 1,2 → env	$(H_1^i) H_1^{1,2} H_2^{env}$	
1046S	SW1049	i : 1,2	$H_1^i H_1^{1,2}$			b → 1,2	$H_1^b H_1^{1,2}$	
K	SW1043B2.2	1,2 : b	$H_1^b H_1^{1,2}$		b : env	b → 1,2	$(H_1^b) H_1^{1,2} H_2^{env}$	
49F						env → env		
1074C	✓ N97	b : 1,2	abony env			env → b → 0 env	$(H_1^{1,2}) H_1^b H_2^{env}$	

SW1074.
10496 2 → i? extant

N97 ... Lovers.

1038B	1007b → × miami ✓	b → 1,5	SW1028
1038D	• 1036G1 = N97b' → × TM ✓	b → 1,2 → b	SW1027
1036D	1009(1,2) → × abmy ✓	1,2 → enx (2)	
	N251,2 → × " ✓	" "	
1074A	N97b → × miami n9 ✓		
B	SW1043 → × lanalindy a enx ✓	b → enx	
1038K	1026i → × miami	i → 1,5	⇒
1046 D	SW1031a → × SW1046 (=?)	a → 1,2 → a (2)	
E	b	b → 1,2 → b (2)	

N251,2 → × 666 — 1,2

→ × typeli — 1,2

→ × miami — 1,2 : 1,5

→ × abmy ✓ — 1,2 : enx

→ × TM? — 1,2 : enx

lashter.

1,2 — × ← abmy — b — and 1,2 : enx

× ← TM — i : —

D...

D. Cf. above x FA50 (N25 1,2:-) (A) → 1,2: enx
x - FA71 (N97 $_{SW1009}$:1,2) (B) 1 → 1,2: enx
2 → 1,2: enx
∴ SW1009 is also $H_1^{1,2...}$! The 1,2 phase should be studied closely, and the reversibility to b confirmed.

B. Receipt.
Stock N97 received. Phase in b serum. Alt. phase grew out in 48 hours.
= SW1009 (1,2) = 1036B1
Stock acc. decanted. Recover SW1007 from unpurified inoculum of 1036B1 back in 123 serum. This grew out fairly promptly.

SW1009 purified, s.c.i in 1,2 serum → (about 3-4 da.) a b phase again. [Is SW1007 original N97 or N97: b:1,2:b ?] 1036B2-6 are added. s.c.i of SW 1007 in b serum. 2-5 grew through in 3 days: all z_{33}
6 grew through in 4 " " .

(Apparent monophasicity might be due to b-1 cross-r ?)

~~Test by E~~

E. FA 12 (i:-) x SW1007 /b serum. 4/11 PM. Restrict each phase. swarm: i: → b (sc!)

F. SW1009 s.c.i. /1,2,3.

1 no g. in 24h. (poorly agglut. - Try after motility)
2 turbidity through 24h → 36F2b
3 "
4 "
5 "

not ← b (not z_{33})

b-
b-
b-
b-

F1 probably just poorly motile to start 48h

G = SW1009 /1,2,3 → b. (from stock SW1009, repetition of expt in B. Test s.c.i in b, cf. B2-6. Use one colony = G1 as stock for further experiments, but 2-6 are separate colonies from the first plating of 1009/1,23. ✓ reversibility.

4/13 G7 = F2b, restricted, s.c.i. /b

(over)

4/14: G1, 2, 3, 4. (must be originals)

The hypothesis that para B java might be
$H_1^{1,2} : H_2^{b}$ had occurred to me
(and PA sw1007, G1, was initiated to test)
just prior to reading the result of t!

---

	b	z_{33}	
sw1007	++	− (or $^{v.delayed}_{+}$)	✓ repeated 4/16.
sw1009b	++	++	

∴ these are distinct.

~~of sw1027~~ sw1009 = 1,2 ++
z_{33} −
b −

---

sways, 10001 1,2 ++
z_{33} −

9/2 b ++
z_{33} −

$G3_0$ b ++ z_{33} ++ $\xrightarrow{b}$ $G3_1$ b − z_{33} ++

G2 " b − z_{33} ++

G4 " b − z_{33} ++

G7 " z_{33} ++ b − (roughish)

4/17 G1 " z_{33} ++ b −

SW1026 b:i ?

1036E

4/12/53

E1. isolated from FA12 ✕ SW1007/b in tube =SW1026
↳ i swarm. After s.c.i., to i serum for second phase.
After 3-4 days yielded further swarm, reacting b !
For further verification, restreak SW1026i and SW1026b and
a) plant these colonies in homologous serum
b) restreak for further purif.

a). 1026i 4/4 colonies i +++ b -
1026b 4/4 b +++ i —.

---

a) 1036EA 1-4 4 i colonies from above / i → all four gave b in 24 hours. don't save
EB 1-4 4 b colonies from above. (EB5-8 = restreak 1026b(1).)
EB5 single colonies, b — (faint ?) from EB-1 (mic.) } #2 in 48, 24h. respectively.

4/4
z33+ b+
EB5 → z33 ++ b—
EB2 → z33 ++ b—
EB1 → z33 ++ b ± save
3,6 " " of 1038

That SW1026 is i:b is confirmed.

From the relative stability of EB series, the b phase seems to be more fixed". (EB5-6).
↳ tube agglutination 1:1000 i, b

	sera i	sera b
i	+++	—
b	—	+++

(over)

(Also check SW674 phase 1 and 2.

1	+++	—
PRE 2	—	+++

12y..! Attempt to detect dissociate.

It was noted that SW1009b (1036G1) reacted strongly with ≠33 as well as b, leading to further tests

	b	i	≠33	1,2
SW1026 (i)	−	+++	+	−
1026 (b) } 36EB5) }	++	−	+	−
1027b	++	−	−	−

SW1007 and other isolates of SW1009b should be rechecked.

	b	≠33	
36F 1b	++	±	
2b	++	+	considerable "quantitative" variation.
3b	++	±	
4b	++	++	
G1	++	±	Strains should be matched for more detailed comparisons.
"1009b (thought to be G1)	++	++	

S. napoli 1037

3/25/53 Stock culture (#187 Edwards) appeared resistant to P22, but ff. one single colony isolate found sensitive (and smoother). (marked unc. After /unc → (w) +.)
Attempt two FA preps (P 122 — FA 70, 70A — from phase unc)
70B from liz.
But these preps. have no action on SW666 / nut. agar.

A) 70A ×967 : occasional tracks.
B) 70A ×666 1? swarm: no tracks no Gal+ (b spont?) May have strong lytic action on nut agar; not apparent on EMB. (normal fp)

These preps have no Gal+ transd. activity for SW900 : presume negligible phage content

---

S. napoli rec'd from AMS (also #187). Plate out 4 petri tubes, and test individual colonies / PL722, ≡ 2/11 showed distinct sensitivity. = 887 A1, A2. Prepare FA + P22, P7.
lysis & killing but no FA or phage!

Homologies of javi b.

4/13/53.

A. FA42 (SW942 = N25b) → mainz /a, 1,5 N.C.

B. FA73 (SW1007 = N97b) → " b: 1,5 SW1028

C. FA74 (103661 = N97b') → " C1: (z33 b-) : 1,5

D. FA74 → TM (1035CD mix tube) /i, 1,2 → SW1027 b: 1,2 : b Galt (not z33)

Note previous experiments: (1000:

A	SW588 (FA25) → 942	4	1,2: —	Numerous swarms!	SW945
C	above FA15 → 942	1	1,2: —	1000 C1 saved.	

Concluded at that time that N25b was homologous with #157 1,2:- As these are macrophagic, and in view of C, the conclusion is unsafe. Retest stability.

3/13. Make new PA preparations. 103131 appears to be resistant to FA10, 22 (or 22?) SW1007 - 1009b, 942 are susceptible both. Beccles and Jusay are susc. only to FA10. (possibility that this decay b:- is equivalent to Kauffmann 248?) ? types of N97 N25

D: hypothesis that SW1009b is $H_1^{1,2} H_2^b$ is contradicted by finding SW1027, which implies the homology of b : TM i. → SW1007 should be repeated., as well as by C. For further analysis, the homologies of the b, i phases of SW1026 will have to be examined in a similar way.

E	SW[redacted]7 ←	FA22		E1 → i : b = SW1030
F	" ←	10	2: both ≥33	
G	SW1009b ←	22		G1 → i : [redacted] b)
H	" ←	10	1:	G2 → i : [redacted] b
J	SW1026 ←	serial (FA40)	1	
K	SW1026 i →	mainz	i: 1,5 2	
L	" b →	"	2: no outgrowth	
M	FA22 → SW942		i: —	

~~a : ecc! SIC Rev from not.~~ a : b : a SW1031

Store labelled J + is ecc. Must

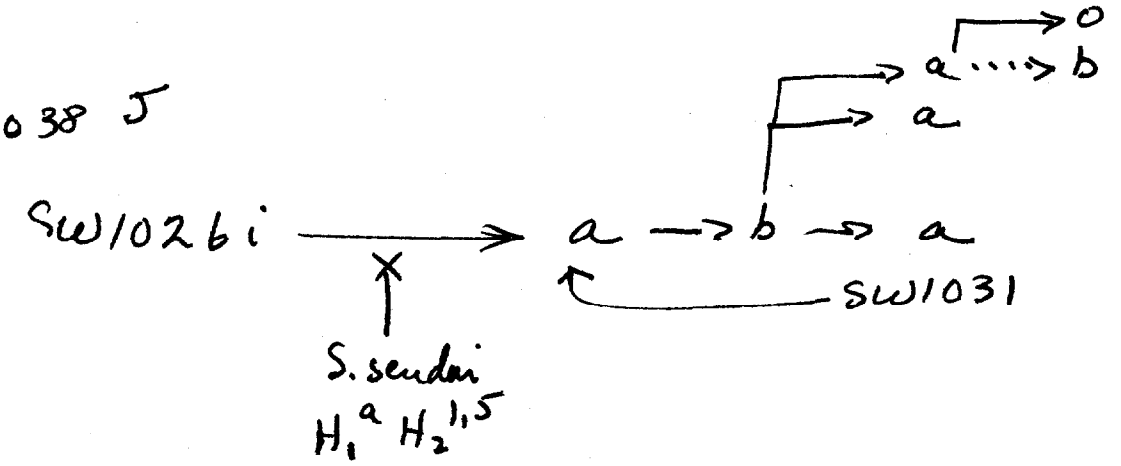
V. slow progression after 9 days, seal off. → still i

assume substitution = 2681 2r save for H1? & type sometimes

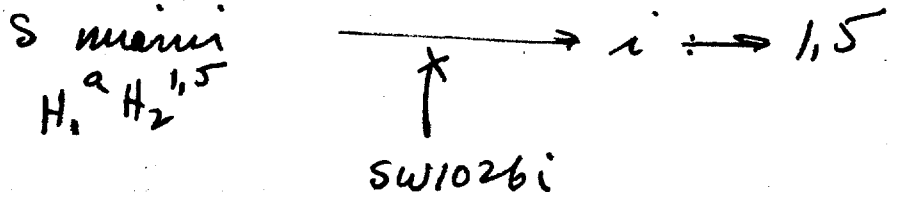
"J1" = ecc assumed contamination. Study as 33 → a. Pure Galt. Pass through ecc

K1 — nonmucoid i's in (i + 1,2) serum.

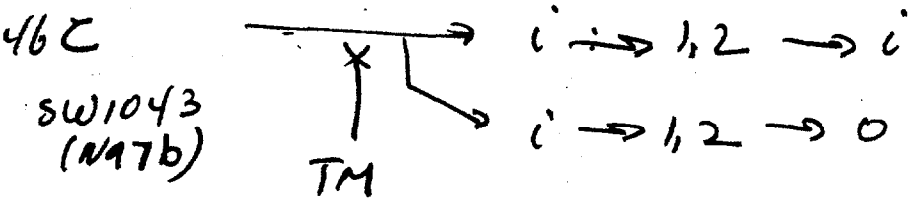
1038 J

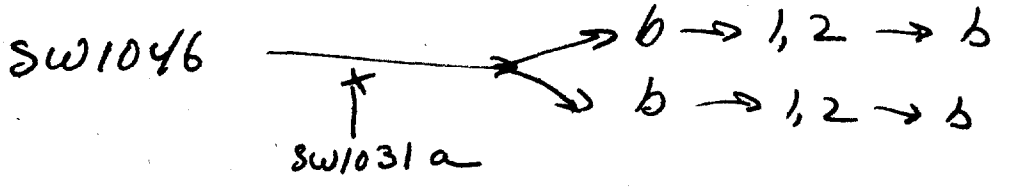


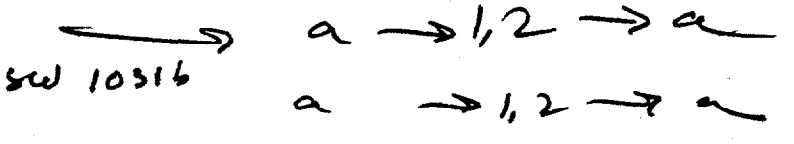
1038K.

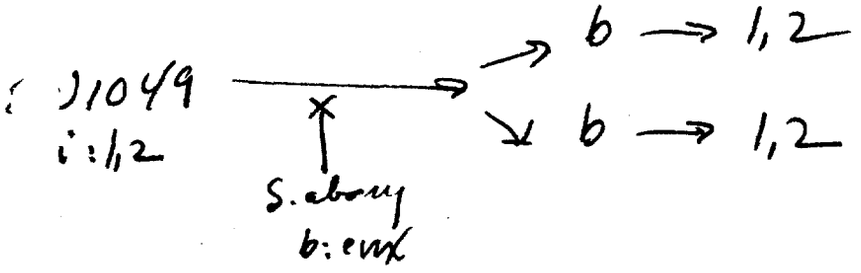


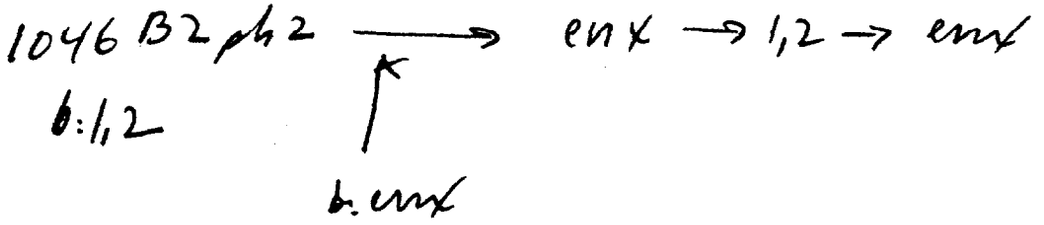
1046C











1049.

SW1031 seems to be reversible a:b:a.

4/29. Retest
1. SW1031 b stock (once pure)
2. Second semis, s.c.i from SW1031b:
21 ~~b~~. → a
22 ~~b~~. → a (after 3 days). Compare 1036F1

1 and 2d promptly gave an a phase again. 22 few light blobs 2 days.
~~Restreak 21, 22 to exclude diffuse in possible stability.~~

Assume that stock SW1031b is uniformly b:a.
Test 38521a in /a for trial reversal test.
5/6. } v. small blobs overnight. → ~~stock~~ b.
5/12 } eventually swarmed → still a.
Also, restreak 521a, then into a (ca 5/9). By 5/12 still no progress. → 5/15 b.

Thus, SW1031 goes a:b:a:b (very sluggish).

B. N97b → S. mainz → SW1028 b:1,5 ∴ b initially $is H_1^b$

i. ditto N97b' but gave z_{33} 1x.
→ S. TM → SW1027 b:1,2 " " .

M. TM → N~~e~~25 → i:— (so far). N25 possibly ≠ N97.

E-H TM → N97b, N97b' → i:b:— e.g. SW1030

J a:1,5 → SW1026 (i:b:) → SW1031 a:b:a ✓ (i, b being checked.)

K1

K 1026i → mainz → (i:1,5):— worked ½ ∴ i of $H_1^{b:i}$

L 1026b " n.g. indecisive. (cf. 1044)

~~need to repeat L.; prepare FA from~~ 77 ~~SW1030 a; b phages for homology tests.~~

From previous results, 1,2 (N97.1,2) → mainz → SW1020 was stable in 1,2,3 serum. (did not carry over likelihood of → $b_{z_{33}}$).

and i (SW1026) → mainz (K1) was stable in i,1,2 3.

Of 1031 is reversible, prepare FA's.

L
1026b →
FA77 i, b →

abony → × TM phases.

3/14/53. Reisolate ~~TM1, TM2~~, TM2^1 and 2, ; SW950^{1,2}. cf. 1035

Inoc for TM^{1,2} and 950^1 from slants ; 950^2 from 1035 broth.
Also Reisolate a 950^2 from plating of prev. 950^1 (1/15 colonies mated i, 1,2,3.)
= 1039 d2

1039-( )-1 : TM2^1 a 1
TM2^2 b 1 → 5 colonies each i − 1,2 +++
950^1 c 1
950^2 d 1, d 2. → " " i ++ 1,2 +++

Restreak motilized (a-d)1 and d2 to imitate fresh inocula for transduction

P16 exp't: All motilized cultures react 1,2.. +++ , 1,5 +++. 2: +++ c and d also react i ++ (delayed). This seems to bear out previous observation that SW950 is either phase-variable or phase-mixed. Restreak c1, d1 SW950 may thus be unsuitable for phase var. study.

1039 d2 → i 1,2 colony → 20 colonies all i: 1,2 ++ sal −

[Inoc i, 1,2 SS. to attempt phase separation = 39 d 2.] over.

abcd 3 = s.c.i. from 1035 ABCD. To 5 ml broth : shake agglutinations:

	a	b	c	d
i	+++	−	+++	++
1,2	−	+++	−	+++
		Gal+		

Try b+c.

(of SW986 which is SW950+ and i: enx +)

P17. A FA14 → × b+c (FA 2:1, large types)
B FA15 → × b+c (FA : 2 small types)

A. 38 swarms: all Gal−. all b ++ # 1, 3, 21, 22 enx ± ?

B. 25. 17 b ~~all Gal−~~ ~~(#1 ? b+ enx ++)~~ Gal+
~~7 enx all Gal+~~ ~~#3, 5, 16 Gal− ± (mixed mostly − + +)~~

All 4/B

Of these, #8-16 were "least crowded swarms",
include 6 b: 3 enx. (not noticeably different)

AB$_0$ (mix) Gal− broth : all i ++ 1,2 − (I was 1,2 ± rough?)
Gal+ " : all i − 1,2 ++ .

1039 d 2 /i → 1,2,3+++ i++ (delayed) (i.e. same as original. selection unlikely to give pure 1,2,3 phase.

1039 d 2 /1,2,3 → i+++ 1,2,3 —.

SW 414: s.c. from stock. 5/33 tested with 1,2 were ++.

These < 2 i - 1,2 ++ ; - . . . ++. (from the s.c.)

	1st test colony	sol →	broth	Removed to broth	NSA plate
1	i — 1,2 ++	—	all i++, 1,2++		
2	i — 1,2 ++	—	1,2++		
3	i + 1,2++	—	1,2++	ditto	all i++ 1,2 ++ or +
4	i ++ 1,2++	—	1,2 —		
5	i ++ 1,2++	—	1,2 —		

In general, i reaction stronger from broth, 1,2 from agar.

1039 e. Repeated: 4/15 1,2++ i++. < NSA i++ 1,2+ ; broth i++ 1,2 —
from SW 414 stock.

TM 2: 5/5 s.c. broth → i++ 1,2 —.

4/21/53

see 1039.A -B.

B 1-8 are Cal+ enx. Brothers also wait i :
9-25 are b Cal-.

pitch out A: 1-8. S.C. wait enx++ i- from agar.

		Cal	exc. #6a: b, i, enx, 1, 2 - pv +.	66: enx ±
1	1	+		
2	9	+	4a: i++ enx-	
3	11	+	b "	
4	15	+	c "	
5	20	+	d "	
6	(25)	+	brother: (= HLB #9) i+++ pnx++	
7	21	-		
8	21	+		b.

S.C. brother 1-5, 8 are all enx++ i -
#6 is b. Original brother (HLB 39-25) is enx+++ b+ i+
#7 is enx+++ i++. pitch out on EMB Cal.
s.c.
Original brothers all stated to wait somewhat i i.
Note: most unstable or mixed enx: i appears to involve → socio-50 (bl-) & previous enx +.
Purify original brothers:
#6 = Cal+/- ca equal ratio. Test Cal-,+ <-: b+++ 1,2 - + : enx+++ i - } save for stability checks
#7 = pure Cal- 5/5 wait strongly i enx from EMB Cal. #3,4 also i i.
4/25 Broc #1, 3 to broth. but as #1, 2
4/4 s.c. from #7 s.c. above, NSA, behave similarly (i- enx+++).
Broc 2 to broth as 3, 4. → all 4 brothers wait enx+++ i ++).
4/26 suspicions from NSA magglut.
Cannot verify here whether i reaction is due to simple vacational instability.
Save as 1041-7

S. abortus equi : further strains 1042

4/25/53.

SW1033-5 rec'd from Edwards

see 1052

As rec'd:
(by slide agg.)

		a	enx
A	1033	—	++
B	1034	+++	+ delay-of.
C	1035	+++	± " .

Restreak for further experiments. Abortus cultures as rec'd in homologous serum = A1, B1, C1. — no motility in 3 days in enx, 9, a resp.

Sp motility not tested.

5/3/53. Received ETS26 and ~~41-D-1~~ from Army. Label single colony cultures as SW726A and SW1042 respectively.
Rec 726A in motility agar: essentially immotile 24 hours incubate.
SW1042 grows slowly, rather rough on plates.
726A appears smoother than 726 (Edwards). Agglutinates strongly in enx, ca 30-40% of cells in broth culture V. active. But spreading is delayed.

5/6 Rec 1033–1035 in motility also.

ca 5/2. SW1033 (S.C. but not motile) — 2 beg. small buds

3 tubes each. ↙ FA22 5/6 1 budding
↘ FA18 " 2 budding + 1 beginning

5/8 5/10
— 2/3.

42A 1 SW1033

2 — x— 22 1 tube
3 — x— 18 3 tubes } all 4 are a/+++ (rough) (smt + c enx). Test in a S.S.
1 2 a :

A2 — 1 48h. A2 a
A3 (1-2-3)/a + few bbls. → A3 {1 — 5/26/53 2 smt 3 enx → } — 5/26/53
A1 —

A2 → a: 5/16/53 5/18
u: —
a: enx: a
a: enp:
a: — enx 5/18

see 1052 ✓

1035 - 1036 - 1041

4/29/53

		Cal+	Cal−	Cal+	Cal−	Total
①	b: enx → i: 1,2	3	43	0	0	all b
②	b: enx → i: 1,2	39	23	9	3	12/74 = 16% enx
③	b: enx → i: 1,2	5		0		all b
④	b: enx → i: 1,2	0	0	21	4	all enx

① / 3 and 3/4 show predominant role of FA, or

$b^+ > 1,2^-$ ① $b^+ > 1,2^+$ ③ $enx^+ > i^-$ ④ $\underline{enx^+ \leq i^+}$ ② in TM

note: i: 12 → b: enx grouped, role of FA also,

i^+: 1,2 → b: enx → mostly i^+ $i^+ > enx^+$ ⑤.

i: $1,2^+$ → b: enx → mostly $1,2^+$ $1,2^+ > b^+$?

contradicts ③

unless b: enx are mostly $enx^+ b^-$

of ⑤ $i^+ > enx^+$ and ② $enx^+ \leq i^+$ discrepancy?

Malt pullorum; S. gallinarum ↓ 1043

4/25/53.

Rec'd SW1032 from Edwards as 2479-50. Restreak for further tests.
Test culture as rec'd for motility, Mal fermentation, PLT22[s]

Restreakout of both culture to EMB ~~Lac~~ Mal, two colony types were noted: typ. Mal- and small Mal+.
These reacted similarly in fermentation tubes. Malt, however, was a coccus. At tubes, 24 hours: Mal- —
Mal+ A
Malt,- AG.

Restreak original stab culture. Rare + papillae noted. Repicks and reisolated to EMB lac, Mal.
FH72 → 1032 and 1032 → SW666 gave non-motile.

see 1029

5/2/53. 48h.-72h.

A Gallinarum' = strain 74. → SW1040/a
1 ++ (gas)+ = SW1041
2 ++ " = 1043A2
3 —

B Pullorum' → SW1040/a
1 —
2 —
3 —

note: 74 did not grow on D(B,) agar. Typical gallinarum?

prepare PA from other gallinarum, pullorum.

C. Gallinarum 1-10 and Pullorum 2-9 → SW666 +
D. → SW1040 /a.

After 60 hours: C all —
D: G 2, 3, 4, 5, 7, 8, 10 are + to ++. P 2-9 all —. (repeat G1, 6, 9)
P2 + after 2 days.

G1 (gas) secondary
G2 (gas)
G3 (gas)
G4 (gas)
G5 (gas) a
as sent

G6
G7 (gas)
G8 (gas)
G9
G10 (gas)

Prepare G6, G9 negative? streak out for S.

G5 a otherwise gm.

↓

cultures were typed shortly from swarms, then streaked out and (hastily) single colonies picked off & rechecked. G5 is a as printed out by PRE

Repeat 6/2/53 ✓ → G5-2 (gp) +.

Entiol SW1040 a/a → no swarm 6/3/53
T.O.

Transductors: TM, miami, abony. 10/4

FA → B

A 1 S. miami → S. abony — a → b; a → enx; 15 → b; 15 . → enx
2
3
4 — b: enx

No transductors T.O. FA (27/miami)
input i FA 12/miami
5/3/53: still no swarms on b-enx.

B 1 S. miami a → TM i+1,2
2 " 1,5 — 46 " i,2

C. 1 S. abony → S. miami — b → a; enx → a; b → 15; enx → 15 .
2
a,15

2/2 : enx.
12/12 : enx
1/1 : enx
2/2 : enx + 1 enx + 1 ?

D. 1 TM2 → S. miami mixed. — i (22), 1,2 (18) } a+1,5
2 — a,5 — swarm

7/7
1 i
1 ? not still ? pr±.

Also note v. small ~~bb~~ blebs in C1 ; v. numerous tracks in C2, C1, mostly subsurface.

#1,2,4 still 1,5
#3,5 possibly b (for aggl.) – struck out
Tracks and blebs became very definite in C1
Two delayed swarms in C4 : 10 tested, all

A2, A4 showed very dense surface blebs. A1, A3 were smaller, less distinct
Surface growth in C was rather sharply restricted. Moderate spread on H, D, most in B.

Repeat C1: on a 1,5 agar 1 swarm → enx.
a 5
save 1-8 several days: → 22/12 : b = C5 – #1 = SW1038.
all b: ⑤

Conclusions: 1,5 serum may inhibit b (colonies 1,5 – of 12 6...)
FA miami n.g? – Remake. (of 970).
Not SW1038 in a:1,5
inhibited for several days, finally grew out → still b.

(over)

E1 isolated from JL. 5/8/53. History from ca. 4/29
ca 95% of culture

E2 5/9/53 ca 30%. symptoms truncated.

typed: IX XII a:1,5
sensitive to FA10
~~it~~ not to 22
SW1004: $22^{s\pm}$.
both 44E1 and SW1004 ~~by~~ ~~isoquin~~ for SW~~1006~~

E1. 5/8/53. Histoplasmin 4/29 ± 1. Symptoms most acute 4/29-30. almost negligible thereafter. ca 95% –

E2 5/9/53. ca 30% –

5/10/53: no PM sample obtainable.

5/11. E3. Throat sample dirty in motility; in EMB; on S-S.

EMB ca 10% –. → a S-S – blank. (few colonies from transplantal of purified E2).

Subculture from motility of E3 (E3M): → pure Lac –

5/11 PM – mild headache + malaise (typ Sunday?)

5/12. V. mild d. trunk. AM ✗/✗/✗ → no Lac – on EMB direct plate

His's method may be advantageous. (Test motility selection: → agglutinates in a serum. Test combining ethaethionate or butyl galactoside. situation: pure lac –

5/13 AM v. mild symptoms continue / plate on EMB, Motility; S-S.

5/14 AM E5 no sympt.

C = E. coli types

pure lac + → pure lac –

lenticular growth pure Lac – → a

not typ. salm → E5a

5/15 AM E6 → SS – V. paracolonies, incl. 1 Blade → blade colony a → lactose –

→ EMB – ca 1% lac – → a

→ motility – fair progression → a

→ other → Lac – E6a

see EMB lac. pure Lac –

5/16. N E7: EMB: pure lact+

Motility swarmed → a

→ E5a } Lac – non-motile inagglut. not assoc. to Ø3 Salm!
E6a }

5/19 E8 – EMB pure Lac + Mal + Motility

SS – No swarm ~~out~~ to 5/27.

↓

2 colonies only a ++

(over)

E9. 5/25/53 1 lact+ in SS → not a → lact+

E10 5/27/53 pure lact+ on EMB.
SS – 3 colonies not a → in EMB lac
pure lact+.

no further Salmonella?

E5a: eventually motile.

no marked papillae on EMB lac (10 days)
but repute → . no +.

E11 6/7/53. (Mild?? sepsis)

EMB lac – pure + two types: Is hum?

SS → pure lact+/EMB lac

MOT – irregular swarm →

5/13.

N25b (several)
in b sem..n } z33 [#157 = —:1,2]

N97b → b → SW1007 b
b → 1,2 : z33 (fragment)
b → SW1009 (1,2 : b)

numerous
1046131
1,2 : — and
1046132
1,2 : b : —

not clear whether diffuse is already inherent in N97b or whether both kinds are $H^{1,2}_1$.

SW1009b (b z33)
(b) : —

1036F

1038G1
i : b several

several (SW1026)
i : b → z33
a → SW1031
a : b : a :

× TM i : — i : 1,2

→ × i : 1,2 or a : 1,5

b : 1,2 (1038B)

b : 1,5 (1038B)

1,2 → × b: error other testers:
#157 : $H^{1,2}_1$

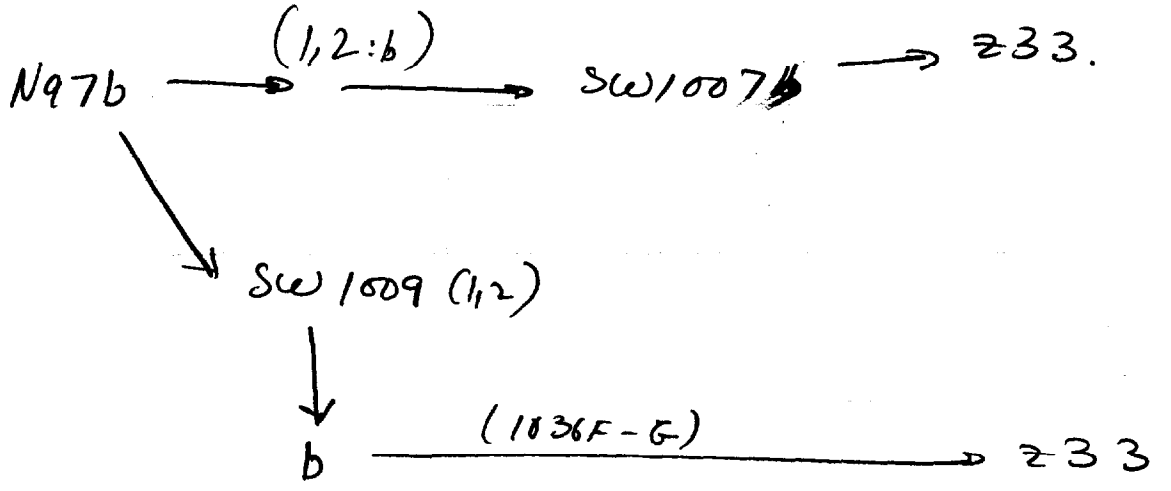
SW1009 $H^{1,2}_1$

S 031 → × TM → b : 1,2
1026 i → × mimic → i : 1,5
Type ?

∴ SW1007b has behaved just like SW1009b (possibly excepting z33 ×)

5/13

1036:



b 1,2 b.

1046 N97 → A1 1,2 : —

- B1 (SW1043) → 1,2 : — (FA86.a)
- B2 → 1,2 → b : —
- B3 → 1,2 : —
- ~~B4~~
- B5 → 1,2
- B6 → "
- ~~B7~~
- B8 → "

single clamis.

∴ N97 b consistently → 1,2. Some of these → b : —
others are 1,2 : —

86a → × ~~102~~ 1022 or above | FA (1046B2,2)

para A transduction

1045

ca 5/1/53.

A SW928hw → x 1033 G2 numerous T+S. see 1033, 1008 (over)
B SW944hw → x 1033 G2

22 → x 1033 G2 s.c. 1, 2, 3 and original) gave almost nothing: rare trailers...
→ x 948

Question of better transduction of G2 is left open. Compare A on s.c., and on SW 948.

3 days.

C. SW944 hw → x S. paratyphi A SW701
D. " " " 702 / a
C. " " " 694 (diauxic XII 2)
E. → x " SW948 a+ (1045B1 improv.) } no swarm

Repeat 22, 944hw → x ...

	overnight
22 → x 948	—
G2-1	—
944 → x 948	1-2 swarmers
G2	5-6
G2-1	5-6
-2	15-20 num. trailers
-3	4-5.

Save G2-2 as SW1048

	24h.	48h	5 da.	
F. SW944 → x 45B1 (pur.)	—	—	—	1045B1 should be transducible!
G. FA22 → x 45B1 and control	—	—	—	Check other similar isolates; might
45B1 /a	—	—	—	be rough.
				5/26 — T.O.

H...

	→ x 948	→ x 1048	
SW944 hw. 1 swarm.	20-30 sw, numerous trailers		2 a
SW967(60) 1 sw	2 swarm trailers.		1 gal = 1045 H1

SW1048 seems definitely more transducible by SW944 than SW948.
But efficiency is PLT22/2 still very low.
F1a⁻ (SW1048) not allelic to SW866 or SW967!

J. SW944 → x SW694 /a after 2-3 days. + b:
K. "
L. "

1

over

A.

18 swarms all a

B.

36 29a 7b

pctr. significantly different.

save a b as 1045T31

us sw / to 5/27.

On EMB Xgl:

	papillae
SW701 grew poorly	+
702-694 moderately	?, ?
1048 fairly well. (& a few self. lysed or rough colonies)	−

use Xgl as transduction marker? cf. SW 702 - 694 - 948 - 1048.

N97 continued 1046

5/3/53. Fresh culture of N97 (b) received from Edwards. = SW1043.

A. Broc in b serum as is. → after 24-36 hours a 1,2 phase (of 1036B). Save as 1046A1. overnight.

B Streak out. Broc single colonies in b serum. 1-8.

All but #6 spread in 24 hours → #1, 2, 3, 5, 8 all b— 12+++

~~most b in next gen.~~ (#4, 7 n.g. acc. killed)

in 48 hours → 1, 2.

Save 1046B1 org. as SW1043 (b) and streak out #1 as SW1043 (1, 2)

SW1043 appears to differ from SW1007 in recurrently → 1, 2. 5/17.

Why is N97 classified as b: — ?

B1.12 / B2 " / B3 " / 12 → — (two trials mid-way) → z33

→ b: — → z33

→ — (1 trial eventually still 1, 2

Prepare FA from each phase.

C. FA22 → SW1043 /b, 12

	24h		24h.
1.	++	→ i:	+ 1,2 SW1049 = 1,2 ph. ∴ i: 1,2: a
2.	++	→ i:	± ~~+~~ 3 days → 1,2. a: 1,2: — org.
0 (control)	—	→	5/16. z33.

B. SW1043.2 in 12 only small blebs. Repeat i: motility of SW1043. SW1043"3

46A1.2 " " " "

D SW1031 b (kw) → 1046 { b: 1,2: b

E " a " → 1046 { b: 1,2: b

24h +:

a: a: 1,2 a

a: 1,2 a

5/11/53 Repeat (3) single colonies of B1 and B2 (original b) a in b agar. F=B1 G=B2

overnight: all — F1 P,M 1,2 → 1,2 G1 5/14 1,2 → 1 mixed → 1, ...

24-36 hours ± to ++ F2 P,M 1,2 → — G2 —

F3 5/14 1,2 → 1,2, + 33? G3 P,M 1,2 → b z 33 5/26

2 days

pr homology test. J ebony (1#) → SW1049 /i 1.2 J1 b: 1,2 all mot in 1,2 N15. exc. B2

K. " " → 1047 B2.2 /b. 12 J2 b: 1,2

b → K1 enx → 1,2 (2 days)

! 1,2: enx

χ phage tests (for diphtheria Fe⁻ testers) 1047

5/12/53

		χ 942D	χ SW 942	22	10
PB	703	−	±	+	++
PB	704	−	±	+	++
TM	714	−	±	++	++
Stanley!	715	++	++	−	−
Heidelberg	716	−	−	±	±
Albany	803	−	−		
TM	1046	−	−		
Loma Linda	874	±	±	+	+
	Miami	−	±	−	+
TM.	LT-1	++	++	++	++
typhi	H901	+	+	−	−
PA	701	−	−	±	±
PA	702	−	−	−	−

	χ
modified 703	+
704	+
SW 422	−

χ may be possible to adapt χ to 703-704.

LT-1 seems most generally satisfactory of these. Possibility of adapting 703-704? ! Check motility: pass through motility.

5/12. Centrifuge & mutant hunt using LT-1.

① Inoculate motility agar for optimum sensitivity.
② streak out on NSA for single colonies.
③ streak out tests above (microscopically non-motile). → 1/5 non-motile = 1047A. 4/5 micr motile; 5/5 " "
④ Inoculate available auxotroph mutants of LT-1.

Note: 2 "LT-1" strains: of #84 and #306 TM1 = LT-1 (84).

	χ
LT-1 84	+
" 306	−
SW 202	+
SW 411	−

SW 411, 422 may be presumed #306 derivs.

However SW 202 stock, grown on D(d) agar. Reckons single colonies! ↳ no phototrophs.

B. Incor 20 s.c. TM1 motile in broth tubes, add diluted χ. Incubate. Streak out. Pick 1 colony each to broth. (see over)

C. TM for bal - 10 plates 9-10 secs. only 20-30 secs/plate. Repeat 8 sec.

(over)

Prepare fresh X: add X/sw592 to TM1 in 100 ml broth

Incubate overnight. Filter (sheat). Test samples for sensitivity to chloroform, heat.. Save (60° 20m.)
aliquot in freeze also.

B. 4/20 were presumptively motile by mucin test.
" " Restreak and test
May have had rare spinners.
directly on motility agar. #1 did not swarm out immediately
#2-4 did (not homogeneously).
ignore these unstable Fla⁻ for the present
all other isolates here examined also swarmed out
(sw703-4/X; 702/X; TM1/X).
T.O.

B1: Retest single colony → swarmed!

D: Plate TM1 various dilutions in
".1 ml X": At 10^{-6} ca 100/plate 20 examined
~~only~~ single colonies in broth. 17 + ~~no~~ 3 occ: spinner
no Fla⁻ !

modifications of motility agar.

5/17/53 ±.

① Summarize HLB expts.

② Add $NaNO_3$ (.1, .2, .4 ml. 25% soln. per tube)

TM-1 not. sl. inhibition of spreading but growth considerably denser at each level.

Consider incorporation of .5% $NaNO_3$ in basic medium (replace NaCl by $NaNO_3$).

③ Add Methylene blue: (.1 ml of .1% per tube): distinct demarcation of bacterial spread, but substantial inhibition.

gas bubbles noted in MB and — tubes above; absent in presence of $NaNO_3$.

MB + NO_3 inhibit as above; decoloration very slight.

5/17 ④ Tetrazolium ± $NaNO_3$ (1)

⑤ glucose to .1% ± $NaNO_3$ (1).

	—	TGN	TG	TN	NG	T	N	G
not.	+++	not colored ++ dense uncolored	+++ dense color throughout	++ dense not colored	± very dense yellow color	++ colored	++± dense growth	++ explode dense

5/19/53.

A SW1031 a × — FA3 (c) 2 tubes 1 → overnight: small blebs only. SW1052 c:b:

B " b × — FA3 (c) 3 tubes. ½ → overnight ++ SW1053 c:a: c:a:

C. " a × — FA59 ($l_{2,3,8}$ to simulate wien). no swarmers 48 hours.

note #1 and #3 running 1% $NaNO_3$, and these did not swarm! Del 1040.
nitrate did not reversibly inhibit motility.

D TM i (22) → S. wien (wien ++ b+) / b best ~~4 tubes~~ no cur

E " → S. [illegible] / [illegible] ++ env − 3 tubes no cur

F " → S. [illegible] / env d ± env ++ ½ → d; 0 → d;

G (= 1046 5...) abnormal (15c) → SW1049 i: 1,2 / i, b, 1,2

H SW 1026 i: b × FA59 (cf. C) still i.

J SW 1026 i: b × FA60 ½ →

K " i: b × FA60. 1. [illegible]

(d). (c)

		/env	1,2/1,2	1,2/1,2 env	env/1,2 env
C →	1	env : 1,2	: env	not env	
	2	" :	1,2 : env	magg ⇒ mag.	
	3	" : 1,2	: env	i ? → i ++ b+ ?	
	4	" : 1,2	: env	no sw. 4/5 f.0.	
	5	" :	1,2 : env	i ? (or rough ?) → mag.	

Isolation 2 5 delayed.

Note: SW 926 and SW 938 (1,2: env) each in both phases / 1,2, env not agg. in 10 days, neither swarmed.

6/3. Rechecks 49G1 d after not + s.c. i.
6/4 " G5 d " (over).

Retest possible i phases of 1049 G: 1, 3, 5...

G 3d is only culture to show definite i-reaction after motilysation. Rechecks after s.c.i.

homologies of diphasic pair B

5/22/53

pair B type	#		FA 10	22	X
A	1	B76	+	−	
B	2	B300	±	−	
C	3a	B62	++	++	
D	~~3a~~ 3b	B97	++	++	
E	BAOR	B2227	++	±	
F	3a I	B624	−	−	
G	Dundee	B3590	++	++	
H	Taunton	B2253	++	++	

all showed X; some ++; most distinct ++ → nonmotile broth strain of 1

( ) Jersey 2 B4182 Zeules = B17472

Isolate 1,2 phase; pure FA for → a:en x → motile broth.

Strain of 1 50A for origins of fla⁻.

50A1. From initial 50A/X test 18 colonies on semi-solid.

1 (initially) non-motile. Restreak + subculture.

↳ swarmed later. Microsc: ~~small~~ ca. 1% motile cells occ. spheroids (L?)

and 1 swimming pair of cells followed in zygotaic 5-10 ms. (cf Hfr x F⁻ coli).

6/3 Ntreals, FA from VV XII types for later study

		FA 10	22
PB#	7	S	~~S~~ R
PB	8	S	R
TM	11	S	S
TM	12	S	S
TM		S	S
TM	159	S	S

1 Nov. 6/have tested X also.

6/6 50H. 10 s.c.i inoculated to 1 ml Penassay + X. after 24 hrs, 2d transfer.

9/10: mostly nonmotile (culture also lysed). 9/10 actively motile.

= 50H1. strain of → not

1050A 10 s.c. in broth + X. Bottles immobile
streak out, test 2 s.c. from each on next agar:
all Fla+!

Need to redraw higher incidence of Fla−?

6/18 Repeat
50A-14 × SW1022 / a: ery

6/1/53.

A ⇒ after recalibration, b.
C ⇒ b
D =
E → b
G =
H =

T.O.

J FA 50 (SW 546) =
K. FA 24 (PB#3 ph 2) —

→ x above / b: extra

most trials, no serum!! A, C, E magnet! Repeat. ↺

para B: 1,2 homologies

6/19/53.

→ SW 1022/9, cmx

A
B
C
D
E
&
H

K
L

FA 24
SD
71
SW 707
D SW 708

b : cmx : b ←

b : cmx
b : cmx
b : cmx
b : cmx
b : cmx

need 9, b, cmx to suppress b.

Save cmx phases (after S.C.i OK)

as 1050 A... N
and b of 1050A as SW 1059

others T.O. 6/26/53

Each preparation thereafter has same FA, but only b phase came through (any reason??). In previous experiments, FA 50 and FA 71 ~~42~~! Present sera may be questionable.

These results do not answer previous questions of homologies of the 1,2 phases. Should be repeated ~~me~~ by me.

Remedy above-2.

7/5/53. Repeat ~~1~~ above A1, C1, L1... (3 each)

b : cmx

A-1-3 L3- gave through (still b); C1-3 no reign to 7/8.

L1-2 : → 1,2 (7/11) melt off limiting covariance

L1 : b 12 cmx —1,2→ b 12' cmx 2/3 :
— ++ — — — ++

L2: + ++ − (+ delayed) − ++ S.C. 4/show weak, delayed b+.

S.C. show same

Not 1,2+cmx reimm.
no swan from L2 or L2'.

Linkage of $H_1 - H_1 - F/a, \ldots$

5/22/53.

A SW666 × SW1049 i / plates — no swarms. 24h. 5/27 6/9

B " " " / b tubes → $\frac{1}{2}$ i: — — — T.A

C " " 1,2 plates : $\frac{1}{2}$ i: — — —

3. — b: 16 (2 l-x c i in 1, 2)
10 ++

D " " " / b tubes $\frac{1}{2}$. — 1,2: 3 ++
4 ++, all x i, b.

E SW967 × ~~SW1049 i~~ / gm tubes / SW 1031 a:b 1. —
2. —
s/gm 3 (gm) v.wk (gm ?)

F " × ~~1,2~~ / gm tubes SW 1031 a:b 1 — 1.0.
2 — / enx

G SW1053 a:c × 15c (abnorm enx) / abc → enx: 1 enx :
2 "
3 "

H " c:a × 15c " " H 1 "
2 "
3 "

HO → [illegible] 5/26 6/5

K SW1053 a → × 666 / b 1 (48h) a:
2 a:

L " a → × 967 plates 1 enx, gm: → gm / gm 1. —
2. —

M " c → × 666 / b 1. c —
2. — c: → b?

N " c → × 967 plates 1 enx, gm: → gm / gm 1. — Rev cannot.

Note: in above experiments, SW967 recombinants (and transcendentals) were very low. SW666 × yields were a few swarms / plate (.1 ml)

G.-H.

		isolated a	b 5/24	6/3	c	d
		enx/enx s.z.	enx/a enx		a/a	a/enx.a
G	1	enx : a	wk. enx	48h →	· enx	· c? ? rev not.
	2	enx : a	magg	a.	· enx	· magg.
	3	enx : a	magg (not)	48h → magg.		
			enx/c enx		c/c	c/c enx
H	1	enx : c	— enx		· enx	· C (wk.)
	2	enx : c	— magg		· enx	· enx
	3	enx : c	— ~~magg~~ → magg.		· enx	· ~~magg~~ ~~no~~ no swarms 6/5

save some recombinable derivatives.

No evidence of a:c (:enx) variation.

Perhaps 1053 insufficiently viable to start. Review $H_1 : H_1$ available

Rev old after not; s.c.i.

(over)

Q SW1054 (hexoffate) ant → TM2 1. ph1 still i
2. ph2 ant :

R " 1055 " " → 1. ph1 still i
2. ph2 c: 1,2

SW105

After re-metabolization (second passage)

Q2 still ant+++, immobile in ant screen.

However, reacts slowly + lightly i → i, not b, in 1,2

Compare SW986. (Edwards calls this i:ant)

1041-7

1041-7 (ebony → TM ant:i) moved overnight through ant

Q,2 - immobile !! somatic?

1051C		1051M		
1	21	1	12	24
2	23	2	13	25
6	26	3	15	26
9	30	4	16	27
11	32	6	17	28
12	1051K	7	19	
16	1	8	20	
18	2	9	21	
19	3	10	22	
20	4	11	23	
	5			

1051
QR

6/18/53.

Q1 SW1054 enx → SW1046 /i: 1, 2 — 3 tubes.
R 1055 " " " "

Q3. (4,5 no swarm) → staff i+1, 2.
R3 (" ") → enx or rough. S.O. fizz smoothest colony and nitest
T.O. others (no swarm) 6/26.

Q2 repeated had not swarmed in > 1 week. T.O.

enx +++ i ++ a - c -. Remotely and pass through enx, i ...

single colonies i; enx resp. Check on both from these.

Thus SW1055 is (at least) $H_1^c H_2^{enx}$ and SW1054 is confirmed tentatively as $(H_1^a) H_2^{enx}$

Recheck: R3 s.c: (by slide aggl.) ∴ confusion of phases may again be involved. (cf SW 986)

		i	enx	b	-
R3	1	++	—		
	2	++	+++		
Q2	not	++	+++		
	stale	++	+++		

R3 o moved rapidly through i, largely blocked in enx. T.O. in view of evidence of mixture. enx smears may contain secondary agglutinins: wait for shipment of minnesota strain

cf SW1061!

S. abortus-equi stains 1052

5/30/53

1042

Received from Edwards + Moran: ca 24 hours swarming. + = 1cm and density

	A	WH_2 vacc. 147	++	+	
	B	Penn 818 ← SW1058	+		
PRE	C	" 827	±		
	D	" 830	±		
	E	1967	++		
	F	1255			
A.M.	G	715B	+	+	
	H	Pellico 7-404	+	+	
	I	Thomas	±		
	J	McLaren	+		
	K	M66	++		exp: b ev. not ab-equi
	L	M1	+	+	
	M	1966	++	→	" " "
	N	Coe	−		
	O	Schofield	−		
	P	703B	±		
	Q	C	+		
	R	D2	±		
	S	Penn 2	+		
also save	T	WH1 Rough			
	U	Platt "			
	V	PM764R			
	W	D7 Rough			
	X	D "			
	Y	Bonetmin556 "			

∴ choose among A, E, K, M. Melt off and reinoculate; streak out.

	By record run: Motility	"Smoothness" Edwards NA	14 cr Mot	Aggl. (exp by a)
B	+	±	+	++ ✓
E	++	±		++
K	++	+		++ ✓ diss?
M	++	++	++	++
A.M. N	±	++	+++	++ ↓
N	− n plates	} microscopically almost immotile (occasional spinner)	sic.	− b ✓
O	− "			−

726 Use M particularly. s.c. after mot = SW 1056

22 → O dense slow swarms; edge of : immobile
(no trails seen initially)
↳ still slow after 24-36 hours.

N and O are essentially O-forms / but why don't occ. motile cells swarm? Temp?

Fixation

(over)

PA22 × O 20 swarms: all enx

No swarms on controls (2 plates, 48 hours)

Purify 4 for dysbacteria test. enx → absolutely immobile to 6/9/53.

N gave one. Late. very rough, slow swarms spent

+ PA22 → 20.

M taken as SW1056 for later study.

However, in enx serum → b! Repeat with single colony reisolates! (and purify somatic antigen ∴ PRE)

In recheck of Edwards' cultures, B = Penn 818 served best. After mutilization, use as SW1058

SW1056b in b serum 1. → —, later rough
2 → ≠33.

∴ enx → b: —

Resume. 6/10/53.

IVa. (N97)

1. 1046 | various single colony isolates for consistency in extent of variation : of B1–B38.
But 3) s.c.i from B1, B2 (which had gone b:1,2:— and b:1,2;b:— respectively) → 1,2 all :— (1?6?33).

46 FG. Some misfluctuable phases. B2-1 is only example of b:1,2:b in this series.
Of 1046B1 = SW1043 and SW1007 ; former is b:1,2:(—)
latter is b:—
Suggests possibility of "extinction" of variability.

46 C : FA 22 → × SW1043 → 2 : i :1,2: i / i :1,2:— SW1049

DE SW1031 b → × TH 1046 → 2 cases { b:1,2:b (not tested further) / b:1,2:b } D

a → × " { a:1,2:a / a:1,2:a } E

∴ SW1031 is interpreted as $H_1^a H_1^b$

JK. Erythrosin or homology test
J. abony^2 → × SW1049 $(H_1^i H_2^- H_1^{1,2})$ < 2 b:1,2 not tested
1046
K " → × SW 1043 B2.2 $(H_1^b H_1^{1,2})$ → K1 enx:1,2:enx / :1,2
not tested in 1,2:enx

1049

Misc. tests on S. urei; dar-es-salam; salinates inconclusive.

A-B FA3(attenuated) → SW1031 a:b → SW1052 c:b : not tet.
→ SW1053 c:a : tet

49G abmy^enx → SW1049 /i;b;1,2 (H1^i H1'^2 H2^-) → H1'^2 H2^enx
see 465
5 isolates enx : 1,2 : enx

Either phase in 1,2+enx → either enx, or inagglut.
Some are still very reluctant for i.
enx:c:enx SW 1054

151.G-H. abmy^enx → SW. 1054-3 → G 1-3
→ H 1-3
enx:a:enx SW 1055

Efforts to demonstrate c:a:enx, a:c:enx resp. in c+enx, a+enx have given inagglutinable forms. Cf. 49G.

Q SW1054 (a:enx) → TM2 ph2 → enx: monophasic!

1055 (c:enx) → → c:1,2

∴ While H,H, structure of java seems to be justified by SW1052-3 (c:b c:a), the final proof of a triphasic is not settled. Homologies of (enx → H,H,) → still to be settled. (Q-R large scale).

(over)

A more readily variable example of $H_1 H_1$ would be desirable. (SW1031 is perhaps the most distinctive).

Tests 1050 perhaps should be repeated. Try on a:enx.

Linkage $H_1 - H_1 \rightarrow P/a_1^-$

1051 Tests very limited : Total actually only 5.

SW1049 (i: 1,2 $H_1^i H_1^{1,2}$) $\rightarrow$ SW666. 2 i: —

ph 1,2 $\rightarrow$: b: 26 1,2: 7

SW1031 $\rightarrow$ SW967
1053 $\rightarrow$ SW967
) note: very low yields + apparent lysis Why?

$1053_a \rightarrow$ SW666 a: — a: —

$1053_c \rightarrow$ " c: —

dontes-eggi and paratyphi A.

1052. Screening of Hoan strains leaves doubts on several of these concerning identity, as some are ent: b. E (1967 · relation to 1966) not yet tested.

Fix on 1052B (Edwards) as authentic strain.

1045. Several attempts → a:− unsuccessful.

However, SW1048 seems more transducible (for F/a+) by FA(PB)., though not by TM.

Note: F.K. records SW1048 as I−, 948 as I+.

(1045J1) SW1047 as I+ SW694 as I−.
Transduction?

Perhaps should check other transductions → 1048 for restoration of I.

Gal – Lac diploids.

105 v

6/3/53 (y EML)
W2281 x H245

40 colonies streaked out., ~~replicate to~~ pick 1 apparent Gal v. to EMS Gal. Replicate to EMB Gal, Lac, Mal, MH.

Pick 2 as most tentatively still Mal v., Lac v.

of original 40, all were Lac + (v) exc. 2, 8, ~~32~~, 40.

	Lac	Mal	MH	Gal	
4	v	+v	+v	+v	
7	v	v	v	+v	
14	v	–,v	+v	+,– ?	} not –. not Gal v.
23	–,v	v v	v	+v –?	
27	v (+/–)	+v,–?	+v	+v,–?	
28		v	v	+v	
29	v	v	v	+v	
39	–,v	+	+	+	

14, 23, 27 must be ~~redone~~ re-to verify whether Gal v or Gal +v (latter is assumed Gal +/. or +, modified by other segregations)

Estby later isolated H324 from this cross. T.O.
This material

6/6/53.

by PDS - W2284 from W1895 / motility passage.
In his hands, F⁻ and not re-inflated by W1817 or by W1895.
(However, controls not certain).

1. W2284, W1802 grown with W1305 3 hours. Mixture (F) then streaked out (→ F1) and plated directly with W677, W1896:

			D/O) 48h.	EMBLac	
1	W1802 ×	W677	—	—	
2	W1802	1896	++	++	+>-
3	1802F	677	2	3	-
4	"	1896	+		
5	2284 ×	677	—	—	
6	"	1876	++	++	+>-
7	2284F	677	+±		+± ->+
8	"	1896	+		
9	W-6	677	+	+	->+
10	"	1896	+		

Thus mixture of W2284 (or W1802) with W1305 is clearly F+

F1. Isolate Lac+ from initial mixtures with W1305. (ca 10-20 colonies from EMBLac pooled in broth + used after incubation overnight).

W1802'	×	677	+
W2284'	×	677	4

F2. Lac+ from mixture overnight. Pool incubated 4-6 hours.

1802'	×	677	+
2284'	×	677	+

(over)

Restreak on EMB. Use to check purity
2284F1-2 pure Lac+ some ?mucoid
1802F1-2 may have some Lac-
Repurify and check single colonies of 2284F2

55A W2284 + W 1941 in broth overnight.
S.O. EMBlac ca =. Isolate, test, rep. Lac+
x W677
streak

B. to W1802. Came out ca 100:1 -:+, rep. Lac+ and test as above.

Repeat 2284 ... F x 1936 Reprinted (pool still)

1802 x 1956		—
1802F1	"	++
1802F2		+++
2284F1	"	—
2284F2	"	+(few)

may be utter streak or mutable

Try passing back to W677.

10552: 6/10/53.

2284 x
1057

prot. test as Hfr, F⁻ ...
4 Mal+
3 Mal⁻ test by SRP

On retest 6/14/53

	W677	W1816	
2284F2 pool	—	++	∴ pool has become F⁻!
sc. 1	—		Test for transfer to
sc 2	—		W677!
sc 3	—		
W6	++		

6/19/53..

H. W2284 "F2" + W1956 overnight, s.o. on EMB Lac agar to recover "exposed" 1956 as Lac⁻ SR. Incorporate to Tuesday.

I. (W2284/F2 + W1305) × W1956 10 prototrophs
2284F2 " 0.
∴ 2284F2 does not become grossly infectory after being F⁺ quality.

JK J (W2061 (MTL-Hfr) + W1956) × W1802 40 prototrophs
K. (W1305 MTL-Hfr + W1956) × " 80 " .

J. Transfer from Hfr? Temporary or heritable? or recombination.
Lac⁺ V_1^R Mal⁺ S^S Lac⁻ V_1^R Mal⁻ × Lac⁺....

no other markers at hand.

Repeat: (W2061 + W1607) × W1956
Lac⁺ S^S (Lac⁻ S^R) Lac⁻ S^R

or (2061 + 1956) × 1607.

Reinoculate K.

L. (W1956 + W2061) × W1607 D/O) heavy growth → v. low yields.
Test all 8 prototrophs on EMS Lac.

M. (W2284F2 pool + W1956) W1956 recovered, + W1607 no prot.
infer no P⁺ in 2284F

Controls W2061 × 1956
× 1607
1956 × 1607
} no prototrophs

H24[redacted] × W.

6/9/53

A. H24[redacted] from S. c'sanE M[redacted]Lac in Benassay (TLB,-) × [redacted]2. gave ca 50 prototrophs mostly Lac+ ✓stock: H24[redacted] ca 1% lacv.

6/9/53 B. aerated in D(Lac, HC) } 1. × W1321 H⁻F⁻S^Lac⁻Gal₄⁻λ^S
C. Standing in " . } 2. × W1486 F⁺ " "

yield
B1 no prot. — —
B2 ++ → Replate to Gal no -
C1 ca 10 prot. Lac - streaks on EMB Gal. all Gal -
C2 + < Lac+ to EMB Lac / Lac- to " Gal

✓stock: B 90% v Rare +
C 70% v 1% +

This stock therefore seems to behave as F⁻, especially when aerated.
Lac⁺ should be uniformly lacv.

C1. 3 Gal? 9 Gal-λ^S Restreak 3. plaques noted on 1.

C2. Restreak possible lacv or Galv.

	Lac	Gal
33	—	v?
34	v	v or +v?
35	v	+v
36	v	+v
37	v	+v
38	v	+v

(over)

C2

	Lac	Gal
1	v	+v v?
2	v	v?
3	v	+v
[redacted]	v	+v
5	v	v H327
6	v	+v
7	—	+
8	v	+v
9	v	+v
10	v	+v
11	v	+v
12	v	+v
13	v	+v
14	v	+v
15	v	+v
16	v	+v
17	v	v+v?
18	v	+v
19	v	+v
20	v	+v
21	v	+v
22	v	+v
23		
24		
25		
26		
27		
28		
29		
30		
31	—	v +v?
32	—	—

(over)

459: ✓

	Lac	Mal	Gal
1	v	—	
2	v	—	
3	—	(Mal v?)	— λ^v Lp 3/4
4	—	[redacted]	v λ^v
5	—	+ Mal v	

Re v single cols.

	Lac	Mal	[redacted]	Xyl	Gal	MR	D(o)
	v	—		v	v	v	+
	v	—		v	v	v	+
#3 is Lacv Gal- (4 p.c. heat)	v	v		-	λ^v		+++
	— ? or intrac.			v	v	+^v	+
	—	+ faint middle?		+	—	+	—

Observe 5 as segregant. Save #3 as Lacv Malv Gal= Lp^v H326
#5 may have been misplaced as fem Gal= Lp 3/4, or may be crossover.
(over)

Next experiments are

1. Heterozygote rearrangement
2. Lp^s Lp_2^s rearrangement. (perhaps better from a Gal^+ reversion ??)
3. Gal^+ reversions (*cis* and *trans*)
4. Gal^+ transductions ....

Most Lacv from C2 are Gal^+ mottled. → H327

Retain #5 as Galv Lacv and check other markers

also check "Galv" from 1,2,17,34.

H327: Lacv Galv $Mal+^v$ S^s (S^s/S^R [illegible])

Lp_2^s Lp^+

↓ no S^R with heavy inoculum on EMB-lactose

No unusually overt lactose: does this segregate Lp^s?

↳ Esther finds Lp^+/Lp^-, $\underline{Lp_2^R}$.

? Lp_2 status must be rechecked.

C. 1-5 : #1,4 are evidently Lac^{v}; may be useful later as Hfr IF^{-} ?
#2,3,5 show peculiar mottling is definite evidence of segregation.
Could look for evidence of mutational segregation. Store in NA stabs.

		Lac	Mal	
A	1	+^{v}	+	E1
Residuals of presumed	2	+	+	
	3	+	+	
	4	+^{v}	+^{v}	
s.c.i	B 1	+^{v}	+^{v}	
	2	v. light +$^{v?}$	−	
feamplating	3	+^{v} or v ?	+^{v}	E2
	4	+^{v}	+^{v}	
on EMSlac	5	+^{v} and -	+	E3

presumably Lac^{v}.

to EMSlac.

C	1	+^{v} and −	+	E4
	2	+^{v}	+^{v}	
	3	+^{v} -?	−	
	4	+^{v} +,-?	+ -?	
	5	+^{v} -?	−	E5
	6	+	−	
	7	+^{v}	−	
	8	+^{v} +-?	−	E6
	9	+^{v} +,−	−	
	10	+^{v} +	− occ+	
	11	+^{v} , -?	−	
	12	+	−	
	13	+	+	
	14	+, − $^{v?}$	−	
	15	+, -	+	E7
	16	+^{v} −	+	E8
	17	+^{v} -?	−	

have presumably been reisolated

Remarkable mottled appearance of all of these. Save EMS originals protem; choose for further study:
A1; B5; B3; C1; C16; C15; C8; C5.

Repick single colonies and spot EMSLac, brush EMSLac/sm; streak out EMSLac again. Handle as E1-8

Mark 5 b c 2, 3 4

2 Lacv Malv Bal +v +v

3 v "

6/9/53

A. H310 (from 3.2. EMBlac to Penassay)

× W1801 EMBlac Pred. lac −

Pool plated on EMB lac, Mal. Ca 5% Mal−, 70% lac−. Made possible lac, Mal v for later pick. Plates may have been moist. growth very rough + spreading.

H: mostly lac v.

B. × W1802 EMBlac. Pred. lac +

Pool: almost pure lac +

yields very high. >> 1000/plate pool growth and retreak for sampling.

SE 57E

C. H310 × W2209 ($P^-G^-F^-$) Dilute plating. >> 100/plate. Streak rest weaker lac+ on EMB Mal. 24 tested: 16 Mal− 1+,− 7 Mal+

Repick ½ Mal+ which might be Mal v. (2 from rare + among −; ~~2~~ Mal v).

Replicate to check lac poss.

	Mal	lac
1	+v?	+, +v?
2	+	+ +v?
3	+ and −	+ and −
4	+	+

Replicate streak plate (24 cols - C):

	lac	Mal	lac S
1	+		S
2	+		S
3	+ only each +,−		
4	+	(+ 2 days too)	R
5	v?	−	R
6	+	−	R
7	+	−	R
8	+	+	S
9	+	−	R
10	+	−	R
11	+	−	R
12	+	+	S
13	+	−	
14	+	−	
15	+v?	−	
16	+	−	
17	+	−	R
18	+	−	R
19	+	−	S
20	+	+	S
21	+	−	R
22	+	−	R
23	+	−	R
24	+v?	+	S

Replying penalty?

No Mal v indicated.

Recheck #1, 2, 5, 15, 24 on EMBlac, Mal, M+l; spot on Slac.

Remember C1-5.

some lac+ might be v.

C: Repeat: 20 ~~tested~~ streaked on Blac; save 12. = 6-17

	lac	Mal	M+l
1	v	+v	v?
2	+v	+v	+v
3	+	−	−
4	v	+v	+v
5	+v	−	+v

D H310 × W1911 EMSlac v. heavy seed (Numerous spiked lac⁻ T+Q!

Replate H328:

7/8/53. all colonies prototrophic, including
<100 lact, fuc lac - segregants
on two good plates.

SRP crosses ♀ 1057E (J.L.)

Penassay (7.5 ml) cultures of the following were made and grown up overnight:

1817 1177 57E 1, 2, 3, 4, 7

Half a ml each of the 1057E and 1817 or 1177 were mixed in Penassay (5 ml), incubated 8 hrs, centrifuged, washed twice in saline, resuspended in saline, plated on S lac sm 7/7/53

	♀ 1817	♀ 1177	control	S.O. on B lac
57E 1	lac±	lac−	—	lac+ (light + dark)
E 2	lac+, few lac−	lac−, few lac+	—	lac+ (light + dark)
E 3	lac±	lac−, few, against solid background	—	lac+, lac− (fewer)
E 4	lac+, few lac−	lac−, few lac+	—	↓
E 7	lac±	lac−	many colonies, all lac−	↓
control	−	—		

E7 unstable; E1-4 evidently Hfr. Test lac − segregants.

H310 x ...

1057E

6/16/53.

Restrich S.c. EMB lac

			MH (from haploid)	Mal (from haploid)	SM
x1801	1	+ (rough) only	+	+	S
x1802	2	+, sectored or mottled +, and occasional — (similar to H310)	+	+	S
x1802	3	" v. rare —	—	+	S
x2209 =1678	4	" occ. —	+	+	S
H328 ←	5	", definitely sectored +/—	+	—	R
	6	app. pure +	—	—	R
	7	+, s, m, occ. —	—	+	S
	8	pure mottled +.	+	+	S

all prototrophic on EMS lac

no evidence of R/S.

#2,3 2n from H310; Mal, S from F⁻

4,7, 2n from H310; Mal, S from F⁺

5 2n from H310, Mal S from H310 (TL⁺ from F⁻)
+ mutations; heterozygosity. H328.

Replicate each to D/O for nutritional segregation.

No auxotrophs noted in diploid cultures (very few segregants sporulated)

Are these diploid or unstable lac?? (H310 + ?)

5 Mal+ from H328. Streak on EMB lac, Mal. of H328.

↓

All appear to be lac ∨ Mal+ " ( ). Suggests H310 + ? is Mal-1.

Attempts at H310⁺: plate on EMS Mal+ 1/100 hydrol. casein: ca 20 papillae after 3-4 days, but these are not Mal+!

Repeat — now streak papillae: all Mal+ — ! Repick papillae (over)

Proof that H310 is diploid: Lac is only segregating marker!
(pure TLB_1^-!)

a) Hfr 2n vs. F^- 1n.

b) On a single occasion α 1895 gave Lac+/Mal+ Su.
(cf. 1057.) H313

---

for test for its own papillation:

1057E1.-2 papillae of H310 on EMS/Mal HC.

↓ Mal+ Lac+. save 1057E2[illegible] = Mal+ Lac+

∴ Mal heterozygous

Reverts ~~57~~ 57E21 as Hfr.

and segregants.

57E21 x W1607 → same equal # recombinants
parent controls =

6/10/53

A. (aerated H290) × } { 1. W1394] nEMSlac
B unaerated " × } { 2. W1918]
(D(lac,M))

	yield		
A1	6 colonies	1 lac+	1 lac+
2	10 "	rare lac+	20 rev.+
B1	2 -		
2	rec -,+	0.	

1058A. Streaked A1 (1-5) B1 (6-9) nEMS lac
In repetition, none were lac, Mal V. T.A for more

Reconstitute H- ...

1058a

6/9/53.

H290 from lyophil: direct streaking → to EMS lac. (7/30/51) grow in D lac HC...)

H302: $2iO_2$ tube 1 → grew out slowly: pure lac- inviable
tube 2 → " " "
lyophil → heavy mixed growth, ca 50% lac+.
→ 90% lac+ ✓

H313: susp. inviable
lyophil: (3/53 → found all segregated)
6/53 → EMS Mal: → mostly Mal-, Mal+ Some lac+.
EMS Mal

H226 occ. lac+ from vial 9/52 → ✓ (some lac+!)

H267 Almost all lac- " " . Restreak on EMS lac; took from EMS lac to D lac →

318 Inviable

319 Viable; rare lac+ (v. light, maybe ✓)

313-14 13: pure lac+ 14. Reath.

H304-5 Mostly +, - T.O.

W1940 x 1956 1059
Hfr F⁻

6/11/53.

A nEMS lac

B Inc together in broth; plate EMB lac., EMB lac ?

A: 30 lac+ from EMS lac to EMB lac. Re- possible lac v others are + (1-2) Restreaks 10: all are Mal−, #14 lac v? others lac+, very rough!

B. Papillae eventually noted. lysis in streaks?
Not scored for lac v. Single + noted - streaks streak, probably reversions.

None show lac segregation. Repeat Expt., also streak for Mal.

59B/B: Investigate lytic appearance in B.

59C 6/16/53 -x- EMS, EMB lac after growing together 6 hours aeration. T.O. mixed inquinity.

BB: W1940 proved to be mixed Mal+ / Mal−
by plating of W1940 + W1956, Mal+ and Mal− lyt. are lyl+ = W1940
Mal− normal are lyl− = W1956.
all lac−.

=W2302 Isolate Mal− as presumed mutant of W1940 (This strain had been recovered from an old, desiccated sheet which grew out slowly). Retrack lyophil and current cultures of W1940. Rescue W1942 also for further experiments.
Renew W1940 stock from lyophil (pure Mal+) and throw out others.

(over)

D	W1956 x W1940	EMB Lac	
E	"	"	S Lac
F	"	W1942	B Lac
G	"	"	S Lac

after growth overnight

Lac+ seen as papillae in streaks of D, F.

ca 1% Lac.+ colonies in E, G.

Hold suspicious colonies for later retest

7/4/53:

---

abandon in view of Cavalli's finding re Hfr - Gal linkage

6/11/53

H324-5. Treat in D(Lac-) c UV, 20 sec. Broc. mainly in Serratia saccharin, and plate on EMB lac. Replica to EMB Gal, Mal, D(c) for discordances. ca 15 Lac^{v}/plate : 5 plates

A = H324 deriv. B = H325.

			Lac	Gal	Mal	Lp	Lp^2	D(c)	
A	1	Gal- Mal-		−	−	−	R	−	
	2	Aux.		v	v			−	
*	3	"	H332	−	v	−	v	−	
	4	"		v	v			−	
	5	Gal- ✓	H331	−	v ou v^{+}	−		+	H331
B	1	Mal- Gal+		+	−			+	
	2	Aux		v	v			−	
	3	"		+	v			−	
	4	"		+	v			−	
	5	Mal-		+	−			+	
*	6	Gal- ?		−+?	v	+		−	
	7	"		+	v			−	
	8	Aux		+	v			−	
	9	Aux		v	v			−	
	10	Mal- "		+	#v			−	
	11	Mal-		+	+			+	
	12	Gal+ Mal+		v	−			+	
	13	Aux		+	−+			±	
	14	"		v?	v			−	
	15			v?	v			+	

all are + or v

S (R?)

Isolate as possible: ✓

all Lac^{v}

Lac, pick, pos and plate papillate Lac^{+} which may not have registered on replicas. ~~recent papillate were apparently~~ Add 2 possible discordances to above as A5; B15

On initial restreaking A1, A5 gave light + Lac^{v}. Transfer to EMB lac plate and replicate for characterization. * No overt lysis in A1 streak; present in A5.

C. H326 Gal^{+} revertants picked from Lac or Gal, streaked on EMB Gal, Lac. — to EML. Some typ. Gal^{v} noted; others are slow +. For further study, streak out H326 on EMB and obtain Gal+ from separate Lac^{v} colonies by replica to EMB Lac Gal ...

over

for replica of streaks: v / v^{+} not certain

Lp^2 tested on EMB/D)

streaks may segregate

Repeat λ-2 tests on A1, 3, 5

1 apparently R
3 RS?
5 S-R?

(over) Try infecting A5. c λ.

λ-2 tests on #1, 3, 5

EMB1Mal D(6)

1 R

3 Mal+ S Mal R

5 Mal+ S and R? S!
(Can't tell background action)

λ

S H332

∴ presumably S/R but Mal+/+

H324. In EML tetrad segregation #1-7 appeared to be segregating Mal+/−, but #8-55 were all Mal+ (including many Xyl−). Fermx confirmed 6/17!

Check with T6: #1-10 ~~Mal−~~ Gal− were T6S. H324 T6S
H325 T6S/R

H324 as now available is Mal+/+.

The bulk of EML's data must pertain to this "secondary".

Except for auxotrophy, H332 is most suitable for infection of Lp^S/Lp^S ..... Now need to obtain Gal+ version.

H331 also OK; not segregating Mal

G1 H325. v. slow lac v? — restock + test prototrophic λ^- $\lambda 2^R$ Gal$^-$ Mal

H H329 1 Malv lacv Galv lysogenic auxotrophic
2 " " "
3 " " "

G1 might be Gal$_1^-$ lacv, but is apparently bp_2^R and presumably unsuitable for present purpose. H1-3 might be used by crossing to Gal$_4^-$ bp^S.
On the whole Gal$_2^-$ (H326-331-332) seem technically most suitable.
Check G1 by transduction to Gal+; if insusceptible, probably unusable.
don't bother

↓ H325 appears to be as given. (See 1062) Grow on D(Glu)

6/15/53.

A H302 grown in D(M, Lac) × Y10
B " → aerated (aerated), into D(M Lac) 7 hours not aerated × Y10
C A) aer. × W1918
D → not aer. × W1918

plate on EMS lac.

A. 2 plates No prototrophs

B 20-30 lac+/plate 102 ? lac-/plate (H-302 paralytic)

C 3-400 lac+/plate 1-2 ? lac-/plate (H-302 orthotypic)

D very heavy background 5-6 ? prototrophs/plate.

B. Streak 72 lac+ on EMS lac for lac V. C. Hold!

Pick to EMS lac. Not all grew out.

	Lac	Mal
1	V or V	V
2	V	V
3	V	V
4	V	V
5	V	V
36: 6	+	+
7	V	+
45: 8	+	+
9	+ V?	+ V?
10	V	V
54: 11	+	+
12	+	+
13	+	?
14	V	V
15	+ V?	? + ?
16	+ V	V
17	+ V?	+ V
18	V	V
19	V	V
20	V	V
21	+ V	V
22	+	+
23	+	+
24	V	V
25	V ?	V
26	V ?	—

Picked as probable lac V

∴ in sum: 1- Mal
23 probable lac V < 3+ ? 19 V
(not fully characterized)
33 lac+ < 1- 22+
(6 were not prototrophic)

27-56 picked as supp. lac+ 25

lac+: 27, 28, 29, 30, 31, 32, 33, 34, 36
Mal+or- + − + + + + + + + +

lac+ 37+ 39 40 +-? 41-44 46-48
Mal+or- + + +,− + + + + +

(lac 37? 38 V 45 V 54 V
Mal V V V

lac+ 49-52 53, 55, 56
Mal+,- + + + +

Re-collect possible V and streak with sm, etc.

Mal V usually crossed on basis of +, − being present.

Elimination evidently does not occur in 2nd F+ × 1st F−
Remember 38, 45, 54 as 6-8-11

106113

previous

Conclude 1-23 are likely Lac v. (Malv). ~~Re-~~ ~~[illegible] the following~~

Restreak entire screen of 23.

EMB Lac, Mal

Streak on EMB Mal / SM.

EMB MH

		Lac	Mal	→ sm MH	~~MH~~ sm Mal	
1	E	+	+ −	+S	+S −S	
2	A	+ −	+ −	+S −R	+S	
3		−	+ −	+S −R	+S −R	
4		+	+	+S	+S	
5			−	−R	−R	
6		+	+	+S	+S	
hap 7		−	+	+S	+S	
8		+	+	+S	+S	
hap 9		+	+ +	+S	+S	
10	A	−	+ −	+S −R	+S −R	colonies picked may have segregated.
11		−	−	−R	−R	
12	R	+	+ −	+S	+S (-s 2) blue	
hap 13		+	+ +	+S	+S	
14			+	+S +R	+S −R	
15	A		?	+S −R	+S −R	
16	A	+	+ −	+S −R	+S −R	
hap 17		+ +	+ +	+S	+S	
18	F	+ −	−	−R	+R −R	+S also pur?
19		+		+S +R	+S −R	
20	E	+ −		+S	+S −S	
21		+ −		+S −S	+S −S	
22	A	+ −	+ −	+S −R	+S −R	
hap 23		+?	− −	−R	−R	

A) Lac v Malv Sv MHv number : 2, 3, 10, 15, 16, 22 — 6

B) Lac v Malv Sv MH+ " : 14, 19, — 2

C) (Lac+ Mal+ MH+ S^S) : 4, 6, (7), 8, 9, (12), 18, 11 — 8

D) Lac+ / − Mal − MH − S^R : 5? 11, 23. — Ds H30 2 MH− or MHv? 3

E) Lac v Malv MH+ $S^{SC?}$ 1 20

F) Lac v Malv MH− S^R 18

(7) Lac v Mal − MH + S^S

Recheck: ~~[illegible]~~ / on MHv. B)

C): haploid: find diploid for D.

G) Lac − Malv MHv S^S 21

1061C

	Lac	Lacsm	Mal	Malsm	MHL	D(o)	S (from EMS/Mal → Lac, Lacsm)	
1	++	+	−		−		R haploid	∴ all either S R or S ✓
2	+ ✓	+ −	−		−		R	
3	+ ✓	+ −	−		−		R	all "Mal+ are Mal ✓ S ✓
4	+ ✓	+ −	−		−		R	
5	+ ✓	+ −	−		−		R	
6	+ ✓	− +	−		−		R	
7	+ ✓	− +	+	− +	+	✓	✓	✓ are probably painted H2O2.
8	+	+ −	−		−	− −	not prot	
9	+ ✓	+ −	−		−		R	
10	+	+ −	−		−	− ✓	not prot	∴ 35 prototrophs
11	+ ✓	+ −	−		−		R	all evidently segregating
12	+ ✓	+ −	−		−		R	
13	++ +/−	+	+ −	−	+−	±	R (+R?) ✓	
14	+	+ −	−		−	− ✓	not prot	
15	++ +/−	+	−		−	±	?	
16	+ ✓	+ −	−		−	+	R	
17	+ ✓	+ −	−		−	+	✓	
18	+	+ −	−		−	− ± ✓	not prot	
19	+ ✓	+ −	−+	−+	+ −	+	✓	
20	+ ✓	+ −	−		−	+	R	
21	+	− +	+ −	− +	+ −		✓	✓
22	+ ✓	−	+	− +	+ −		✓	✓
23	+ ✓	−+	−		−			R
24	+ ✓	−+	−		−			R
25	+ ✓	−+	+	−+	+	✓ ±	✓	✓
26	+ ✓	−+	+	−+	+	✓ ±	✓	✓
27	+ ✓	−+	−		−	±		✓
28	++ +	+	−		−			R haploid
29	++ ✓	+ −	−		−			R
30	+ ✓	+ −	−		−			R
31	+ ✓	+ −	−		−			R
32	+ ✓	−	++	−+	+	✓	✓	✓
33	+ ✓	−	++	−+	+	✓	✓	✓
34	+ ✓	−	++	−+	+	✓	✓	✓
35	++ +	+	−		−	±		R haploid
36	++ +	+	−		−	±		R haploid
37	+ ✓	+ −	+ −	✓ + 1	−		R	R?
38	+ ✓	+ −	−		−			R
39	+ ✓	−	++	−+	+	✓	✓	✓
40	—							

most lac var. mod. +/− +/+ some are +/−

all either pred.

from EMS/Mal → Lac (excluded plus: + EMS lac ± sm)

+ = +or +, −

Mal ✓

7, 13, 19, 21, 22 are evidently MHL ✓ Mal ✓ S ✓

25, 26, 32, 33, 34 may be (MHL+ or MHL ✓) Mal ✓ S ✓

37 is Mal ✓ MHL− S?

1, 15, 28, 29, 35, 36 may be lac haploid.

- Replate to EMS lac / EMS lac sm to verify S^R pur.

only exceptions for ✓: 13, 15, 17, 27

10613B

Conclusions from tests

Comments.

	Lac	Mal	MH	S	
1	·	v	+	[redacted] S	
2	·	v	v	v	
3	·	v	v	v	
4	+	v	v	S	
5	v	v	v	v	
6	v	v	v	v	
7	v	+	v	S	
8	·	v	+	S	
9	+	+	+	S	haploid
10	·	v	v	v	
11	v	v	v	v	
12	·	v	+	S	
13	+ v?	+	+	S	haploid — Lac+ mutant!
14	·	v	+	v	
15	·	+ [redacted]	v [redacted]	v [redacted]	
16	·	v	v	v	
17	+	+	+	S	haploid
18	v	v	−	v [redacted]	Mal+ S^R / Mal− S^S
19	·	v	+ ~	v	
20		v	+ ~	S	
21		v	v	S	
22		v	v	v	
23	+	−	−	R	haploid

some probably lac+

18 seemingly Mal−, Malv no mal+ segregants. Restreak apparent Malv for more material: 4 colonies gave same pattern. Most Mal− are S^S Mal+ probably include pure +.

Summary: 56 prototrophs. 19 ultimately diploid (lac+ or lacv)
Most probably lac+/− rather than +−/−+.

Lacv	MHv	Malv	Sv										
			Sv :	8	2	3	5	6	10	11	16	22	
+	v		S :	4	1	8	12	20					
−	−		R_S :		haploid only (9, 13, 17, 23)								
v	+		S :	1	7								
v	v		S :	2	4	21							
+	v		v :	2	14	19							
v	+		v :	1	15								
−	v		v :	1	18								

2n "F-" x n F+ 61cc

1/2/53

Recap. of types (provisional class. of 13, 15, 17, 27 as S^V).

A. 16 LacV (+-/-+) MH- Mal- S^R : 2 3 4 5 6 9 11 12 16 20 23 24 29 30 31 38

B. 1 LacV (++/--) ~~V~~ ~~V~~ ~~V~~ : 13

C. 10 LacV (+-/-+) V V V : 7 19 21 22 25 26 32 33 34 39

D. 1 " MH- MalV SV : 37

E. 1 LacV (++/--) Mal- MH- SV : 15

F 2 LacV (+-/-+) Mal- MH- SV : 17, 27

31

haploid : 5 1 28 35 36 40

aurotr : 4 8 10 14 18

9

36 prototrophs tested. 31 diploid LacV : 29 (app.) Lac+-/-+

2 Lac++/--

(may be incorrect).

MalV : 12

MHV : 11

SV : 15.

#2 maybe ++/-+ #3 +-/-+

Compare:

(Major types only, Mal – S).

			B		C	
1	Mal V	S^V	11	58	12	39
2	Mal –	S^R	0		16	52
3	Mal +	S^S	1	5	0	
4	Mal V	S^S	6	32	0	
5	Mal –	S^V	0		3	9+
6	Mal +	S^V	1	5	0	
			19/ep +		31/ep +	

$B = 2nF^+ \times Y10$

$C = 2nF^- \times W1918.$

H302 = H-Lacv MH – Mal-S^R.

References: 1. 2n parent functions as such whether F^+ or F^-. If $2nF^+ \times nF^-$ depended on polarity reversal, there would at least be some Mal$^-$ S^R. Split (B:C)(1:2) is certainly significant.

2. "No" Mal+ S^S in B, suggests that "none" of these diploids are hemizygous in this region. If elimination occurs from the F^+ side, it must involve only 1 of two strands, and the eliminated one may be discriminated against in zygote formation. It is possible that elimination does not occur at all from the F+ side of 2n. Note high incidence of Bd. (Mal/S) crossovers!

3. High incidence of C2 suggests usual elimination from $1nF^+$ side. ? are these homo- or hemi-zygous? Need tests on these random C5. Nearly half are S^V. Note: in B, only S^S and S^V; in C only S^R, S^V.

61C hemagglutinin tests: (EMS Mal)

17: numerous Mal+ (pinty??)

2: 2 papillae
3: 5 papillae
} → Mal v.

15: 2 plates vs Mal+ 7/10
2 plates: 3 Mal+ 7/18: → Mal v.

#17 initially a few Mal v. Reisolate Lac v Mal — and test
(mutants?)

None of this is inconsistent with pre-elimination, if it occurs. Would need a Mal/S crossover (Mal-S>) or a Mal-hemizygote in B to substantiate it. Presumption of elimination is based on polarity differential.

Immediate requirements: hemizygosity tests on C1, C5 classes.

Later: Look for Mal-diploids in B. (Should occur by crossover).

→ x Val⁻ 1062

6/19/53

A. N1–x H326 on EMB Val

B N1–x H331 " ".

Several thousand est. Val+ from 1 exp. Poured streak out on EMB Val
Background ca 4-5 papillae

check for lac v Val+: pick lac v to EMB Val. mostly lac⁻, but most Val⁺ are definitely variegated.

2 lac v Val+ selected from A, B resp. Spots of lysis in org. from each. Further screening indicated.

C. N1–x H325A (Val⁻ lac v) → UV Val+ (Lp_2^{x} !)

of EML

→ × S. aboriferi aegui
SW1058.

6/16/53

A. SW1058 (mot.) in eux serum. See 1042, 1052
Pasteur tube had not moved in 4 days.

6/16 1
2
3

B. FA 18 → ×
6/17 1
2.

C FA 22 → ×
1
2.

} 7/9. All immobile! T.O.

6/26. no swarms in any of above! (Try IV serum!)

6/26 SW 1058 mot (fermtant) = D and TM2 = E.

1. mot
2. IV
3. IV V XII (/ Typhi O)
4. IV XXVII XII

24h.

D 1 ++
2 +
3 ++
4 +++ stimulation? or further medium (definite inhibition)

E 1 24h.
2 +++
3 +++ → still ~~IV XII +++~~
4 ++
+++

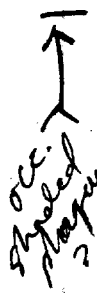
Remove O2 after 48 hours. Shake not and test for transd., /eux etc.
Mor + purification 7/1/53. /eux i,i FA 18, FA 22

6202 /eux ±FA 22 ~~Repeat IV~~ no mot. 7/1/53 T.O.

7/2/53

Cross-streaks on EMB bac, ϕ stock ϕ as received from WBC.

B: ϕ:	FA10	PB-1	PB-3a	PB Taunton	PB Bendee	TM ϕ PRE
TM2	++	—	—	—	—	++ lytic
1057A (PB1)	++	++ semi lytic v. few plaques	—	—	—	—
SW730	—	—	—	—	—	— (Sudeney stock)
SW887A1	—	—	—	—	—	+ not lytic (Napoli stock)
SW957	±	—	—	—	—	+: inh? (0901)
Sudeney: 1074E1	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	—	—	—	—	—	—



titer? shoulder on PB1

ϕ perhaps should first be grown to higher titer. Try TM ϕ PRE / TM2 SW887 SW957.

PB1 / PB1.

Try other ϕ on Napoli, Sudeney, etc.

Grow Taunton ϕ, ~~Ex~~ PB2 ϕ.

Test ϕ preps → SW666

FA22	+
control	—
TM / TM2	—
/ 887	—
/ 957	—
PB2	—
PB1	—
PB Taun	—

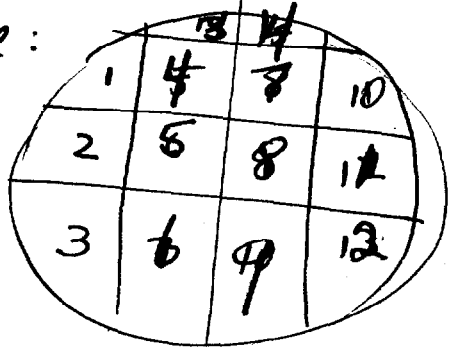
ϕ maybe ok.

7/5/53 From results over, ϕ's have not been made to adequate titre.

Use PB2, PB BAOR, PB Bendee as basic stocks. (over)

Test loopfuls for φ activity.

PB φ:



Bact. = (PB1, PB2, 666 (Jersey), Taunton)

For later study,
2
BA0K
Dundee
represent types of phage.

	PB1	~~Aber~~ Taunton	SW666	~~Toddington~~ PB2	TM2
1 φPB1 (Ch.)	++	—	—	—	
2 " "	~~+~~ ++	— sic!	—	++	
3 " Taunton	—	isol. pl.	±	— sic.	
4 " BA0K	—	—	—	—	
5 " Dundee	+	± mephages?	++ fine plaques	± ? v. fine	
6 FA10	++	++	++	+ 0.	++ 0
7 φ1/1 S.L.	+ isol pl		—		
8. φ2/2 S.L.	+ isol pl	—	isol pl.	few pl.	
9. φT/T S.L.	—	isol. pl.	isol pl.	—	
10. Jersey (Ch.)	++	—	++	—	
11. 3a (Ch)	++	—	—	—	
12. 3a1 (Ch)	++	—	—	—	
13. 3b (Ch)	++	—	—	—	
14. Bedee (Ch)	? fine pl.	—	+	+ fine pl.?	

Ch. pups??

φ's not prepared to adequate titer.

In first preparation, PB2, PB BA0K cleared; Dundee did not. Re-grow Dundee on 666, PB2 and Dundee

7/5-6/53

Cherry phages.

Ch:	TM2	SW688	SW566	PB1	SW730	SW887	SW957
1.	−	−	−	±	−	−	++ + (lytic ++)
3.	−	−	−	−	±	−	−
4.	−	+	−	−	−	−	+ lpl. lyt.
5.	−	−	−	−	−	−	−
7.	± doubt	−	−	−	−	−	−
8.	−	−	+	−	± sh.	+	− ±sh?
9.	−	−	+	−	+.	−	±
10.	−	−	−	−	−	−	−
(12) ~~11.~~	++ T.	−	−	++ T	−	++ T	−
17.	−	−	+	−	+	−	± lytic ++

From point of view of survivorship, #12 seems most likely.
Label on this vial reads: S. luciana #12. Cherry's letter refers to S. luciana #18 as v. specific for group XI. Presumably not these ϕ.

Grow #12 on TM2, SW887 to test heteroduteonic. Also grow
#1 /957; 9/SW730; #4/957; #17/666, 957.

Need to find better hosts for 3, 7: try smotopes strains (enterides, citrobacter and gallinarium).

#1 seems to differ from 01 in host range. Called para A phage!

Ch2 grown on TM. spot: OK. / TM
on SW887 " "
on 730 " "

Stocks 6/5.

v. small plaques
Titres not too high.
but worth trying on
01010; SW967
etc.

PB2 /2 large + small plaques. Moderate #
BAOR 1 ✓ small plaques. "
Dundee 1 ✓ " " "

/ PB1

Regrow for higher titer.

Ch stocks repeat spots: Taunton, BAOR, Dundee: not discernable / PB1.
May not be host sources.

#7 SW764 (enteritidis) – +++ (lytic) and scattered survivors
967 (dublin) –
957 (0901) + (overgrown)

BAOR PB1 + shadel
666 ± shadel
730 –
887 ± shadel
957 ± shadel.

Transcel. BAOR – x 666 → b, cal –
~~=1063 AT~~ in 6 serum 6: –

Repeat 7/8: BAOR – x 666 → 6
→ 967 → (gm) +
– x H901 → —.
strictly checked.

Repeat, looking for trailers on plates → x 666

Groups: test strs. 7/764 1/H901 strs.
(Hostel) PB1 – Bender-BAOR (new) } but – x 666 possible trailers. ++
7/8/53. 12/TM2, 887, PB1 } not trailers. ① melt off and test
4/H901 ② Chloroform and
17/H901 ~~test~~.

---

7 – x 666, 967, 957 all – in 24h. Titres?
ch2 – x ~~957~~ 957 (ch2/TM2, 887, 730) all – in 48h.

B1 (Ch12/PB1 not str. × sw666 not) → Gal+ doubtless contaminating bacteria in 4
B2 Ch12/TM2 " "). → Gal+
B3 " 1887 " "
7/8. Repeated after $CHCl_3$ treatment → × 666, 957, 967 all —.

A. BAOR → × sw666 / motility → v. numerous swarms + tracks
B. = FA90 / EMB Gal → ca. 100 Gal+ / .1 ml
Purify.

controls: each 0, 0.

(A) ① Pool motile transductants (>730) all Gal−.
② 8 individual " " : all Gal−. Pick to broth for subs. lysogenicity test.

(B). 1. 20 streaked out: none unstable, all pure Gal+. Test pur. for lysogenicity, motility.

∴ as before, no Gal: H, Fla, linkage

(C) FA90 → sw950. / Gal same (as 5-10 plate).
/ D (Meth) ± FA. no evidence of transduction).

Ch2 TM2 → × 666 } ≡ also obtain spirit H+ sw950.
/730 → ×
1887 → ×
1B2 → ×666 —
Tundee → × 666 —

(over)

Lysogenicity tests on 63A-B.

A:1-8 all are lysogenic on BAOR ; sensitive to FA10 ;
SW66 not " " " ; " ;
SW928 " " " " ; resistant ;

all are resistant to FA90.

no control, BAOR/FA90. By 90 → SW66 these have evidently became lysogenic for BAOR indicator. In transduction platings, only rare plaques were seen. Save A1, B1 for further study.

B: 1-20 15 lysogenic on BAOR. Suggests that BAOR might also be lysogenic on many of these.
5 may be sensitive. In initial streakings a few were noted as possibly mixed lysogens.

BAOR lysogenicity. 1063

7/18/53. Reciprocal lysogenic cR...

	BAOR	PB2	SW666	FA90.
1063 A1	++ ·	—	—	—
B1	++ ·	—	—	—
SW666	— ·	—	+—	— (shaded)
SW918	— ·	—	+	—
BAOR	—	—	—	+
PB2	— ·	—	—	+

note difference of 1063A1/PB2 and FA90/PB2. Suggests 63A1 may be lysogenic for a phage other than BAOR!!

· v. small plaques in A1, B1.

BAOR lysogenic for 4 caused by 1063A1 = 63C1.

Streak out BAOR/90, PB2/90; and 63A + BAOR.

BAOR may be lysogenic for another phage which attacks PB2, C66..., but this is probably distinct from the lysogenizing phage in 63A-B.

Lysogenicity of SW1060 (Cherry, 305-50): cross-str. on ETMB Lac

	SW1060	PB1
Taunton (Ch. strain)	—	—
Bucles (" ")	4 plaques	—
Dundee / Dundee	11 plaques	ca 30
Dundee / SW618	—	++

After 48 hours, SW1060 streaked in 1, 2, 3 zones → SW1060' (

Try to grow Dundee, Bucles on SW1060.

63C1 FA90 × 63B1 / not
63C2 FA10 × 63B1 / not — Hold 6 colonies for later lysogen. test

Tom's ?? Mal V.

7/1/53...
TCN 174-95 for further study. From W1325 × W1394
Streak out 2 colonies from EMS lac. 1 → pure lac+ Mal+; poor growth on Stac
2 → lac, Mal +, − and ? ↓
Restreak 4.? on Stac: many satellite pinpoint colonies
1,2,4: variable colony size on EMBlac; no −
3: Mostly lac−, Mal−. Occ. small +?

7/4. A. Restreak + colonies from 1,2,4 on EMB, EMS Mal /sen.
→ no overlap of sensitivity on EMB. of EMS; e.g. on EMS! edges of many colonies look lysed (or lac v?)
~~all pure lac+!~~
Test parents, intact +, − for sensitivity to "ϕ".

B. Restreak 6 clones from 3 (pres. + or v, not −).
all mottled + on EMB lac → pure lac+!
Replica of 1-2-3-4 above: 1, 2, 4 and lac+ 3 are S^R Mal+
lac− 3 = S^S Mal−

C. Tests for ϕ: using parents, 2+ 3− and 4+ as indicators, and parents, mottled + as donors: no signs of ϕ in cross-streak on EMB-O

maybe diploid or more likely, contaminated prototroph + pseudomonad satellites.
Try Mal + prototrophs on EMS Mal /sen.

Write p.v.?

7/8/53.

N97 s.c. to broth

A1. —
B ~~A~~ UV 30 sec (washed cells) in b plates (java b, and minnesota b)
C ~~B~~ Δ - 48 ~~10~~ 10 min.

Numerous swarms - more in B than A ? - in b (java, Edmonds)
None in b (minnesota)

wte A4: b, 1,2 ++ ? Rebuilt pen. soil.

7/11/53. (UV - 40 sec.) Standard size inoculum.
swarms/plate 24 hours. (two batches)

A 8, 3, (5)
B 2, 10, 2
C 1

Too variable for any clear result!

D:
after 48 hours, A and B 1 each in 6 min.
b + 1,2 ++ ? v.s.c.

Inoc 7/14 in 1,2.

A1, A4
B1 B2
C1 C2
D1 D2.

7/19. a few beginning to swarm, very unevenly

7/20. C1, D1, D2 } → inocc.

Inoc 7/19 in 1,2

No prompt swarms through 1,2 screen in all tests!

7/26 Buffig.

Serine assay for Singer. 1066

6/18/53 W-1975, 1977 in D/O) + glycine or serine r/ml in 10 ml tubes
24 hours P17.

		1975	1977	
0		−	−	
L-serine	1	+	+	
	5	++	++	
	10	++	++	
	100	+++	++++	++++ est 10^9
glycine	1	+	−	
	10	++	−	
	100	+++	−	(+++ est. ca 3×10^8)

confirms previous conclusion that W1977 is specific for serine.
In both cases, 100r serine showed early inhibition – serine itself or impurity?

6/20/53. W1977

X.	~~100r~~	·
	14r	·
	40r	· +
L-serine	1r	·
	10r	·
	20r	·
	100r	·
DL-serine	200r	·
	—	.005
Small samples	100r (X)	·
	50r (X)	·
	10r (X)	·
serine	50r	·
L-	10r	·

(over)

X in sol 2 mg/ml
10r/ml in 10 ml tube
≙ .05 ml

(Later) Singer's compound has about 10% activity of L-serine.

200r dl-serine ≒ 100r l-serine.
Inhibition of early growth at serine > 50r/ml.
Might be possible to select resistant mutant, not tried.
Occasional (ca 1/10) tubes at low serine adapt.

Full wild growth not achieved for either W1975 or W1977 with 100r serine, or 100r glycine. Mixtures not tried in this expt. Recrystallized L-serine used for these expts.

Szent-Györgyi later stated his
compound was mostly
aspartic acid! (Activity frequencies ?!
asp decarboxylase?

TM2 monophasic ph 2

1067

7/17/53. SW1061 received from Edwards 7/16.
Both it and silent mutant TM2[2] did not migrate in 1, 2, 3. serum.
=67A1

Cf. also Tube-stab "TM2" ph 1,2 5/12/53., and TM 2 phi
=67A2 =67A3

67A1
67A2
SW1061 } mutable in 1,2 serum!
all 1,2 ++ i −

Save SW1061 for further study.
Prepare FA 22/1061.

67A probably came from 1039-1; confused history, but likely a single VSA colony i → 1,2, while looking for absence of cross reaction.

67A3 = TM2[i] : i ++ 1,2 −

67A3: 1-4 (s.c.) all react 1,2 +++ i ++ promptly!
i serum
Repeat 67A3-1 in i

Put the 1 → 3 s.c. / on NA
to both } all 7 1,2 +++
i − or ±
1,2 ++
1 ++ −
b −
from NA with

1061 in Tube → 1,2 +++

67B7 = 22/SW1061 → SW666. No mut b in tubes though numerous swarms in plate, and not in tube. Not to be repeated! ∴ H_1^- $H_2^{1,2}$

In 1,2 serum, A1, A2 and SW1061 are each immobilized.
TM2 (A3..) passed readily through both i and 1,2 → i ++ 1,2 +++
22 × 1061 also immob. → i +++ 1,2 −
esbatore

S. bunzendorf transduction

7/18/53.

SW 1060 from Cherry reported to be susceptible to PB phage ~~Bucks~~ Bucks, Taunton. by my hands, Dundee ++.

① vir 1,2 ~~susc~~ → c phase. This was tested as follows.

FA10 (122B)	— lpl?
FA90	—
FA18 (227M)	+ sparsity of plaques
Dundee	+
Dundee/618	—
PLT7	+

680 1 Dundee / 1060c
2 Taunton / "
3 Bucks / "
↓ T-0.
no activity on 1060c.

This strain seems to be ~~susc~~ susceptible to PLT22 and PLT7 as well! Attempt to grow these phages on it.

Slide agglutination SW1060 (from E.M.B. Agar, dmit):

—	—
IV V XII	+
IX XII	—
VI VII	+++
VI VIII	++
II XII$_2$	—
II XII$_3$	—
IV XVII XII	—
c	—
1,5	—
ρ	+ grain
Vi	—
V	—

definite reaction with S. paratyphi B. serum

cf. other bunzendorf and cholerae suis.

No overt lysis of PLT7/1060c; PLT22/1060c. "Lysates" still active on TM2 (carryover?). Try ~~×~~ SW666.

7/20
68A1 7/... × 666) no motility
68A2 22/... × 666)
↓ not! → i (probably carryover of PLT) ~~SW1060c itself partly motile: motility~~ type proceeding

SW961 also reacted slightly with B serum, C_1 ++
SW853, 732, 737 reacted poorly with both in first test.

853 also showed plaques (?) vs. P7, P22.

· T.V. and start fresh after summer.

68B.

in C_1 (somatic) or C (H) serum as indicated

7/21 – 7/26. Initial motility not checked. Initial check in C_1 (.01 ml or .02 ml/tube), about same.

7/26:

1. SW1060C /c → 1, 5
2. " ← PA22 /c → 1, 5
3. SW1060C /C_1
4. " ← PA22
5. SW1060 /C_1
6. " /C_1 2×
7. " ←
8. " ←

Vi + Typhi ×—×.

1069

Misc. ϕ —× 0901

9-10/53. See 1071.

2a 9/10. Motility SW 759, 760 through plates of semisolid.

after 2-3 passages, both cultures were actively ~~not~~ motile ~~but~~ but reacted Vi + d−, except for an original SW 760 which was Vi −. Passage strains of SW 759 M2 tested × in semisolid tubes ± d antiserum.

A. 1 no serum +++ in 24-48 hours.
2 d " 2 tubes Complete inhibition.

3 FA 10 ×, " no outgrowth
4 FA 22 ×, " " ".

discard after two washes.

B. Use A 1 as inoculum — found not very motile.
Repeat passages.

9/15. C. Various det't phage from Anderson, rather low titer. Test × SW 957 in tubes: (820, 63, d 1, d 4, d 6, 25', 28', 26', 30', f 2) and no ϕ control each
10/19. negative. Retransfer to fresh tubes for recheck.
29' swarmed. Repeat test.

However, "control" FA 22, FA 85 gave no swarms: former a few hours?
On recheck, 28' and 820 swarmed in second tube test not the 1st. Appearance of multiple sites of initiation??

Try other markers × Vi.

Hfr – Gal.

Hfr – Wj 28.

1070

7/27/53.

W1895 x W1321 in Penassay, then streaked on EMB Lac, EMB Gal sm. 5 plates each.
controls ok.

1 full lac+ 1 full Gal+ (ca 1% each. of all lac+ S^R).

Restreaked on EMB Gal sm. Heldas 1070 A1, A2 – to 11 Lb to test.
(should be M-)

Hfr ... 12

Hfr 3	x 1177	control
1070 A1	800-1,000 cells/ml	—
1070 A2	"	—
control	—	

10/6/53 W1895 + W2333-8 in broth overnight. streaked on EMB lac sm: All showed lac+ S^R ca 1-3%.

W 2333
~~2334~~
2335
2336
2338

Use 2333 for further crosses.

2337 gave few or none Mutant steril lac+.

K —x SW666
22 —x ....

1071

September 5, 1953.

A. SW957 x—
B. SW666.

1. Control
2. FA22
3. K (endogenous strain)
4 4's.
5. FA84.
6.

Plates

A 1 —
2 no sw on plate
3 ✓ numerous sw. = 6 all d.

B 1 —
2 ✓ numerous sw —
3 ✓ 3 separable. streams or tracks.

3: <2-d (all 3: (not kynogenic R / not 51 / Gal-)

4 k
422

Tubes.

no sw.
no sw !!
1 +, 1 —

✓ no sw
✓ sw
1 +, 1 —

✓ streak
✓ streak

5. 5-6 streams ca 100 tracks. Picks and isolate streams = 71 A5.

∴ K functions as transducing phage. FA22 appears to have lost titre in part. Use 71A5 for x—gallstreams in d serum. (Cf. H401)

1071A6. 957/k (not notified). Tested and ✓ resistant to FA51, k.

9/9/53. k —x ... sw. in motility agar 1 plate each

C.

1	550	—	
2	1063	—	
3	1064	—	
4	1066	—	
5	1067	—	
6	1068	—	
7	71A6	numerous T + S.	
8	1072	+ →	1,2 +++ i ++ ②++ ⑤+ !!

DL FA51 (01) —x 71A6 —
2. 53A " " 1 sw ?
3. 53 " " 2 sw
4 ——— " —

streams: significance ?

(over.)

Note reactivity ⑤ as well as ②.

Note ⑤ = S. berlin/S. para B. X-Rx may be related to ③.

September 15, 1953.

58-161 from stock (MLM) streaked out. Pick one colony = 1072-01 (Ety3 Sac)

9/14/53. Restreak 01 for reisolated single ~~cle~~ clones.

9/15/53. Save samples; inoculate motility tubes and plates. 1-10 11-20

A: P19. N20 AB B:

9/18.

	A	P19. N20 AB	B
1	+++	–	+++
2	+	+++	
3	++	+++	
4	+++	–	
5	+	+ ?	++
6	+	+++	
7	++	+++	
8	+	+++	
9	+++	–	
10	+++	–	– agar pretty n.g.
11	+++	–	– P23 +++
12	++	–	+++.
13	+++	++ ±	
14	++	++	
15	+	++	++ to +++
16	+++	+++	isolate.
17	~~+~~ +	+ · ++	
18	±	++	
19	++	+++	
20.	+++	++	

→ streak out +++.

+++ = nearly confluent plate or through tube.

~~Restreak + + to fresh plates.~~ all to SP18.

Hold tubes addl. day.

P23: A10 only non motile culture. Restreak from top.

Store streak plates to 10/5. Pick single colonies at A, B. (inoculum and after growth of second motility selection) for complete tests. Note sectored nucleoids at ~~A~~ 16, 19 A-B, 20 B (some A) ~~A~~ #1, 17, 18 B. Relation ship to selection? Restreak variegated colonies from 19B. Note noticed in series A.

not apparent with

10/8. Test large loops of concentrated mixtures
± W1177, W1896 on Bl/Blac.
for compatibility scoring

10/10. Most X ~~W1896~~ overgrown! check this parent!
W1177. (contaminated ??)

	× W1896 yield	predominant bac+or − .	
1	XX		
2	X		
3	XX	−	
4	+	+	
5	++	ca = sic	
6	++	+	
7	XX	−	
8	XX	−	
9	XX	−	fuw +
10	XX	−	"
11	+++ ?	−	
12	++	−	sic.
13	XX	−	
14	++	+	
15	X		
16	X		
17	++	+	
18	++	+	
19	XX	−	fuw +
20	++	+	
W1607	++	all −	
78 A1	XX	−	

note ratios might be distorted by growth

XX confluent
all × 1177 are XX.

Repeat 11/1/53. (test — B)

	W1177	W1876
A1	+ heavy streaking.	+
B1B	2?	+
2	4 5?	+
3	−	+
4	−	++
5	−	+++
6	−	++
7	+	+
8	−	++
9	−	+++
10	−	+++
W1607	−	++
—	—	—

of 1-10, 1, 2, 7 may be F+ still. Rebuke 3.

Some platings may have been too heavy.
unless Hfr!

5, 12 should be rechecked after compatibility is contained.

Motility selection.

1072
summary.

11/7

58-161 streaks streaked out. Individual colonies = 72A (1-20) grown (at 37°) in motility tubes (1-10) or plates (11-20). Mass inoculum for second passage. Also streak out these inocula and save s.c.i. = 72B(1-20)A. Streak out second passages and s.c. = 72B6(10)B-, for F test. Tests on 1-10 by JF (allogram separately, washed and plated D(0)) 11-20 by TCN (grown together, plated on EMSlac Th sm, DO Th sm).

Results on B×B series: All F− except 1, 2, 6, 7, 14, 15. 14 may be fersi Hfr (cf. W2206 = 1022 C3). Showed up by reduced fertility ♀ F+ as well as conjugability. Four prototrophs were recovered on EMSlac: 1: 1+/1- /2 2 1+/3- /5 6 1+/1 7. 3+/3- /.. and thus probably bona fide. Should be recovered, and further selected.

Series A should also be renewed.

S. gallinarum → x.

1073

A. FA84 → x H901 not. B " → x 71A6 not. / 1 sw 1043.

A: 4/5. 9/18. → all gm+.

B: ditto ....

Previous failure may be attributed to insufficient motility of the H901 used.

Brantford: not. T.O: a/19.

Brantford → j+++, pv− gm− A4: may be mixture of j, gm.

rx: pv+++ j++ gm±

A5: j ++ pv ++ gm? d?

B5: gm ++ pv+++ j− d−

purified A0: j+++ d− gm−. = SW1097

A1: s.c. = SW1097. 6/6 gm± j+ (gm or j or xzw?) !

A4: s.c. 4/4 gm± j+

But note: A1 reacts ++ w/ pv
A0 −

in camp test. j: sw 1041

Region FA85/SW775
84/SW774

9/17. 84A, 84B } S. gallinarum; 85A, 85B } § S. pullorum → x 0901.

gallinarum: few trailers. 4T; 1S.

pullorum: few trailers. 2T

Tracks too short to isolate for selected higher transconjugants

0901 motor S.

later checks i fresh dilution of gm serum (1/60 of 1:5000)

Jamboree SW1041, 1097 gm ++ pv++ j −

" 1096 gm − pv ± j ++.

Further trials on H, H, H_2 from N97. 1074

10/10/53.

A. Test SW1028 = orig. N97b → S. marini, b: 1,5 in b, 5 serum for occurrence of 1,2 phase. 1: stock culture 2: motility passage

B. SW 1043 FA86b (Long Beach) → SW874 (Loma Linda) / a, enx [for b(12): enx]

1 2 3 4 } stock culture 3/4 migrated → b (whl. growth). #4 late – still enx

Rec s.c.i / b. benx

vb ✓ → enx

see over.

C. SW803 FA15 enx (abony²) → N97 original / b; 1,2 for (b:12) enx

(cf. SW926 1051–1055)

6/6 tubes migrated in 24h. 10/11 PM stock out.

all enx: Rec s.c.i. in enx, benx

(poss. contamination abony in phage? Re-FA15B 15C) stability. → all b.

P11. Purify B1-3 C1-6.

A17. A1. → 1,... → s.c.i $1,^{2}_{r++}$ 5 r+ 2− 1,5 r+. Probably had some 1,10

A2 nomvgn. yet

C2,3. slow buds. otherwise still almost stationary.

in b, enx

B, C / b, enr.

10/16 — C3 b+? z_{33}+? 1,2- enr- somewhat rough.
C4 stillenr+, b- ~~1~~ 1,2 -

These were enlarging buds, not swarmers. scheduled s.c.i.

10/17
swarmed to bottom B3
C1

Retest single colony isolates:

10/19 CB : b. z_{33}? + 1,2- enr- Remotely.
c~~3~~ : enr+ b; 1,2; enr-
c4 : enr+.
B3 : Rough. b?

1074

A) 976 → x main → b; 1,5 /b,5 → 0 ✓

B) sw1043 → x (δ) lama hindi → b → eux ✓
a : eux

C) abnyeux → x N976 → eux → b → eux.
b:eux ✓ → 0.
no; 2
(tighemi)

---

pure eux of eux → x $H_1^{1,2}$ H_2 eux is still H_2?

---

pullorum, gallinarum → ×

1075

ca 10/10/53

Note: 1043: pullorum was tried extensively on SW1040/a and failed. Other pullorum ** should be tested × 957 for highest activity.

A. ↙ →×957 Test n.g. 3/4 owing to overgrown contaminant. All others gave numerous trailers. Also swarms in P2, 9, 12. P2 probably most active. (Test each on ½ small plate)

× B. P, G1 → × Lonsdale 874/9, enx no swarms. test 2·P and 1·G another 10/18: still no

× C. P, G1 → × Eastbourne 770/chx; 1,5 2 each. No swarm.

13 D P, G1 → × SW967 (dublin 0) G1: few long trailers, 1 swarm. P1 single trailer!

× E P, G1 → × SW989 (TM 0) no T or S in either

× F P, G1 → × SW991 (dublin i)/i 2 each

× G. P2 → × SW1004 (Swinton)/a, 1,5 ca 10/14. } 3 each no swarms 10/19.

× H G1 → × " " " }

J P2 → × SW1040/a No swarms 10/19. Held to..

C, F poor migrators, rough. C still 1,5... F still i.

× ① SW1028/b+5. → slow bud: b- 1,2 +++ 1,5 ++ 2- 5÷ 1,10??

② (motile) v. slow spread noted 10/19.

(on next transfer → 5++ again)

S. typhi seemingly only receptor for phage from gallinarum. Results with pullorum equivocally negative.

N. not finished.

11/5/53.

A. Mix ca 4 hours in Penassay. Plate out on EMB Lac.
Pb. (C.M.) Pick and streak Lac⁻ colonies (ca 1% of total) on EMB Lac

Morphology (1 or 28)

	Lac	Mal	S	MH	Xyl	dil'	Lac	Mal	S	MH	Xyl
1	+	−	S	R	−	+	+	−			
	+	−	S	R	−	+					
	+		R	R	+	+		+			
	+		R	R	+	+					
2	+		R	R							
	+		R	R							
	+		R	R	+			+			
	+		R	R							
3	+		R	R							
	+		R	R							
	+		R	R	+			+			
	+		R	R							
4	−		R	R	+	+					
	+		R	R	+	+		+			
	+		S		−						
	+		S		−						
5	+		S	R	−	+		•+	•+	28	28
	+				−	+		+	+		
	+				−	+		•−	+	1?	
	+				−	+		−	+		
6	+		R	R	+						
	+				+						
	+				+						
	+				+						
7	+		S		−						
	+		S		−						
	+		R		+						
	+		R		+						

Lac⁻ Mal S MH Xyl
22 Xyl. Xyl. as Mal 28 as Mal as Mal
counted

Morphology

W2057 = wg 1 Hfr TLB, - lac+ S^S Mal - Xyl - MH -
W2333 = wg 28 F - lac - S^R + + +

B. Repeat:

1076 A.
Summ.

W2333 × W2057.

IF— : Mtl-Mal-Mgl-S^S / +++ ^{R} completely linked.

13 Lac+ Im tested.

8:	Lac+ Mal+ / Lac − Mal+	R1/P1
4:	Lac+ Mal− / Lac+ Mal+ / Lac − Mal+.	R1/P1/P2
1:	Lac+ ~~Mal−~~ / Lac − Mal+	P1/P2

P2 type was morphologically distinguishable: lighter colour.

Note (as before) absence of ~~Lac~~ Mal − recombinants. Recovered P2 should be included for HFr (incl. ~~Hfr~~ Stellae). Also ~~with~~ morphology.

Hfr × F⁻ unselected.

November 5, 1953.

W2057 + W1894

24h. parasexual cultures 1+1+5ml Penassay 4:40PM — 9:40.

Dilute & plate on EMBlac ± sm.

Test XYL, MAL, GAL, LAC/lac⁻ for analysis

EMBlac sm. No SR+ at first. Ca 36 hours, weak lac+ appear (presumably Gal⁻).

A. EMBlac: from ca 2 plates, only 4 possible lac+ at first. Two gave lac+/−; two pure lac+ Test components as A1-2.

2 Gal+/−. Later, more found and streaked out. Little likelihood of colony admixture, especially with lac+. lac⁻ → lac+.

B. after ca 36 hours, weak lac+ appear, almost certainly diagnostic of lac+Gal⁻. Test s.c. from A, B.

all pure Gal⁻ in spot tests. → 17: all S^R Mal+ Xyl+ Lac ± Gal⁻ # 2 MAL⁻ times + (15/16 ?, ,,)

A1.

	Lac	Gal	S / MAL / XYL	
1	+✓	+ +	+ +, −R	−
	−, +·	+ −, +	+ + R	−
		+	+	−
		+	+	−
2	−	+ −	+ +	−
	−	+ −	+ +	−
	±	+ −	+ +	−?(R)
	−	+ −	+ +	−?(R)
3	−, +·	+ −, +	+ −, +R	−
		+	+	−
		+	+	−
		±	−	+R
		±	−	+R

some v. tiny lac⁻ colonies.

A2.

	Lac	Gal	MAL / XYL	S	
10	−	+ −	+ +	− +R	−+(R)
		±✓	−	+	+R
		±✓	−	+	+R
		+	+	−	−S
		+	+	−	−S
11	−+	±✓ +	− +	+ +R	+R
		±✓	−	+	+R
		±✓	−	+	+R
		+	+	−	−S

Added: lac+,− from 2, 4, 6, 7, 8, 9, 11.

On 11, lac± verified. Others either ++ or − test. Check on applica to EMB Gal.

Aim of expt. is to determine the incidence of parent parotype and of Mal/Gal recombinants. These seemed more frequent than Lac/S.

A1 cont.

	Lac	Gal	Mal / Xyl / MH / SM	
4	−	+	−	+ R − S
	−		−	+ R
	+		−	+ R
	−		−	+ R
	−		−	+ R
5		+	+	−
		+	+	−
		+	−	+ R
		+	−	+ R
6	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −
7	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −

	Gal	Lac	Mal / Xyl / MH / S	
8	−	+	−	+ R −
	+		−	+ R −
	+		+	−
	+		+	−
9	−	+	−	+ R −
	+		−	+ R − + MH, Mal
	+		+	−
	+		+	−

Numbers of 4-8 clones of A2 4 6 7 8 9 11, except in #11 are lac+, were Gal+ S^S lac−, Gal− S^R

Notes: 5: No lac⁻ clones in master but retrieved poss.: 3 clones seen on retrieval → Xyl to EMB Lac ± ones

Better procedure in lab notes for records. Might be to replicate as in 78A.

✓1: P1+P2−

5: P1+P2− seen +R1

4: Lac+ are Gal+ S^S (i were Lac+ Gal− S^R prob see

DATE: 11/14/53. REF: 1077 SUM.

A. Test for symbionts. W2057 S × W1321 R

Hfr TLB, Lac+ S^S Mal- Xyl- MTL- P2. Lac- Gal- S^R M-F- P1 and R1 = P1Lac+.

11 lac+ colonies: ✓ further

1	P2, + P1 ✓	Purity of A1-1, 2 + P1 + ?	Probably P+P2 ✓ + some P1/P2 lac clones?
2	P1. P2 ✓		
3	P1, P2, R1		
4	P1, P2 ✓		
5	P2', R1	- + P1 ✓ ~~not~~ P1?	~~Feodtest~~ diet:
6	P1, P2 ✓		
7	P1, P2 ✓		biparental 7
8	P1, P2 ✓		biparental + R1 4
9	P1, P2 ✓		outbiparental + R1 0 (not easily sought)
10	P1, P2, R1		
11	P1, P2, R1		

no further record of presence of lac± : may have been present in some of these.

5 Lac- arGal- S^ = P1

B. Lac±. No effort to identify lac- components. 17 isolated were all Mal+ S^R Xyl- Gal- (outbiotypic). 16/17 also MTL-. Class B might have been favored by less restricted conditions for picking. [Gal and Mal-S generally concordant, but exceptions not yet looked for.]

Notes: defer more detailed analyses for single cell, Gal- lac+ x Gal+ Lac-.

These results are now interpreted as symbionts from which recombinants may or may not ensue.

Hfr F -

11/10/53.

Pre-sodayovernight (ca 48 hours). 1:1:10 Incubate 2-5PM

A. W1895 x ~~W1177~~. 1956. Plate on EMBlac ± strep (E) 11/12. Check W1956 on EMBMal OK.

B. W2057 x W1321 " "

C. W2057 x W2333

11/11 (D) W2058 x W1578. P12: no lac± noted. (Hfr??)

C:) Gram. 10^{30} A11. 12 plates EMBlac + ~~1~~ strep. No SR+ noted.
These plates have lac- > lac+, well separated colonies. Only well-isolated lacs/ picked for further study.

1- irregular margins. dark center, ~~irregular~~ ragged edges. No definite sectoring. No likelihood of contamination unless noted.
4/5 merely puggy. 6 mean lac- but not touching: 7 11 ~~[illegible]~~.
9: def. sectoring 10 touching +. Also remember.

P11. +2: small, puggy 13. C.M. struck out from my pick
(cont. c+) These colonies generally smaller than others.

B) - hold E). lac+ >> Lac-. SR+ ca 5% of Lac-. Lac+ too numerous for present purposes. Hill Sigal - by replica test,
9/19 one lac+ V_1^S, 10/19 lac+ V_1^R

P11 B): Preferably lac±, whether weaker staining +/-, that are not likely contam. & lac+. Separate some possible adjacent +/-.
Preferably lac± that are not merely simple sector.
14 picked, all of type ◉ 15-20 are simple conjunctions.

A. EMBlac Lac± of two types: ① and ② Nicolsen EMB Mal
(vW1956 Mal!) 9/14 type 1 and 1/3 type 2 had Mal+ (presumably the (1-9) para-type parent. Restreak to analyses: spot s.c.i. lac+ and replica to EMBlac sm. (1 plate inadvertently L.C.M. 3 streaked on EMB sm: note several phenotypes!). (over)

Mal (in order)

A: + 3

− 1
− 2 + 5 − 14
+ 6 + 13 + 15
+ 7 − 16
+ 8
+ 9
− 10
+ 11
− 12

✓ i Carol sequence used in streaks.

As EMB Lac, #2, 6 apparent

3, 6, 8 Lac+

13 – 16 as EMB Cal. 13 Cal++ > Cal− > Cal÷.
14. Cal± > Cal−
15. Cal± > Cal−
16 Cal÷ > Cal±

	P2	R1	P1
	Lac+ S^S	Lac+ S^R	Lac− S^R
1	~~~~ No	Rare ✓	−
2	No ✓	✓	✓
3	✓✓	occ.	occ.
4	No ✓	✓✓	✓✓
5	✓	✓✓	✓✓
6	✓ [Rare (secondary ?) →] No!		✓✓
7	✓✓	✓	✓✓
8	✓✓	Rare see.? No	✓✓
9	No	✓✓	✓✓
10	No	✓	✓✓
11	~~~~ ✓	✓	✓✓
12	No	✓	✓✓
13	✓	✓✓	✓✓
14	No	✓✓	✓✓
15	~~~~ ✓✓	No	✓
16	No	✓	✓✓

Notes:

(Lac+ only possible in orig.)

∴ assume there are in order by C.M.

Note v. many + to test ≥.

Recheck for presence of Mal+! Do not grow on O

If we accept 9 as P2− we have:

5 ~~4~~ P1+P2+R1 : 3, 5, 7, (9), 11, 13,
3 P1+P2(+R1??) : 6, 8, 15
7 ~~4~~ P1+R1 : 1, 2, 4, 14, 16, 10, 12

A9 = Lac + Mal + S^R Lac − Mal − S^R
+ Lac − Mal + S^R mut

(P1+R1 not detectable), S^R mutant?

Retitle

1, 2 | Lac +
9 | Lac + → mostly Mal+ or mutants

! check types on other markers!

11/16/53 Clean up A, B, C.

A9 Mal+ >> Mal− (1, or 2 - noted as streaks). Replica streak plate to EMB Lac ± sens. Mal− are lac− S^R (No S^S noted!) Mal+ are lac+ S^R lac− S^R

A1 Mal− only lac+ = S^R. P1+R1

A2 lac− only. (+ papillae) Mal− only. P1+R1

E: Mal Xyl−. Replica to EMB Lac T1.

B. 1 streak out possible Lac+ Sal− (11: ~~8~~: 1-4; 8:4)
both → lac±.

C. Plates heavily inoculated and overincubated hinders scoring lac+/−. 4,5 pure lac+ S^S 6: Lac+ S^R
inoculum mix scores: Repeat replica to lac, Mal, [redacted], Xyl, Mtl.

Concordance of Mal− Xyl− Mtl− S lac: Lac F
+ + + R − −
− − − S. + +

Mal →

	Lac−	Lac+	
1	3−	1+ 4−	R1 P1 P2
2	1−	3+ 4−	
3	4+	4−	
4		4−	
5		4−	
6	4+		
7	2+	3+ 3− (weak lac+)	
8	2+	3− 3 weak lac+ Mal+	
9	4+	4+	
10	4+	2+	
11		4−	
12	3−	3+	

	Lac− (orthotrip par.)	Lac+	
1	4+	3− 1+	P1 P2 R1
2	4+	3+ 1−	P1 P2 R1
3	4+	4−	P1 P2
4		4−	P2
5		4−	P2
6	[redacted] 4+		P1
7	[redacted]+	3− 3 Mal+ weak lac+	P1 P2 R1
8	~~4~~ 2+	3− 3 Mal+ weak lac+	P1 P2 R1
9	[redacted] 4+	4+	P1 R1
10	[redacted]+	4+	P1 R1
11	[redacted]	4−	P2
12	~~3+~~	3−	P1 P2

∴ 4 tritype + [redacted] < 3P2 1P1.
2 kiper
2 bitt ortho
Cf. R1 1,2 vs 7,8.

(over)

1078A9. (c) Lac+ Mal+ : Gal+ Xyl− MM− S^R

(b) Lac− Mal+ : Gal± " " "

(a) Lac− Mal− (1) : Gal± " " "

(a) = ω1177 = P1

(c) = R1 Mal+ } Mutation ?

(b) = P1 Mal+

Synbaryon tests.

1078

DATE: 11/14/53. REF: 1078 S4M.

A. W1895 × W1956. Final lac ± 10/17 had a Thal+ component.
Test isolates for fraction also showing an SK+ component:
Tentative conclusion: of 16 lac+/-, 8 are biparental; 4 are biparental + orthotype recomb. and 4 are orthotype parent + recombinant.
Nutritional markers not tested.

B. W2057 × W1321. 14 lac ± isolated and types picked. [Adj. mixtures showed no lac ± on streaks of this set!].

lac+ segs as following: (not recorded for 2 days!)
4 lac+, 4 lac- from isolates: #3 has lac- only.

	+	±	−	
1	·		·	P1 P2
2		·	·	P1 P2 #2
3		·	·	P1
4	·		·	P1 P2
5		·	·	P1 P2
6	·		·	? P1 P2
7	·		·	P1 P2
8	·		·	P1 P2
9		R		P1 [illegible] R1P
10		·		"
11	·		·	P1 R1
12		·	·	P1 P2
13	·		·	"
14	·		·	"

Parentals only except poss: #11 lac+ Cal-
#8/4 " Restreak for check

Test: 4 bipar + orthorecomb. / 2 bipar only. / 1 part ortho rec. see 77A ⇒ 4:7

Total 77, 78: 6/19

In view of ambiguities, test replicas/but do not pursue
ripe of forthcoming Cal-lac × Cal+ lac−
What tests? No record except for lac, Cal, S.

C. W2057 × W2033 Lac+ and − except in 4, 5, 6, 11 (# spots +)
(4, 5, 11 had not been lac ±). 12 n.g.
14 n.g.

P1·P2 colonies suggest that, picking from colony morphology units probably single cells are synbaryotic. Occurrence of recombinants strengthens this assignment. (Look for V1/S recombinants?)

11/16/53.

ca. 1:1 in Synd broth 1[15] PM – 4[45] PM.

A. W1895 } x W2033 on EMB lac ± sm.
B. W2341 }

Lac+Gal− S^S × Lac−Gal+ S^R no easy way to detect genotype parent or Gal/S recombinants.

AA. lac+/− control AB: sectorial and pinpoint

A.C. fractions of parents. Replicate strokes to lac sm.

SR+:

AA. 46 colonies. 44 SR+ ~~~ 1 lac+ rare 1 lac+ inf. mostly S^S. Not certain whether secondary SR+ are completely controlled by AC. Note low proportion of P1+P2 in this entire experiment!

AB

10 probable P1+P2 (no or secondary SR+) / 36 total.

AC. 16 Spotty +/− only.

B. 9EMB lac (accurately plated .1 ml from 10^{-6} dil.) and 9EMB lac sm

On EMB lac, score all lac++ : almost all variegated with lac− or lac±
Scores may include some lac±/lac−. On EMB lac sm score lac+ and lac+/−. May include some S^R lac± but probably not.

BB.

	lac+...	lac−	Total	
Lac	1 23		551	
	2 24		534	
	3 ~~18~~		592	
	4 18	255	569	{255 are Gal+}
	5 22		563	
	6 34		545	
	7 19		576	
	8 19		516	
	9 22		560	
Mean:	22.1			

Σ 199

BA

	lac−	lac+	lac+/−	Total S^R
11		1	23	343
12		1	14	331
13		0	2* 22	308
14		0	33	302
15		2	22	295
16		1	33	320
17		1	18	335
18		0	23	328
19		0	24	345
Mean:			24.2	323.0
		6	212 / 218	

(only 2 under others →)

Averages not appreciably different. Unlikely that any genotypes are confused. I.E. SR+ are almost certainly lac+ Gal+ S^R. To identify Gal− would require separation of lac+ components and get total counts. All appear ++. Check pure + = 79 BB. (over)

EMBtal: low count of fuzzy tal+ or +/- colonies. 1-4/plate
= BC. scored, but not very distinctive

BB. On enriching, 2 were found to be pure lac+, others had rare lac-

Parent (means).

lac-	= 323.0	lac+ =	(545) 323
	- 14.2		-
	298.8		222.

BC: 15 tal +/-? or tal ±. Streak on EMB lac

	P1	P2	R1	
1	✓	✓	✓	
2.		✓	✓	sic = F3
3	–	–		
4	✓			
5	✓	✓	±	
6	✓	±	✓	
7	✓	✓	✓	
8	✓	✓	(±)	
9	✓	✓	±	
10	✓	✓	±	
11	✓	✓	±	
12	✓	–	✓	
13	✓	±	±	
14	✓	✓		
15	✓	✓	±	

1 R1+P2
3 P1+P2
10 P1+P2+R1

(1 P1)

EMB lac sen. Prop. yields of SR+ similar to 79B. 17/17 are sens lac+/- colonies
lac+ sectors usually small. Save for picture.

EMB lac. Numerous ⊕ colonies. All that can be learned here is the incidence of SR+ among these. Distinguish type 1 (A1-2) —, central + & radiations but surrounded by lac- and type 2 (A-3) — multisectored purple/lac+/- . On EMB lac sen, type 1 : type 2 = 7:5
12:9:1+
(Type 2 are less characteristic on EMB lac). Do not use this series (in preference to 79B) to test persistence of parapartial component.
Save some plates for photography. Note that lac+ recomb. are distinguishable here from W1895 lac+ also.

11/18. In replica from strokes, A3: virtually all had numerous SR+.
A1-2 not yet tested; also confirmt P1/P2 colony tests to be done.
Ca 9/36 had a lower incidence of SR+ than others. But this test is essentially too crude.

BAEMBlac. Mark clear +/- — and fuzzy +/±/- —
Pick only clearly isolated colonies. Among scorable, isolable colonies:
— are fac less conspicuous as
(and are lac±/- with
possible lac+ component)
isolable colonies.

BA 2	11	3
4	2	8
3	7	2
4	5	2
5	3	2
6	7	1
7	5	3
8	7	3
9	6	3
40		

an addnl. unnumbered plates:

11	2
1	1
6	3
7	7
4	2
1	0
2	0
7	1
3	0
5	2
1	2
3	0
5	4
9	4
5	3
2	1
4	2
9	2
7	2
92	38
D	E

Gal/lac "interaction" (EML Thesis) a prominent feature.

Parents: W2431 - pure Gal- Lac± W1895 Lac+ Gal+ W2033 lac- Gal+

E = BA yellow P 1

D = BA ~~red~~ ~~1-60~~ 1-136 (6.0.)

DATE: 4/18/53 REF: 1079

D. 101

	P1	P2	R1	4
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
110	✓	-	-	✓
	✓		-	-
	✓		-	-
	✓		-	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
120	✓		✓	-
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓	✓	✓	✓
-	✓			✓
	✓		✓	✓
30	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓	✓	✓	✓
	✓		✓	✓

∴ 12 are R1+P1

40 8 R1+P1+P2, though the low incidence of R1 suggests some of these are secondary.

1 R1 only.

1 P1

50

5	2 second 6	P_1+P_2 7	R_1+P_1	$R_1+P_1+P_2$	R_1+P_2
D (uncon.)			12 6	8	
E.	57	7	5	43 (20?)	2

D:E had been estimated at the sames actually found.

D+E		7	131	51	2

but there is a probable bias favoring the detection of R_1+P_1. (R_1+P_1) is evidently distinguishable from ~~R+P~~ ($R_1+P_1+P_2$) on original plates

P_1 = ~~Hfr~~ F- parent

P_2 = Hfr parent

R_1 = Recomb

Should tabulate incidence of R1 from conjugat $P_1 \cdot P_2$ colonies.

6/29 E: Pick 2 zones at junction of P_1 and P_2 in (and streak) (4). deistruct colonies. An EMBlac, no R1 in deistruct colonies. EMBlac con- SR+ types are papillae in their streaks. Thus deistruct from types refered to above as ✓ in R1 column.

Scores of streaks of 1079D on EMB Lac

DATE: 11/18/53 REF: 1079.

	P1	P2	R1	EMB Lac sm SR+ = R1	5	A1	A2	R1	9	10
D 1					51					
10					60					
20					70					
30					80					
40					90					
50					100					

P1 = F- Lac- wg 28

P2 = Hfr Gal- Lac+ wg 1

R1 = Lac+ Gal+

1079E
scores.

DATE: 11/18/53 REF: 1079D.

	P1	P2	R1	S+
least is marked × n 1.				
1				
10				
sec. = F1				
20				
sec. > F2				
30				
40				
50				

5	P1	P2	R1	9	10
58.					

∴ This series, selected for the segregation of P2 in the colony, shows 6+1

43 (23 certain + 20?)	P1 + P2 + R1
5	P1 + R1
2	P2 + R1 (= F)
1	P1

F: (1, 2, 3) (Streak for BC). Save after verification of absence of P1. F1-2 also checked — on EMB lac ind. No Lac− or Lac+ here

(too effective indicators of recombinants)

✓ if Lac+ is considerable all harvest least papillate + very few colonies in R1 tests 1-33

1076-
1079.

	Notes.	P1+P2	P1+R1	P1+P2+R1	[redacted].
		1	8	4	Lac+/-
1076A	W2057 x W2333 sample colonies tested Mal MH Xyl-S concordant.				
Possibilities of syntheyon analysis concerned from this					
1077A	W2057 x W1321 EMBLac. Mal Xyl MH S concord.	7	0	4	Lac +/-
B	EMBlac sens. 17 all S^R Mal^+ Xyl^- Gal^- $Lac^\pm$ {16 MH- 1 MH+}.				
1078 A	W1895 x W1956 EMBLac. Test on EMB Mal for Lac+/- 9/16 [redacted] had Mal+	3	(+A9)	7 5	Lac+/-
	#9 had P1 & Lac+Mal+ S^R & Lac-Mal+ S^R				
Lac, Mal, S tested only.	(additnl. Recomb type : Mal X Son Mal+ recurrent?)				
1A: SR+: 9/9 V_1^S					
B.	W2057 x W1321	11	1	1	Lac+/-
Limited sample of colonies tested					
Lac, Gal, S.					
C.	W2057 x W2333	2	2	4	Lac+/-
Mal Xyl MH S concordant					
limited sample of colonies from each.					
1079 A.	W1895 x W2333. Test Lac+/- for SR+:				
	of. pinctatis.				
B	W2(4)31 x W2333. Also 2(R1+P2)	7	131	51 *	Lac++ associate
? * Maybe biased. Some R1 might be secondary, or many such colonies missed as not uni-cell origin.					
BC.	(P1+R1 of course not picked!). (+1P2R1)	3	0	10	Gal+/-

11/19/53

The general conclusion is that the Hfr parent is frequently associated with the F- in recombinant containing colonies. Etched question still whether these are unicellular in origin — considered like from the colony appearance ~~of~~ and from EKL findings.

It is difficult to calculate exactly what proportion of lac+/- colonies have Sk+ recombinants.

Single cell isolations of
Spirochaetes.

1080

11/16/53.

ca. 11/10 ff. Preliminary trials.

A. Prep'n mercury droplets - overlooked Topsky description. Had altered pipet to vary needle diam by squeezing. Later found simple technique, and could pick droplet off glass plate by sliding needle + drop across the edge. Droplets ca 1/2 - 2/3 diameter of 43X field found adequate and could still permit study under phase contrast.

B. Quartz o/k - Plastic coverglass shutting opaque. #1 coverglass: no killing even for 5 mins! Kodak film base allows partial transmission — and 3-5X diurnal dose, but poor optical quality. Visiting doubts with too readily.

C. Troubles: with several blocks: growth along and up sides
Peril of moving completed work by accidentally touching edge of coverglass

B/B. Plastocel 10 mils showed partial transmission (killing at 1 min dose). May be too restricted?

D. 11/22/53. after various casual efforts, try the method using 5 cm squares of agar blocks & plastocel squares about same size. 6 tests: 2 controls, 4 droplets. On controls, found 2, 1 colonies. On others, total of 26 but only 1 even close to a droplet. Complete failure!! UV dose may have been inadequate.

wg 28 Lac- Screen for test crossing markers.

1080

DATE: 11/18/13. REF:

W2341 × W1 × 1:1:5 12-N18.

A W2334

B W2335

C W2336

D W2337 - Strain already had lac+. (reversible?)

E W2338.

Recheck of these on EMB gal!

	lac V	SR+	EMB gal	EMB lac.
A	✓✓	—		—
B	no	no		flat, v. small colonies
C	✓✓	✓✓	no V	—
D				already some +
E	✓?	no		rather small

Use either A or C for future work. W2333 has the disadvantage of showing slow +.

Must be confused ↑. W2336 is Gal −, + mixed (of D)

Rec		Gal	lac
	W2333	+	− → ±
	4	+	− → ±
	5	+	−
	6	+, −	+, − Mal +, −
	7	−	− thin
	8	+	−

Use 3, 5 or 8. Try 8.

(over).

Motility.

W-2333-8, W1208A, W 2059 (wg 51) are non-motile under microscope, ~~43~~ 33 also by motility tube.

W1208 (hyaphil) is motile. W1258 (obl oval) is motile + (non motile) ?

8 A. W1258 o.v. small cols. (11/19 - 11/20)

1 B " " large.

"wg 28"

1081

11/22/53

W1258 = wg 28 = NCTC 123 as received from Cavalli. Is now microscopically motile; grows poorly on EMB; Lac+. Also should be S^S; λ^S; ... I. Re-isolate from lyophil 11/20/53.

W1258A = wg 28A. Recovered by EML from an old vial ~~#~~ 12/11/..?

~~Recorded~~ as prototrophic S^R. Mutants have morphology similar to that of wg 51 and are likewise also non-motile. Present stock wg 28A also non-motile. 2 types on EMB lac A1 = gummy. A2 - not

---

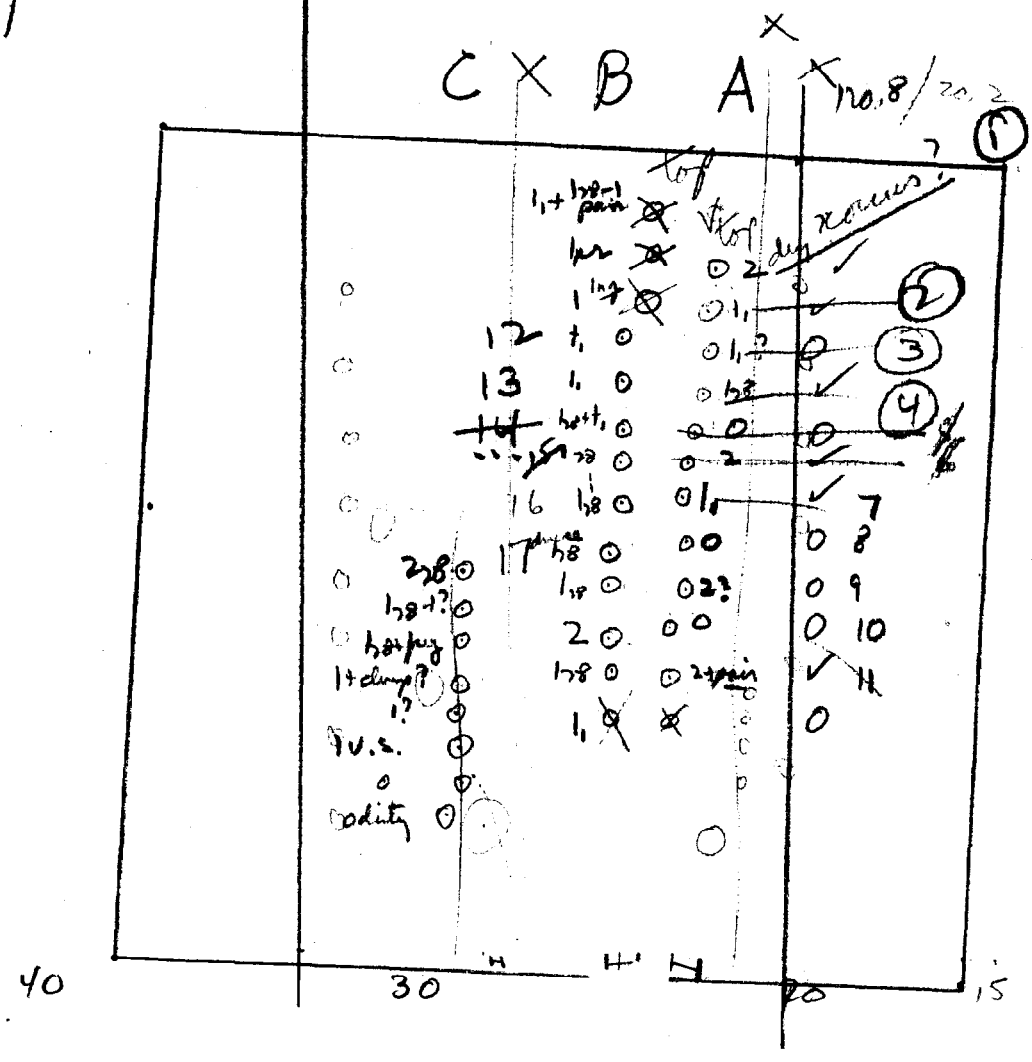
Old vial. Streaked out directly gave only lac- colonies (81A, B. both are distinctly motile. (Reisolated for presence of lac+ = 81D).

Added both, streaked out after growth mostly lac- S^R, as above. Occ. some lac+ S^S = 81C.

	Mot	lac	SM	T1	
1258	+	+ slow (s)			
A	+	−	R	R	R
B	+	−	R	R	R
C	wk +	+	S	R	R
wg 28A 1	−	+	(R)	R	R gummy.
2	−	+	(R)	R	S
2338...					S

1081

24 24-25 22-23



DATE: REF:

	1	2	3	4	5	6	7	8	9	10
1-10	2 -	1 -	1	1/8	—	—	1	0	2	0
Expo-sition			0		—	—			0	0
Growth (table)		?mud		—			—	—	—	
type loc	P2	n.g.	P1-R1							R1-P2
11-20	2 -	1	1	—	1+1	1/2	1/8	1/8	2	1/8
Gr (table)	—						—			—
type loc	~~P2~~	P1-P2			P1 only			P2	P1	

Sequence again is acceptable

~~bio~~

some single reductions

This suppl. only explanation of results n.g.

without

nodge

2338?

1/24/53.

A. W2341 (×W2341) 10:30 – 6 PM 1:1:10 Penn

(also use for all classes of isolations (1, 10×8))

processed as fully.

1. EMBLac

2 EMBLac Str (2×)

3 EMBLac Str + T1 (2×) (20×)

4 EMBGal + T1. (2×)

B. Single cells. Droplets 10^{-3} dil. in oil chamber. Study and consider 0, 1, >1 cell types. Allow to grow in chamber overnight. Pick

2: Brush lac+ S^R colonies (mostly also lac- S^R) / T1 on EMBLac

50 tested, 26 R lac+, 24 S lac+. All but one, if lac- present this was lac- V_1^S (unless masked by lac+ V_1^R).

1: lac+ V_1^S / lac- V_1^R = #18.

→ 1, 2, 3, 4, 5, 12, 13, 16, 17, (18), 19, 23, 24, 27, 28, 29, 31, 38, 40, 41, 43, 47, 48, 50

∴ ca 50% of SR+ are V_1^R. (independence of Lac, V_1 true?) [Neither of the factors is known to be allelic with lac, V. of line 1].

Rel 1117 B

DATE: 11/25-26/53 REF:

A2. SR+ /plate = 22, 19, 23,

A3 20X 233+, 130−

2X [9+ 14−; 10+, 3−; 5+ 11−;] Includes 3 vertical columns.

Therefore there are an appreciable number of lac−V_1^R S^K., but no (Gal−lac+) $S^T V_1^R$ were noted in this experiment.

A4 (no T1) fuzzy colonies (total 18). Streak EMB lac for enzymes

	lac−	lac±	lac++		
1	✓	✓			
2	✓	✓	✓		
3	✓	✓	✓		
4	✓	✓	✓		
5	✓	✓	✓	12	P1+P2+K1
6	✓	✓	✓		
7	✓	✓	✓	2	P1+K1
8	✓	✓	✓		
9	✓	✓	✓	1	P2+K1
10		✓	✓		
11	✓	✓	✓		
12	✓	✓	✓		
13	✓	✓	✓		
14	✓	✓	✓		
15	✓	✓	✓		

+T1. Gal+ counts/plate 23, 21, 14, 12− (386 Gal−)

Phage probably inadequate for total immediate lysis. Cf #2/A3.

A2: lac+S^K = 21/plate., expect 11 to be V_1^R.

A3: ca ½ V_1^R formed, not in disagreement so no evidence of lag. But phage amount needs to be checked, also character of the lac−V_1^R S^K. Seems Gal+ V_1^R, comparable (perhaps low or heterogenotes) in S^T+.

Are notes 11/27/53

1/12/54

1082

T-do. a) Haploid crosses in independence of lac, T1.

b) Het diploids for ind. segr. of lac, T1 (unless linked to auxotrophies. Use SR+ if necessary)

Try S × M7

c) Transfer lfor to this stock by bal linkage. I.E.

→ find a bal⁻ V1^R recombinant in appropriate setups.

d) Study the bal⁺/bal⁻ ratio among lac+V1^R recombinants (direct scoring!)

e) look in frig.

There are 1/2 as many lac+S^R

There may will be many lac⁻V1^S recombinants (conducted along and with P1.

Some "P1+P2" might have such recombinants. (Either test at random or replicate cross plates.) For present work this means testing lac isolates on SM7 T1. Later study "P1+P2" in plates, and try to find P1+(R2). [R2 = lac⁻V1^S recombinant]

Also do (d) above for the record.

1/12/54 au1082

No data on V_1 distr. among lac^+ / lac^- .

$S^R lac^+ V_1^S$ usually accompanied by $S^R lac^- V_1^S$ $\left(\frac{20+}{24}\right)$ = P1

rather than $lac^- V_1^R$ = recipr. recombinant.

Should now test Gal^+ $\frac{lac^r}{ctg}$ colonies for comps. Cf. 1076-1079 Experiments probably nothing saved.

More likely to be with lac⁻ parent. I.E. $\frac{S^R Gal^+ lac^- V_1^S}{S^R Gal^+ lac^- V_1^R}$.

3 = recomb. classes 2 are lac+, now detected.

1 is $lac^- V_1^R$.

If associated with P1. not now detected as seg. colonies.
not readily detectable in segregates.

∴ all 1-cell isolates must also be scored on T1.

W2338 $V_1^R S^R$ (2344).

×

PH- Het $lac^+ Hal^-$ (Gal^-) m

allelism 2338 lac.

1/12/54

082. ? How many zygotes are mixed

a). Are there recombinants not detectable as $Lac^+ S^R$
b) Are there segregates other than Lac^+/Lac^-.

a. $234Y \times 234I = Lac^- S^R V_1^R \times Lac^+ Gal^- S^S V_1^R$
F^-

$Lac^+ S^R$ essentially all Gal+. 2 V_1^R 2 V_1^S.

S^R/V_1 recombinants = (A3) 20X showed { 33 Lac+ / 130 Lac-
1 X showed $8 V_1^R Lac^+$, $8 V_1^R Lac^-$

(A2) $S^R Lac^+$ (2X) numbered ca 21 purplate.

∴ One should have predicted that ½ these would be V_1^R = ca 11.

(A3) Found $S^R V_1^R$ lac^+ = 8 purplate.

1/12/54

Per 2X plate: S^R a Lac$^+$ < V_1^R 11
V_1^S 10

S^R a V_1^R < 8 lac$^+$
8 Lac$^-$.

These experiments suggest that here lac and V_1 are unlinked to each other and segregate independently one of the other. ∴ 3 groups indicated S – Gal (almost always prototypic) Lac; V_1. If parents are

Hfr S^S Gal$^-$ lac$^+$ V_1^R
× ——— ———
F$^-$ S^R Gal$^+$ lac$^-$ V_1^S

Then recombinants are generally S^R Gal$^+$ · $\frac{\text{lac}^+}{\text{Lac}^-}$ · $\frac{V_1^R}{V_1^S}$

but one should test the Gal S character of V_1/lac recombinants for final verification.

The missed recombinants are therefore probably lac$^-$ V_1^R S^R. Do these occur in association with either parent? Would be detected now with the lac$^+$ parent. P1/P2 combinations should be reviewed for other [illegible]

Preliminary mating tests

EMS: #655

A)

W2338 F- line 28A.

(Lac- S^R)

~~W6 F+ line 1.~~

~~W1607~~

W1618

W1655

Mix 10^8 (young cells) each in 10ml broth for 1 hour. Plate out on EMBLac. Test 20 Lac- colonies for F status (x W1607 W-6 ^{180Z} ?).

B) The same in large droplets under oil.

C) Then I will touch single cells together.

Use 1:100 for mucin clogs.

L = hyaline

	A	B	C	D	E	F
1	+ L	+ L	46+ ?	0		
2	0	+ L	(1) 116.8 27.6	L		
3	+ L	+ L	(3)	5 + (3) + 3 ?		
4	+ clump	L + L	(2)	1		
5		1 div	L 3 + pair	vacuole 1 + (4)		
6		~	5 + pair + clump	1 vacuole		
7			3	— 0		
8		L		9 (1 pair) + pair		
9						

D - 8 two cells
recipient pairing
1 v 8
definitely ~~came together~~
(not nec. mutual)

Single cell method.

DATE: 11/25 - 26/53. REF: 1083

11/26.

	1	2	3	4	5	6	7	8	9	10
	W2338 x 2341.									
C exp.	3 +frag.	1? +frag	0	1?	## ..	..	..			
found	2- 1 lac + (3 frag?)	1	1	1	3 2+	4- 5+	0			
D exp.	0+debris	2+ large	++	? v. l.	3	..	..			
found.	0	4L- 1L+	no vis.	1	## 0	5L- 4L+	2L-			
C	4+ ?	1	3	2	3+pair	2+pt+clump	3			
found	2-pt 1+?, 1-?	1-	1-	1-	3- 2+	3- 4+ 1 pt.	0			
D.	0	..	5+3+3	1	1+ 4	1 snake	0	9+pair		
found	0		1+ 4-	1	0	4+ 5-	2-	?		

(same pattern ?)

whence 7 cells?

C exp-lac

sequence ?

sequence probably destroyed but argues for moderate eop.

Need to use fewer, better distributed droplets and a marker dye!

No cells in both side tubes. This can be omitted

Single cell Hfr x F-
W 2338 x 2371

1084

DATE: 11/20/53.

REF:

	1	2	3	4	5	6	7	8	9	10

12[1/2] — 4+:00

5 picked from 10 dropouts, dmit.

† = daughter pair attached

	cells seen.	EMB lac.	
1	1 + †	2+, 2−	may have a green, or not blood?
2	1	0	
18	1 pair + doubtful debris	0	
4	7 (clump)	3+, 4−	
5	1 pair.	2+	

all parentals + lac.

(Use eosin, small drops 1:200. Culture too dil!)

Cells observed in oil over chamber.

Try & chamber dmit.!

ERLively 4/53.

$Lac^+ S^S Hfr \times Lac^- S^R F^-$

Summary of microscopic manipulation experiments with W-1895 × W-1956

I method: Single cells separated from mixture; micro colonies plated on EMB lac.

purpose: To detect recombinants as plates having both Lac^+ & Lac^- cols.

proportion of parental cultures:

expt.	W-1956/W1895	Lac^-/Lac^+	# single cells isolated	plated on:	# plates: cols. Lac^-	Lac^+	missed
5/13/52			6	EMB lac	2	4	0
5/14	(by assay)	3/4	14	"	5	9	0

S character not tested

II method: Small numbers of cells (1–50) were deposited on complete medium agar in holes cut from filter paper. Early growth was observed; then the piece of paper was laid on an EMB lac sm plate.

purpose: To detect recombinants as Lac + or Lac − S^R colonies developing from a known number of cells at a given spot. (& have no record of separating or testing the components of a col.)

expt.	mixture W-1956/W1895	Lac^-/Lac^+	approx. number # fields of cells	total viable cells	plated on	colonies $Lac^- S^R$	Lac^+	$Lac^- S^R$	approx. no. S^S cells (by subtraction)
5/16 & 5/19	omitted (growth failure?)								
5/?/52			7	9	EMB lac sm	3			6
5/21			9	52	"	5			47
5/26			18	140	"	1		1	138
5/28			16	67	"	4			63
5/30			16	79	"	2			77
6/4	1 cc culture/.5 cc		15	80	"	3		1	76
6/7	"		7	45	"	2			43
6/7	"		8	31	EMB lac	1?	8		30
Red Tz added to W-1956									
6/24	1 cc/.5 cc		13	not observed	EMB lac sm	2		2	
6/26	1 cc/.25 cc		13	73	"	3			70
6/30	1/.5		26	95	"	3			92
7/2	5 cc/.5		15	48	"	9		5	34

Summary W-1956 x W-1895

II continued	mainf. by assay		no. fields	no. viable cells	plated on:	Lac⁻ S^R	Lac⁻ S^R	S^S not recovered
exp.	Lac⁻/Lac⁺ (T2 labeled W-1956)							
7/3/52	4.5 cc/.5 cc		14	34	EMB Lac Sm	2	1	31
7/4	4.5/.5 cc	4/5	14	39	"	1		38
7/7	4.5/.5 cc	3/4	8	36	"	0	2	34
7/8	4.5/.5 cc	4/1	8	24	"	1		23
totals			207	852		42	12	802

III method	Single cell isolation of rec marked W-1956 from mixture							
	Lac⁻/Lac⁺		viable cells	plated on:	# plates Lac⁻	Lac⁺	mixed
7/10	4.5 cc/.4 cc	3/1	4	filter paper transf. to EMB Lac Sm	2		2 S^R colo.
7/15	4.5/.4	3/1	8	spread plate EMB Lac	5	1	2
7/17	4.5/.4	7/1	11	EMB Lac	11	0	0
7/22	4.5 red/1 cc + blue T2	3/5	2	"	2	0	0
7/24	4.5 red/1 cc blue T2	1/1	9	"	8		1 * (1 col Lac⁻ S?; +2 col Lac⁺ S^R)
7/29	4.5 red/.4 unmarked	7/1	12	"	12		
Totals			46		40	1	5

#3 only grew. Found them 11/28/53 and tested.

* colonies saved

No trace of 7/15 cultures, more critical

UNIVERSITY OF ILLINOIS
DEPARTMENT OF BACTERIOLOGY
362 NOYES LABORATORY OF CHEMISTRY
URBANA

Nov. 24, 1953

My dear Dr. Lederberg,

Did you really think I would remember? I'm afraid I can't tell any more about the experiments than what is recorded, which isn't much; is it? This is the best I can do by way of summary.

I do remember that in the filter paper transfer experiments, before I started using fz and selecting marked (Lac-) cells, I was plagued by a persistent excess of Lac + and/or S^s cells, those which started to grow under direct observation but failed to produce colonies on sm agar. Several times I assayed the parental mixture to affirm that this excess was greater than might be expected from a higher titer of W-1895. I also tried inoculating fresh broth from the mixture at the time the cells were deposited in the micro-chamber and reassaying at the time the microcolonies were plated, but something always happened to make these assays unreliable, and I don't know what happens to the proportion of the mixture in broth. Do you?

The single cell isolations, 7/15 -7/29/52, seem to have been plated on Lac without sm. I recorded that the Lac + colonies from the mixed plate, 7/24 were tested and found to be S^r. Probably those from the two mixed plates, 7/15, were also tested and found to be S^s. I don't know about the Lac-. It could have been the result you suggest, but I wouldn't base any conclusions on it. I think I saved the cultures, but if you can't find them I don't suppose I could. The Lac + plate in that (7/15) experiment probably arose from an unmarked cell that stuck to the needle and got pulled out by mistake (see drawing of isolation).

Good Luck & amd Happy Thanksgiving to you and Esther and Seymour.

E Helen

Conclusions (11/28/53) apparent morphology of zygotes (normal)

Ethelyn's experiments were directed at a different objective. A few cases of cells giving SR+ recombinants are recorded. Unfortunately most of the experiments involved plating directly to EMB Lac sm. Part of series III was plated on EMB Lac. There were two occasions of Lac+/- from 1-cells. But the two from 7/15 (presumably sisters) were not saved and there is no explicit record of tests for S. ERL thinks they were both Lac^+S^s/Lac^-S^r (bi-parentals). 7/24 were saved, presumably P1 + R1, are being checked now. Her work is therefore not too useful. [My recollection agrees with ERL on the 7/15 expt.]. Do I have any reference in any correspondence?

1-cell exp. cons.

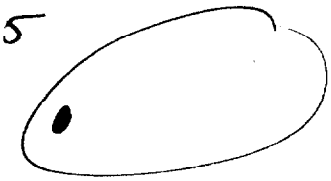
11/17/53.

A. W2344 × W2338. -

1:1:~~5~~5 and dilb 10^{-1}.

B.

5:25 D1 

D5 

E2 

12 others, 2 not formed 1 dirty others o. hold for clones. in chamb

8PM. D1 dried up. D5 dried up E2 dried

∴ n.g. Same with G-H series. no chambers. Legendre weak.

ABC- 4 1-cell drops [ca 15 laid down) But no egg transfer. [egg was rotten egg].

Comments: These experiments i v. flat drops on slides or coverglass. Observation is quite as satisfactory, and keeps the outer surface better (optically). ∴ Dispense i chambers is short term.

These cells are being used in small cell phase, not so good for observation. Onset mitosis in syn is noticed in diplets.

Recommend: ① Use ~~too~~ younger cells ② Use flat depths but add fluid before incubating. ③. Note that in this series oil and slide had been heated and probably desiccated. Water may

11/20/53.

Reinoculate 85B 1:20, 1:100 in 1 necessary Apl —
Technique: Heat 3×1" slide with inoc. wire. Cover other surface
with mineral oil. Add droplets (culture + soil), moderately flat. (after incu warm
add addnl fluid?) Pick up cells or clones by pumping addnl.
fluid back and forth in pipette and then expelling this
onto agar.

DATE: 11/2 REF: 1086 D.

	1	2	3	4	5	6	7	8	9	10
A.	dirty	dirty	dup 4+?	0+debris	0	* 1+diff ←	* 1+diff → ?all	* 1 v. diss nuclei	mass destroyed forms no cells.	* 1+ broken? ←
	* add fluid and incubate in jar 2:20 ← after.									
B.	0	3 v. flat 1 refr. + 1 ~ mobile ±	1~?	0	0	2. + ! sic	0	1 small	0	0
						no check.				
	we 2:50. we added small dose fluid out 7:15									
	B 715PM	1 ghost only ng.				1 ghost?		ng		
						something prob. inhibitory even dye control drops showed little growth.				
	slide was stained in mineral.					(even in heated oil?)				
A.						2??	ng	0	0	0

added duplets with remainder cells each to test substrate

Dec. over night

5 ci, 5 si dozin

20-30/dups.

These chicks grew very well (over night) but cryth. duplets still empty. Cells invisible?

DATE: 11/29/53. REF:

	1	2	3	4	5	6	7	8	9	10
Remonitate dilutions 1:1000 10AM - 5PM. Pick eosinated droplets, moderate size, immediately.										
A. Pichard. Recovery on Embden	8+ bubble	6	9, dirt	2 ✓ 0	1, †	8 singles	③, ②, 6	4, †	3	1, †
					1−	2−		2+	0	0
B	++	++	++	1, 1 dry?	4	10±	4	3, ③	1	2
				0			1	0	0	0

all parentals

Why such poor recovery? Flat droplets deteriorate?

~~Transfer these notes on single-cell isolation to cytology notebook and renumber.~~

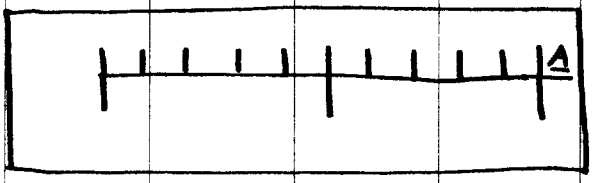
Main problem: Get large young cells. Use ~~Mazola~~ oil droplet as source?

DATE: 11/28/53. REF:

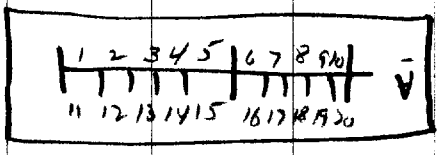
Mix W2344, 2388 .05 + .05 + 10 10AM.

Make large deglits 11:50AM as source of large bacteria. There deglits noted to have large cells at this time! Also remove broth tubes.

Make 1x3" slides with india ink:



no brucse:



number:

Flame to sterilize. India ink is resistant to oil but not water.

1/1000 dil. at 2:45 ca 10 cells/drop dil. 1/10 in H_2O; in buffer. No dye really needed.

B

1-10	5+ 6!					3	0	2	
11-20	✓	0	0	0	✓	0	0	0	1	0

use 20 x 10 objective and look to B 4, 5, 14, 19 for clear

Pick B 1, 3, 6, 8, 11, 15.

Incubate 4PM

11. growth limited probably contam

DATE: 12/1/53. REF: SGM.

12/1

Single cells allowed to form vegetative:

B4	—	P1
B5	—	
B14	NG	
B19	+	P2
E2	—	
E9	+	
E13	+	
G5	+	
G10	+	
G14	+	
G20	+	

all pure parentals.

Plates en bloc from 1ml 1/100 assay after 4 hr. further mutation. P1.

B14?, E15, E16, G12 did not develop.

From duit pairs: — = cells seen

B1	0	5 (Resist)	E1	3	4+ = 7	3 !
B3	0	1 small		6	2 − 1+ = 3	3
B6	4+	3		10	1+ 1− = 2	2
B8	4+	2		11	2+ 1− = 3	4
B11	1+	2		14	0 = 0	3
B15	0	2				

all pure tet+

Score = 24/31 Note excess of clones found in some instances! Might be due to subsequent divisions before plating! Repeated washing might be more effective than pumping back and forth!

DATE: REF: 88

	1	2	3	4	5	6	7	8	9	10
D	4 (mild +)	x		—						
E	3 (+)	1	+ +	4+	6	3	10	1+	1	2
			inf							
11-	4	0	1	2+	2	3	0	0	0	X
							dump			
20				✓	✓	~~✓~~	v.s.			
	add fluid to E			2, 9, 13.		Try to push			1, 6, 10,	
				15, 16, 0					11, 14,	
	Qwe 4:40			0						
30										
F 5:05	1 Go large dep x0	x	x +				capillary too large			
11										
1	x	+	~~+~~ 6	0	1?	4	5	4	1?	
11										
G	++	1? v.short +	+	1	0 on v.s.	0	0	0	1	
			✓	✓		✓				
5:25	add fluid to	0	5, 10, ~~15~~,		14, 20!					
1 M				fluid						
50										

DATE: REF: 1089

	1	2	3	4	5	6	7	8	9	10
L 945	0	+	+	0	0	0	0	+	0	X
	+	0	0	0	0	++	++	0	0	X

4.9. paired!

N. acc. discarded.

① Need better control of numbering directions.

0. Set up sterile drops. overnight.

12/2/53 Plating results: Emb Lac.

H. (direct pick)

1	7+ 5-	1 ++/-	9	1+ 2-		
2	5+ 1-	1 ++/-	10	2+ 2-		
3	4+ 1-		11	3+ 2-		
4	7+		12	5+ 1-		
5	8+ 3-		13	1+ 1-		
6	2+		16	2+	1 ++/- ?	
7	4+		17	4+		
8	2+ 2-		18	2+		

These drops initially were too large for careful observation. Not excluded that rather long cells or pairs were zygotes. However, syngamy may have been after picking: note high yield.

streaks + plates:

I	18	pure lact+	ca 10²		5 : N.G.
	17	"	"	"	
	3	"	"	"	(serially grown cultures show overgrowth of the M-Hfr parent)
	12	"	"	"	
H	14	"	"	"	

88 B. (streaks) 4- 5- 19+

1089

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
4:57-										
L 5:05	1	0	0	0+dirt	0	0	0	1	3	0
–	0	2	0	2+?	0	1	0	0	2	0?
5:10 –										
M	1	2+	1+	2	7	8	3	0 dirty	2	4
N	4	3	0	1+	1	1	0	1	1	1
5:30	0	0	0?	0	1	1	+	0	0	0

too dense

inadvertently thrown out

12/1 — 12/2.

0 20 stitch drops. 24 hours: 18 stitch

1. had a few plumps of highly pigmented matter
2 had a dimer, non coli. Crowcroft.

group A

V 1 N ?

DATE: 12/1/53. REF:

		1	2	3	4	5	6	7	8	9	10
Examine drops begin ca 8PM											
H.	3-8:20				14 ca 100 drops v. large.	15 1022 bacteria!? hold room.				19 X	20 O
9P20:	H14	+++			plate ca. 5 cells seen as yeast!						
	H15	–									
	H20	now ++ with a ~~co~~ cocci. May have been noted in first examination! 1-cell ~~conclusions~~ appear justified.									
I.	8:45										
	1	*1	None dv.	ca 1/4 10 clumped	0	*10	0	0	0	0	0
	20	0	0	✓	0	0	0	0	0	0	0
	EMB					~~H5~~					
	11	0	>200	0	0	0	0	✓ 10^2 clumps	✓ $<10^2$ clumps.	0	0.
		0	✓1 ✓	0	0	0	0	1	1	0	
drops are too large for observation.											
	30	Plate 3, 5, 12, 17, 18. Replenish fluid.									
J	9:30	1. ca 100 pathy. contam. 1 group	0	0	0	0	0	>100	>100	0	0
	46	0	++	0	>100 at one edge			drops			

Remember date 24 hours. 12/2: I5: a few "bacteria" but no further growth. others (3, 12, 17, 18 are +++). I8- indefinite shape; others empty by low power.

K.	1	x	++	0	>100	0	++	0	0	0	0
9:35	46	0	++	++	0	0	++ at edge	0	0	0	++

order must have been omitted!! hold overnight

K, order reversed, >24 hours:

	1	2	3	4	5	6	7	8	9	10	
I	+++	0	0	0	+++	0	0	+++	+++	comes	
II.	+++	+++	0	+++	+++	+++	++	0	+++	X	
	(coccoid?)	?	0	1	2	?!?	4	0	1		

6 may have coli +? Plate K5, 8, 10, 14, 16, 19.

Culture #10, 11, 12,

Lac

5 ±

8 ±

10 ng

X 11 ± fewer than others ∴ probably mixed

X 12 ng

16 ± 1 colony

" "

19 ±

why no lac-?

all fits well. why 14 not plated

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
	Fresh cross 10-12N. Remix 1:100/12N – 2:20 = 89-1.									
	Also remix 2:20 1:100, 89-2									
H				5 all rather small						
Tot.	7 incl 1 pt.	3	4							
	deeps too deep									
II					0	0			0	0
	3PM Plate all these at init. but add fluid to and hold. 14, 15, 19, 20.									
I 4PM.	0	0	1	0	0	0	0	0	1+debris	
same as above shallower deep.	0	1	0	0 on ?	0	0	1	1	0	
	add fluid each time. 4:15									
J	1	X	X	X	2	X				
K 4:30	2	0	0	0	1	0	0	1? 2	1	1 ?
	0 deep.	minute + on deep	0	1	2	1? at edge	(4)	0	1	Δ
	add fluid 4:50									
	Try filtered medium?									

12/3/53.

Fluid must be added!

Growth by Pyll
EMBlast
12/5 Plate

2 ±
6 ±
9 ±
10 NG
2, 6, 9, 10, 14, 16, 17

14 all −
16 ±
17 ±

Rd! ±, −

why 2 not plated?

DATE: 12/6/53. REF:

		1	2	3	4	5	6	7	8	9	10
4PM		Add cocos # 2338 x 2344.				1:1000 Penassay #12			Dilute to 1:10 fecth wood.		
D.	I	1 : 0? 8		0	x						
	II	0									
A	1d	0	0	0	0	0	1++	1++	0	0	8 0
	II	x	x	7	>10	0	2++	0	1 v.s.h.		0
		add broth to	6, 7, 10, 16.		(4:30)	Plate A6, A7, A16					
	4d	Coalescent 2-3 ur dead									
BC	2d	2++	1 0 0	1 1 0	0 0	0 0	0 0	0 0	1 4+	1 0 dirt dirt	
	II	x	x	x	0	x	0 dirt	2 -	0	0	0
		add to	1-10			Plate C1, C8					
		1-) with dirt									
5:15-	30										
	1	1++	0	3	0	0	0	1 pink	3+	0	0
B		1 0 0	0 0	0 0	0 0	0 0	0 0 + dirt	2++	0 0	x++	2:1 ++ smooth
		or oil drop									
		add fluid to 11-20, 1, 7: 5:30									
	40	P1: Plate B1, B17, B20									

Attach cut after growth in broth, EMB/ac

11/9/53. A. 6, 7, 16: all 2344 type = P2
B. 1, 17 P2 only. B20 P1+P2 No Klebsiella
C. 1, 8 P2 only D 18 P2 only.

Zulla.

(over)

1892 –

DATE: 11/7 REF: 1093

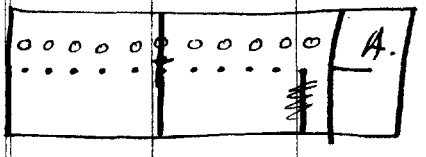
	1	2	3	4	5	6	7	8	9	10
A.	S. fragilis cells overnight. 13, 15 both 1-cell. Latin & bred. both → 10^3									
D B.	1	× dist	~~×~~	0 ?	0	1 ?	1	0	1 ?	0 dist 0
	11	×	0 ?	1	0	0 ?	1	0	1 +d?	0 1?+d?
	10	degenerate; cells small				d (crosses 1:1000 shown)				
E C	1	+	1 +dist	0	0	0	0	?	1	2 ? + +
	11					1+d.	0	×	pair ? 1	+
11/8. E	all but 12, 14 0 by low power.									
	20									
D	only 18 ++ See 1092 results.									
	30	plate A13, 15 for single cell S. fragilis								

#	cells ctd.	types
A 6	1	p2
A 7	1	p2
A 16	2	p2
40		
B 1	1	p2
17	1	p2
20	2	p1 + p2
C 1	2	p2
8	1	p2
D 18	1	p2
50		

DATE: 11/8/53. REF: 1094

W2338 x 2344. ⊗. 8:45 - 10:45. Dilute ca 1:20, incubate large droplet under oil.

2:10 Cells large some clumps, chains, etc. Dilute large droplet and proceed per routine. Use pattern: Top side up.



2:35. F1 6 ++ clumps 0 1.00 +d. 30 x +1 1. + large (3) ++ 8 2 + 0 1 +VS 0 deit +VS 3 +large clumps 8 1 +

9:20 2 d. 00 3++ 0 1 dusty + 0? + 1 +VS 0 1? V. dusty ++ dust 0 0? 2.+ 3+ ++ ++ clumps

2:50 add fluid.

3:30 A 30 ★ 0 1. ++ ++ ++ ★++ 3 0 1 pale short 1 V. dusty ✕

0 0 5 (1 smoke) 3 ++ 2 Sized smoke 6 0

qpm

3:40 B.1 × ++ 00 : 0? 00 00 1. 00 0c 00

40 ✕ 0 +VS 0 with fungi 0 0 2? VS 0 very 3. 1. 20 0 deit 8? 1 at edge probably

11 add fluid A, B 3:50

Room Temp

8 40 p/4. A2 empty ~~except~~: (13) ca 10 misc (14) 60 15 ++

(16) ca 30, some rotten small (17) ca 10 2 18 50 (19) ++ 20 0

50 7 (30) 8:0 9:0 10 1?

DATE: 1/4/53 REF: 1094/result

	#	2 Cells observed visually	3	4	5 EMB	6 Late	7	8	9 Discordances	10
A	13	5	Isolate		P1 sic	P2	R1			
	14	3	incl pair							
	16	2	v.s.		✓	✓				
	17	5	incl isolate		✓	✓	few (2 : 1 intact +/-)			
10	19	6	...	1		24	1 intact			
	7	3			✓	✓				
B	12	1	vs ?	0						
	15	1	v s	0						
	16	0		0						
20	17	1		0	—					
F	3	1				✓				
	4	1			✓					
	5	3	(1 pr)		✓	✓	no			
	6	1			✓					
30	7	1		0						
	10	1		0						
	12	3				✓				
	14	?			✓					
	15	?		0	✓A					
40	17	1	dirty			✓				
	18	2			✓					
	20	1	pr daughter.		✓					

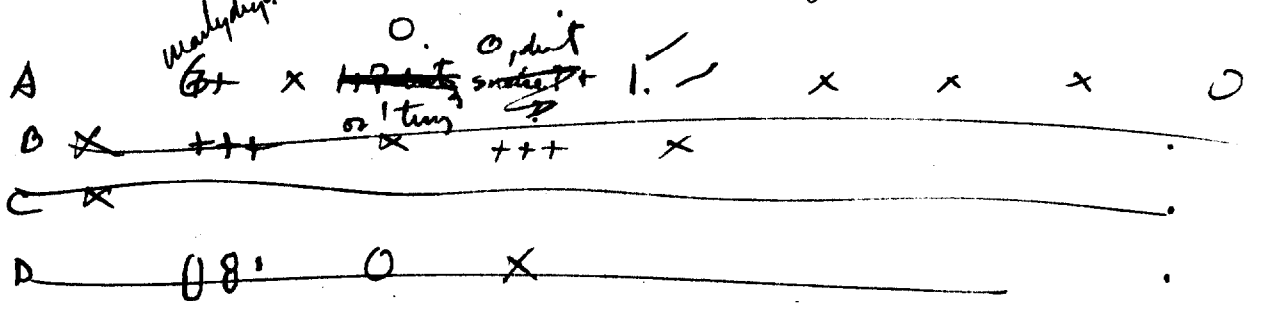
50

clones had massed to ca 10^2 before plating

4/9/53.

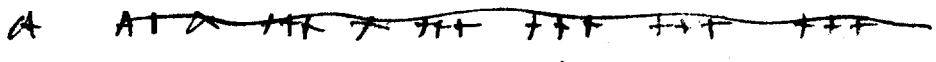
Old cross. Reisol C+ S aerotes. 10-3 Reisol S aerotes. 3-5+

New technique. Some coverglass, examine, break glass and plate fragments (add resuspendable fluid)

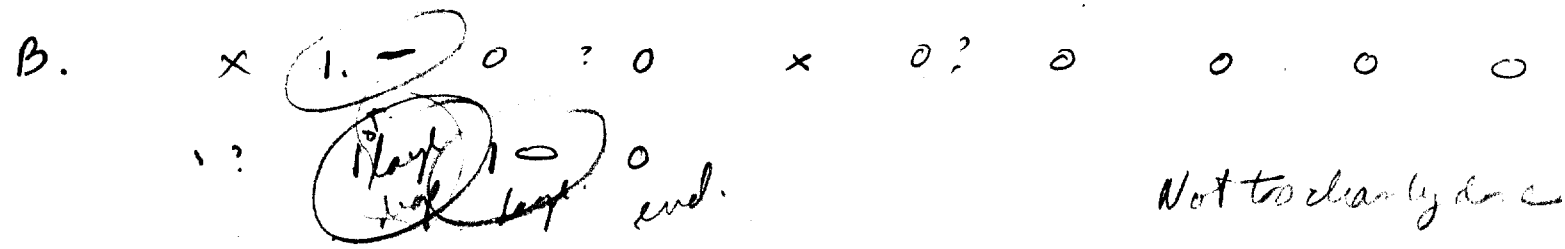


notes: ~~A optics poor~~?

plate density:



A4. A4

B. 

Not to clearly defined

plate 2, 12, 13 separately and remainder collect.

11/12 → A1 0 B— 0

Ell Blac A4 iP1 iP2 iR1/P1 B2 1 P2

B12 P2(1) [illegible]

B13 P1

Technique is therefore OK

A4: possibilities: Long cell divided when plated → segments or (lower)
A1 ⇔ A4 confused. ∴ This one is doubtful.

DATE: 12/11/53. REF: 1096

	1	2	3	4	5	6	7	8	9	10

old work. & together

A/ 235

1 | X | 3 | 8 | 4+(4) | 6 | 1 |

4 | 6 | 4+ (2)(2) | 3 | 0 | 4 |

10

plate 2, 3, 4, 5, 6 (i) extra fluid, 11, 12, 13, 15 (i)

B 10-30

4 | 1+(+)+(2) | 2+(+) | 0 | 0 | 2+(+) | ? | ? | 0 | 3 sh (+)

11 | + | 0 dup ? | 0 | 0 | 0 | 3 sep. | 2+ | 0 | 0 | 0, dif

20

(added finish).

best to separate squares first.

Set up

30

C 1 | + | x | x | (2) | 1 ? 8

40

dupe smooth, e.g. method of Cutting squares tobes to dry - bare steam pressure, plate & Print to

50

DATE: 12/10/53. REF: 1095–1096

Oil cross, 1230 – 3 PM c/o aviatex 1:100

coverglass method. Stripes of 2-5.

A. 1 3? 1+d. 1.0 + d. 0 of d. 2 — third deg. ✕ 0 ✕ 0 ✕

H 0 0 0 0 2 0 0 0 0 0

Plate 11–15, 16–20, 1, 2, 3, 5

N negative.

1096 10 platings. (If unrecorded, not successfully transferred.) Some difficulties
12/12 in handling the coverglass stripes – at first tried to break previously scored segments, but this was too messy. Here used pre-broken fragments of glass. Plastic, if it could be properly cleaned, would be better as it could be cut in stripes.

	Cells counted →		types:	
A.	2	3	3 P2	— each cell counted for
	4	4+④	0	use extrapolated henceforth.
30	5	6		
	3	1 ghosty?	0	
	6	1 large	0	
	11	6	2 P2	
	12	4+2②	4 P2 + 1 P1	
	13	3	0	
	15	4	2 P2	
40				
B	6	2+1	1 P1	
	7	1 v.s.	0	
	8			
	9			
	10	3 short + 1	2 P2 + 1 P1	
	11	1	1 P2	under oil. —
	13	1	1 P2	—
	14	1	1 P1	all under coverglass. —
50	17	2 + ·	1 P1	
	18	0	0	
	19	1	1 P1	
C	4	②	2 P1	

6/12

Old cross, 24h+). Remix 1:20, ~~9:45~~ 10:30; 1PM, 3:15 PM.

3:35 A. (1+?) (11) mm (0) (3) 1 eroded 0 very small × 0 v. fast +

0 0 0 0 0

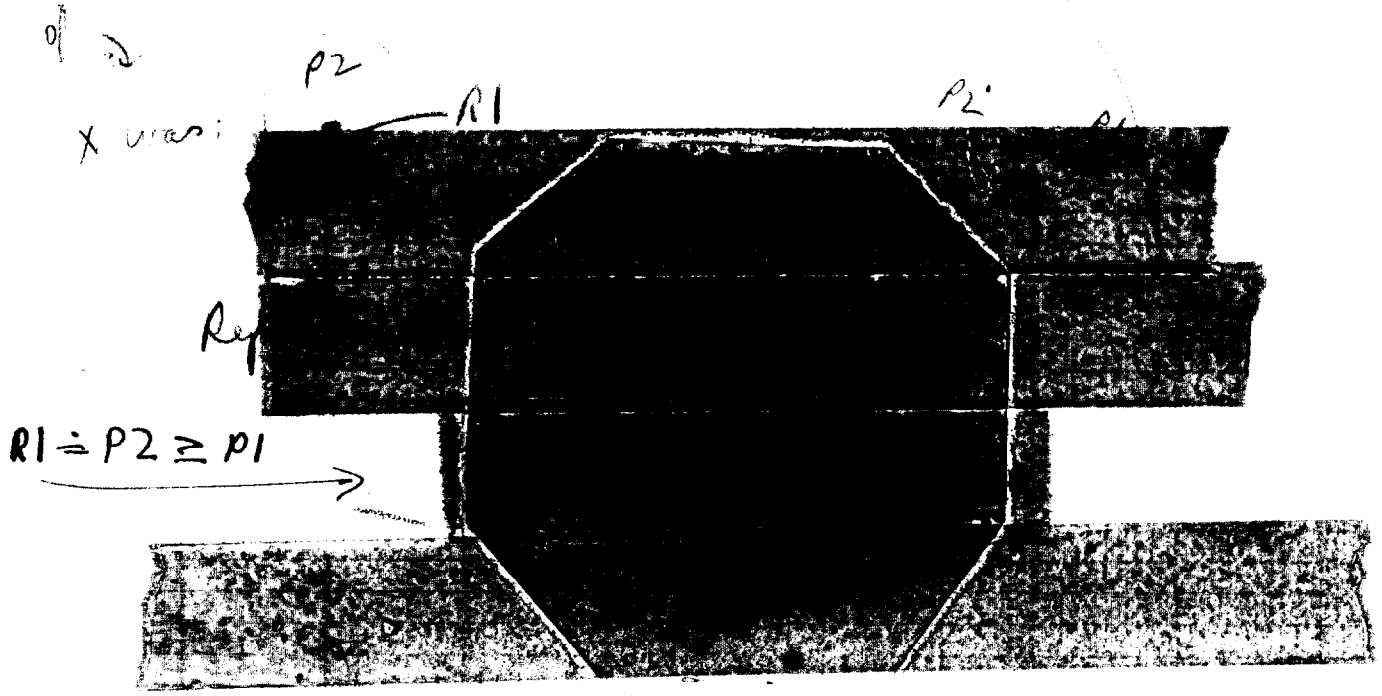
Plate, add'l., 3:50

P10: 1 1 2 3 4 5 6

1 1
2 2(?)
3 4+(?)
4 1
5 3
6 1?

clones
2P2
2P1 + P2
3P2 + P1 + zygote
P1
2P1 + P2
0

Sm C A3, all grits. Shake, pt + A 0 +/- for grits
colonies all dirt [illegible]



12/13

(Ibm)

A

12/14. EMB Lac.

	colo.	colo.
1	3	1 pl
2	1?	0
3	1	1 pl
4	0	0
5	1?	0
6	1	0

12/14/53.

a). New cross .5 + .5 + 10 from old parent susp. 9:05 AM. — 10 30.
set with O.

20

A. 1 x O O O O O O ∞ ⊗ X 1

B 1. 3+ pair O O ✱O x O O O ∞ P2 ?+
(dropped) thick
(P1, P2) (?)
?

(O v. small. rather long. possibly not a cell.)

2.05

C 10 0 0 4+. 4 mixed. 3+ 11 5+ 2+ 11 O 2 1 O O

D 2:13 — (stopped to photog) 12:27

O 1 1 + dirt? clump. loosen. 1 O 1. O 1.0 1.1 O O

E 1:51 —

5+ 21 ✓ 1.0+ ?✓ O O 1v.s.? — O O ? ⌣ x O —

5:21

O+. O / x O = 4+ (11 dirt)

DATE: 12/15. REF: 1099

Platings.

A.

7	1	1 P1
8	+	2 P1

B.

1	4	1P1 + 3P2
9	2 +	2P1
10	1	1P2

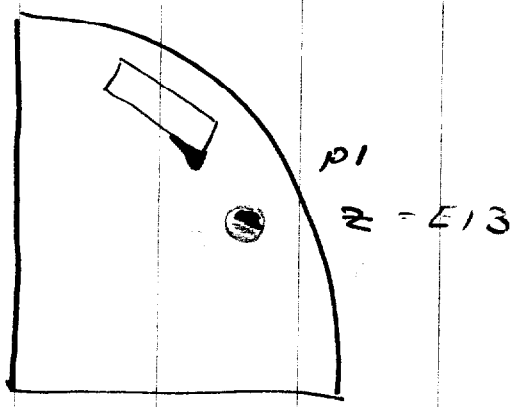
C

1	4+	1P1
2	4	1P1
3	+	1P2
4	5	1P1 + 2P2
5	4	3P2
7	2	1P2
8	1	1P1

why yields so much lower?

D.

32	1	1P2
3	1	0
5	2	1P1
7	1	0
4	3-clump	0
8	1	1P2



E

1	7 + ~~blob~~	3P1 + 3P2
48	1	P2
5	1?	0
8	1?	0
10	1	P2
11	1	P2
13	1	P1 + Z!
17	+	0
16	1	
17	4	4P2

50

large cells recorded. same components.

E13 → P1, R1 No P2 seen. Same mixture and potential toxins.

12/15/53.

12/14. Received cultures from Pomper — lyophil tubes. Incubate in YEx medium.

WY-			
1	62	+++	Pound cells (occ. oval!)
2	63	++	"
3	62-10-19Y	— did not grow out.	(tryptophane, uracil)
4	67-1	++	" (meth, adenine)

12/17. WY-1 grew well and promptly on yeast-sucrose agar
WY 2, 4 grew very poorly initially, but some large colonies suggest incipient adaptation. (probably temper
Hand over to Rubbo for this (There do better at 30 than 37°
After 3 days, WY3 finally grew. Handle as above. ∑ Reversion?)
Transfer from these initial bottles to slants for "cultures as received

WY 6 = dipl S. cerevisiae WY 8, 9 — acriflavine-induced petites (Rubbo).
SOR reports that petites are defective in metabolism of various sugars (cellobiose, rhamnose, maltose, galactose) suggesting adaptive loss generally.

A. Check β-glucosidase in 6 vs 8 grown in glucose, cellobiose.

B. Most sugars, WY 8, 9... showed mighty very poor growth, occasional larger colonies. On EMB Cal, WY8 showed two types of large colonies (fermenters vs slow fermenters). I... and single colonies from EMB Cal of WY8. Also repeat SOR's stations on EMB...

Further tests, nutrition

RL medium base. add niacin 1mg/liter to mix

WY5 (S. fragilis) +++ (~~not~~ previous failure presumably nic requirement)

± metals ... No effect on WY1, WY5 in liquid & moderate medium.

WY1 +++ s/c metals

WY2 +++ confluent flor!

---

48 hours:

WY3 TR+ YNA faint growth. Uracil + TR — TR, YNB only —

WY4 Meth ±

Meth Ad +

Yx +++

Hyd. Cas ++ (enzym.)

HC+pur ++

Meth, pur ±

Meth YNA +++.
hyd.

∴ something in YNA besides adenine. for WY4. another aminoacid?

WY3 unsatisfactory re morphology cavellas growth requirements.

Try adenosine guanosine....

1100 A

Saccharomyces Cytochrome

DATE: REF:

A. Grow WY6,8 in ~~F~~ Nutrient glucose, cellobiose broth.
Growth in cellobiose v. poor for either culture + harvest v. poor.
12/27: test c ONPG. No immediate rxn but
during several hours, all cultures "adapted" and split off o-n-PGH.

12/29. Retest. Harvest WY6, 8 from YE agar and resuspend with
glucose, cellobiose, 10:50 — 3 ml pH 5 buffer + NB.
maltose. ca M/10

Set up 3²⁵ 4³⁰ for ~~fermentation~~ in
(8, benzyl, cellobiose)

V. poor
β-glucosidase inducible Try S. fragilis / β-gal. Torulopsis
5³⁰ : 6C 6GC 8G ~~nitrate~~ +
8C —
dilute out —

12/26 - 27 in Fries sucrose + vits 29°
Random Pompei mutants.

C WY 1-2 grow well in F(S), + glucose, + 4 supplements ↓
(WY2 extremely flocculent: large clumps). (Also will at 37°).
WY3-4 fail to grow in F(S) ± glucose ± 4 suppl.
(meth, uracil, trypt, adenine each ca .15 mg/10 ml)
after 2 days delay, WY3+4 grow in F(glucose) and more slowly
still in F(S). (presumably dyed of hybrid) Sporulation
WY4 slow grow (pearly) in M, M+Ad but not Ad
Further studies omitted

D (over)

Burkholder Cross Reaction F(s) agar:

WY	
1	+++
2	+++
3	±
4	±

3 x 4 ± mid scattered prototrophs at intersection.
Yields ca like E coli cross, corresponding body.
Replate these prototrophs as 1100D1

12/30. 3 x 4, both F(s) and F(s)+glucose
fully grown in tubes plate these as 1100 D2, D3.

Comparisons c/s MB, anaerobic supplement in liquid acetate
showed " , cond with oil
maltose greatly better growth than glucose, see W46, W48.
Cf. SDR comparisons. This cannot be ascribed to anaerobic
anaerobic diffusion in maltose case.

1100B

DATE: REF:

1 2 3 4 5 6 7 8 9 10

ca 12/26. Replate WY 6, 8 etc.

12/29: WY6 gives faster growing scalloped colonies
WY8 (1-4 cols): slower growing, smooth. on YE agar

WY6 WY8 WY9.

EMB. Glu all give ++ ferm. reaction

10

WY8, 9 and 8 (1-4) all considerably slower growth.

Gal. all grew more slowly and gave virtually ≡ ferm. as
on plating, 1-4 were indistinguishable as to this Gal!
(Fetal reaction more nearly — than on S.A.'s plate

20

	Ferm	Gr	Ferm	Gr			
Sucrose	±	++	−	+	ø		− +
Maltose	−	+±	−	−			− −
			v. sharp difference in growth!				
Cellobiose		±		−			−

30

Sid's plates did finally show moderate growth on cellobiose ~~+ maltose~~
i.e. papillae possibly better fermenters. Do ~~WY~~ WY 8 on maltose

Conclu: Maltose shows sharpest differential.

40

50

DATE: 12/15. REF: 1101

	1	2	3	4	5	6	7	8	9	10
	Fresh cross 1:1:10 12:30 - 3:30. dilute in H_2O.									
A. 3:27 -	1. ++ incl 2 v. large (1 pecul. zygs)	23 no paris no v. large.	21 do.	15. v dist do.	8+ ? pairs	3+ dist	3	1. 0	1+ 2+	3
7 yg:	1-2 ✓	0	0	0	1?	0	0	0	0	0
3:55 - 10	23 (2 fairly long)	++ no pairs	++ one ↑ rather large	++ one v. large	+.. 1 large (paired?)	+.. 1 v. large	++ 1? v. l.	+T 1 pr 1 large but not very large	+ some chromo but not very large } do.	+
B.	∴ If v. large cells are zygotes, expect following to have more zygs: →									
4:20								↑	1 long. 1 not	
	2 pr. 10	0 ✓	1 ✓	1	1	1	?	0	0	0
20	7-10 dud? esp. 10.									
C.	×	×	×	×	×	×	0	×	×	×
(not ED.) for epithelium										
D.	2	×	×	0	0					
30 4 40 -	Streak out diluted suspension also →									

	P1	P2	RIC plates
	13	131	3

- = P1 + = P2

40 plates

12/16: A 1 1 RI/P1, 13 P1, 22 P2
2 14 P2
3 12 P1 4 P2
4 4 P1 7 P2
5 2 P1 1 P2
6 2 P2 + ? c.g.
7 2 - 1 +
8 0
9 - ? no gloss →
10 1 - 1 +

B 1 + + (> 23 !)
2 4 P2
3 23 P2 1 RI/P1 + c.g.?
4 61 P2 6 P1
5 37 P2 1 P1
6 49 P2 2 P1
7 6 P2 + c.g. ?
8 13 P2 : 2 P1 : 1 RI/P1 ...?
9 13 P2 : 14 P1
10 17 P2 : 8 P1

DATE: XII. 16 REF: 1102

		1	2	3	4	5	6	7	8	9	10
		~~6 minute~~ 0 + ~~pi~~ V.S.! 5									
		fresh cross. 12:15 – 2:40		dil in H_2O.							
2:50	A.	++++ cq/2 dil	changed	5	+	2,°	0 8	0	1.	1	0
	10	"		.	.	.	.	.	.	.	.
3:10	B.	X	all O add fluid + lost in deep hold to! hold to 4:40	~~2 + .~~ X	2	H-d.	0.	⊘	1+d	2 d	O but change of pigment X
	20								X		

XII. 17

		Cells	Colonies	
A.	1	3	3–	
	2	12	~~4– 1+~~ 12?	Obs. not precise enough
	3	5	2–	
	30		0	
	4	1		
	5	2	2– 1? (c.g.)	
	6	2	1–	
	7	1	0	
	8	1	0	} order? probably correct but suspicious
	9	1	1+	
	10	0	1–	
	40			
B	2	0	2	
	4	0	2	
	5	0	1	
	50			

DATE: XII.12.53 REF:

	1	2	3	4	5	6	7	8	9	10
	Fresh cross: 8¾ to ca 10 AM					.5: 5: 10				
	8	0	0 + 0?	0?	0	0	0	0	0	0

A. add moderate fluid and incubate. Cell probably re-formed in 1, but these drops are too large for proper counting. Left at R.T 10³⁰–4²⁰ PM.

B

	1	2	3	4	5	6	7	8	9	10
10	moderate large thin		0	0	0	0				

Plating:

A	1 1...	4+ !	(1 fission)
	2 0	0	
	3 0	0	
	4 1	0	
B	1 2 (...)	0	
	2 ++	2−	V. low RECOVERY!
D	1 6+ (2)	4+ 1−	Incomplete recovery unfortunately.
	3 1	1+	
	4 1	1+	
	7 ?	0	yields v. low!!

20

still counted in replicating drop.

30

C

Fresh cross 3:30 – 4:40 1:1:10.

	1	2	3	4	5	6	7	8	9	10
500	x	x	0	0	0	0	0	0	X	0

(resp too small or resp too dilute ?).

40

D. #1 includes 0 1 0 0 1 1 6+ pair ?

	1	2	3	4	5	6	7	8	9	10
#	0	0	0	0	0?	0	1?	x	0	0

50

DATE: XII.18.53 REF: 1104

	1	2	3	4	5	6	7	8	9	10
A.	Freshcross 1:1:10 12:15 – 4.				∞					
B.	+ X	0	0 1. +0	0	0	5, none unusual	0	0	0	0
C	5, +	2 + 1? (sup. drops)	0	0	2 + ?	0	0	0	0	X

Plating:

A 5

B 3 0
6 3+, 1 –, 1 z. ∴ 1 type cell = z. → R1, P1 only do not score.
7 1 –

C 1 2+ 6 –
2 0
5 0

3 types have been seen:

		normal site (pinnately)
0	0	0
↓	↓	+
R1 P1	R1	R1
P2	P1	P1

(→ melun. ↓ R1)

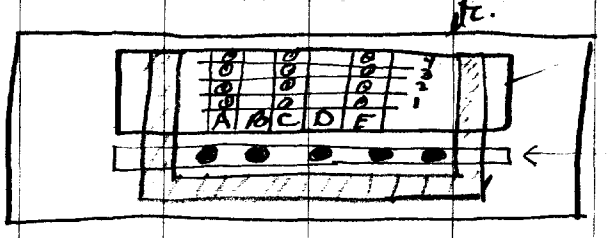
Spend one + four days setting up deFombrine

① Microlomps (Schouten): finally mastered but not very convenient for microisolation.

② Capillary technique: used practical in chambers. Some pul. experiments to isolate yeast (W413)

DATE: 1/6/54. REF:

Developed micromanipulation methods during past few days.
Set up ~~RBC~~ oil chambers:



- Isolation coverglass
- Reservoirs

after isolation to small droplets, add extra broth and incubate overnight.

<2PM 1/6. I 7 8PM II.

A C1 ● C3 / (P2) E1 ○ G3 ⬭ detached in some way
C2 ⬭ large C4 ⬭ E2 ○ v. large
† somewhat elong

B. ~~B~~ A1 ○ large others × stays plating
A2 ○ formerly in pair, between a part ✓ appearance of pair not recorded
A4 ○ ✓ stays ?
C2 ⬭ headdist ? ×
D2 ● ? ×
D1 ⬭ stays
E2 ~~✱~~ ~~✱~~ no change to 8PM. ×
E3 ⬭
E#4 ⬭⬭ long drops stays

One other pair not succ. isolated ? ~~0~~

Plate P7.

C ~~×~~ ~~△~~ ~~×~~ ~~×~~ probably drops
E1 stays?
E2 ×
G3 ✓ stays?

I II

(over)

		plating	appearance
A.	E1	P1	P1
	G3	P1	P1
B	A1	P1	P1
	A2	P2	P1! ~~(had been 2 in pair).~~ Reverts other mutants.
	A4	P1	?P1
	D1	P1	P1
	E3	— ng.	
:	E4	P1 + P2 (+ R1)	(from ∞)

BA2: note: Although vid colonies were all P2 r lac, a few lac++ papillae noted in brush. Streak, replicate to EMB lac sm.

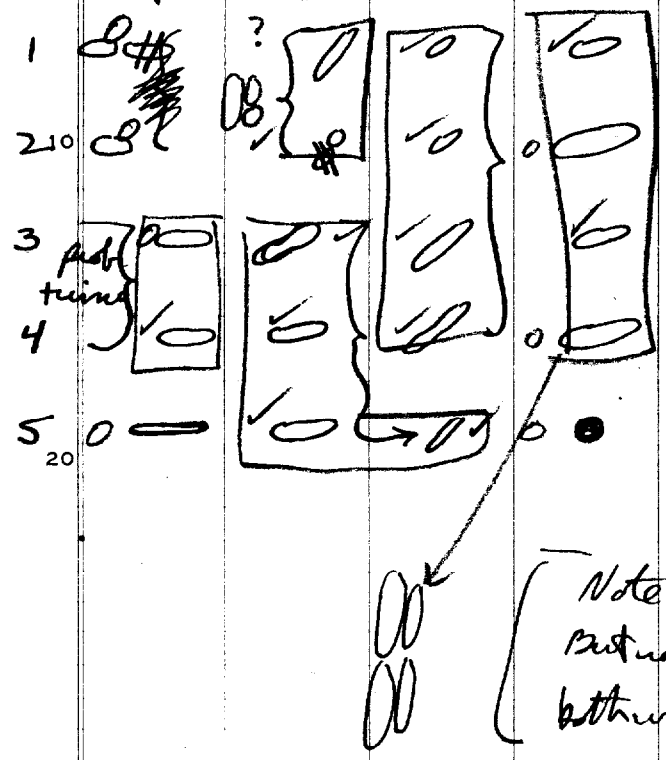
↓

A2 Original plating. One colony was lac- (purposely overlooked). ✓ replica: This is also ~~lac~~ Gal+ S^R . . . P1.

∴ This is a P1+P2 type from single cell. Originally in pair

DATE: January 8, 1954. REF:

P1 x P2. ~~overnight~~ 48hrs. mouth. 1:1:10 12 30 - 2 30 PM. Dilute to 1:200 pre manipulation in Penicillin. 2 30 - 4 45



Note: D1, D3 died (P2 parent?) But note reversal in appearance (enter bottom row P1).

D2

B2 lysed. A1 lost. E, F mixed 1-cell coil. No passis seen then.

B3-4-5-C5 from 1 clump of 4 cells.

C1-4 from 1 clump may include one of the small cell not clearly seen in the original clump.

1/9/53. Growth in droplets ✓ or 0. Series A probably dried out. Droplets not apparent at time of fluid addition. Fate of D2, D4? Not necessarily accident. Keep in mind poss. segregation of λ? (what role of P2).

B1 - probably oil globule only

Transfer to 1 ml broth A9.

1/10/54

Platings (streak out from Imlkoth tenifers, P9) on EMB Lac.
P1 = Lac - P2 = Lac +.

1106.
A4. P1

B1 0
B2 P1

No apparent recombinants in this series

B3 P1
B4 P1
B5 P1
C5 P2

C1 P2
C2 P2
C3 P1
C4 P1

E3 P1.
E5 P2
F1 P1 + few + papillae / No + colonies
F2 P2
F3 P2
F4 P2
D1 P1
D3 P1

Save B2; B3-4-5-C5; D1,D3; C1-4.
mass stab D2, D4 steel empty P10.

Jan. 9, 1953.

W2338 P1 × P2 (old cultures) ca 1:5 at 10³⁰ Remoc ca 1/50 12 N — 3PM
W2344

3 - 4³⁰.

6+, 6- tested:

~~Re Late~~ test, 2/2/54, B5 → Loc+ V_1 R A 2
These are all ✓ as Gal+, Loc+, -. Loc+ V_1 S B 4
Possibility of recurrent recombination here? Or Loc- V_1 R C 1
is this an illustration of a "twin" set. Loc- V_1 S D 5
Re ✓ OK of 111753.

see over

10 P1 Plate. Most had 10^2-10^3 in clumps. NG in B1-3-4 D4-5
P10: Litter

1 clump
A 1 0 P1
2 0 P2
3 0 P1+R1 ~~Loc+ S~~ further (picture as largest in group)
4 0 P2
5 0 P1 (1 col.). Replate from tube (original drop.).

B 2 0 P2 ~~Loc+~~
5 0 P1+R1 ~~Loc+~~ S V_1 R S ~~for the~~ see 111753.

D 1 P1
2 P1
3 P1

∴ exclude zygotes either result from or tend to form clumps ∴ (P1, P2

Nutritional test: 110705 (mess):

O	M	H	M+H	
-	-	-	++	(12h.)

∴ Set up crossing test for A, B, C, D and mess assuming each is M- H- .

Test 1, 2, 3, 4, mess, and W2338
× Y10 (F-) ; W1918 (F+).

all cultures × Y10 [on D(-)] - sterile
× W1918 → [ETHSLac] ++ prototrophs

∴ all F⁻ .

3:30 Start in with dress rehearsal. Make music changes + look for pairs.

① Cluster dresses carried? Reversal (cape) in first transition to deep. Deep end [illegible]

A1 ○ A2 ○ A3 ○

A4 ○ A5 ○ B5 ○ B4 ~~B3~~ B2 ○

○ Dance doubles typed. — 4:30 PM.

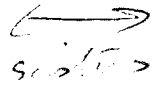
4:03 — [illegible] in recovery → [illegible] D1 →

Personally [illegible]! Rehearsal despite [illegible] system. Great first [illegible] separated. Altitude [illegible] train over [illegible]

after injection of

420 was able to regurgitate. Then all were widely separated

D2 ○ D3 ○ D4 — D5 ○



Y 25

D1 ∫

added fluid Y: 30

ABD

Compulsory mating + ——— w.g. 1108

DATE: 1/10/54. REF:

P1, P2 fresh overnight. 12N – 5:45 in Permassay (sep) 1:100
5:55 – Separate midgps, then coalesce.

Repeat, allowing growth in reservoirs. Cells continued to divide in transfer droplets.

4 Drops coalesced. Manipulate A, B further. Sp B, P1 → 9 host.
Sp A, cells conjoined 00 10^{40} but well separated 10^{45}. Could not force conjunction. Expt. abandoned 10^{45} PM.

Objectives of single cell study:

① Genotypes issuing from primary hybrids < Total clonal separations
② Correlation of zygotes with associations with P2 parent
③ Cytol. appearance of the early hybrid
④ Early stages of the hybrid.
Should do ① first.

1/11. Started a few droplets, but milk broke. Spent day largely on review for Ephrussi &c.

1/12. Class; Hanson apptment. Almost no work.

1/14/54.

P1×P2 1:1:10 5:15 – 7:45 pm then diluted ca 1:100. Difficulties in capillaries. Isolations began 9:30 PM. Pick large cells or clumps (v

A1 ○ 9:34.

A2-3 ∞ separated 9:48

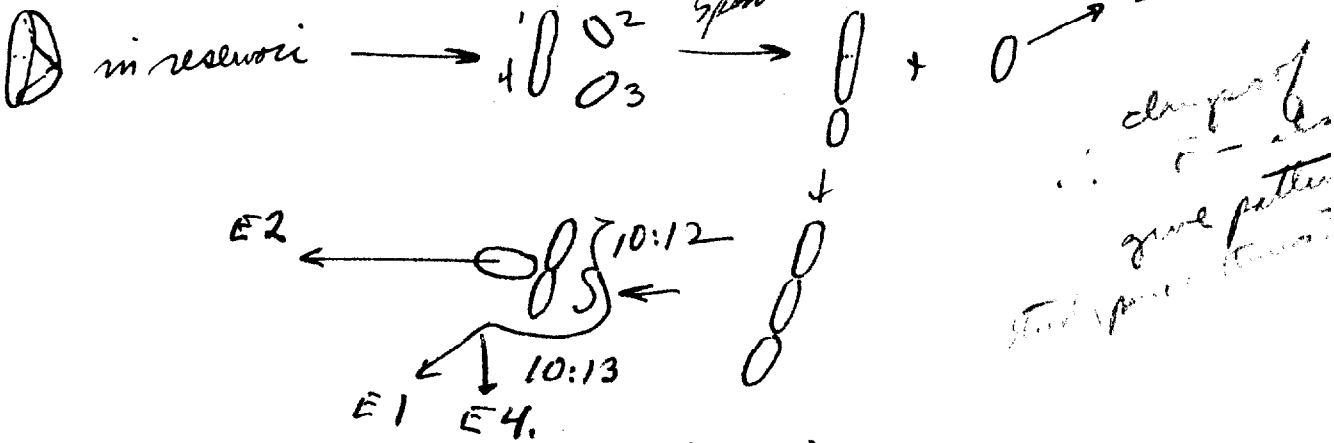
A4 ○ v. motile 9:30. B4 ○

B1-B2 / → ○ , ○

B3 seems apparently ○ but → only ○

C. ∞ in reservoir, → ○ + ∞ in first drop.
9:38 C4 9:43
C2 C3 9:46

D4 v. large 9:58 ○ (later lost.)

E 

Later could not find D4, E1 drops. (evap?

A 1 ✓ 2 ○ 3 ✓ 4 ✓ all other P1 S^R (Loc-Cul + V1^S) P2: Loc Cul-V1^R S^S

B 1 ✓ 2 ✓ 3 ○ 4 ✓

C 2 ✓ 3 ✓ 4 ✓ ○

E 1 2 3 4

8 cultures drops n.g. Growth ✓ n.g. ○ and D1-4 ○

DATE: 1/15/54. REF:

C, Dn·1·E4. P1×P2 1:1:10 12 30 – 2 40. Then dilute ca 1:50. (Keeps prob to dup).
E1-3,5

3:15 80 → C1, C2 – 3:50 and D2, D3 in same pipette. D1

D2 D3 D4.

But all inviable. (dried ?? – tried to add fluid!)

E. // → stayed together → // → 0 + 0 → E3. (seem nascent!)
4:08
E1 E2 separated 4:26 - 4:28

E 0 v. large → E4 → E5.

SURVIVORS. Plate 1/16.

	EMBlac	Gal	SM	TS
E1	–	+	R	S
E2	–	+	R	S
E3	–	+	R	S
E5	±, – very prop.	+ (–)	R (s)	S (K)

∴ has Lac+ Gal– V_1^K (S^s) and Lac– Gal+ V_1^S S^K.

= P1+P2

R1 if present not numerous. Plate crowded: restreak EMBlac. ↳ very rare, pres. secondary.

rare Lac+ S^R also presumably secondary

(Tiny bad E4 may be visible!).

Unless specified, all cultures saved are in purified media when practicable and type is present, saved as well as separated components.

Background for compulsory F-deletion. 1111

DATE: 1/12/54. REF:

Fresh cultures (ca 24h. 1:5) W1655, W2338.
Mix under oil, room temperature, 2 tubes in their own broth
+
A —
B = vol. fresh broth
C ca. 3 x vol. fresh broth.

and plate out.

A20. Grow to EML.
Shd form ca 1/10 - 1/5 F+ in A; 0/10 in B, C.

DATE: 1/16/54. REF:

	1	2	3	4	5	6	7	8	9	10
	P1 x P2 ... 10^{30} – 12^{30} Refr. to 2^{15}. Dilute 1:25, flat legs. 2:50 esolate random, empty. large cells.					N17 (EMB growth)	Gal	Replicate S	V,	Diagnosis
B	5	O				✓	–	+	R	S	P1
B	4	O				✓	–, ++	+	R, R.	S	P1, R1
	30	O	3:20			✓	–	+	R	S	P1
C	4	O	x lost sight			O					
C	5	O				✓	–	+	R	S	P1
D	5	O				✓	±	–	R	R	P2
D	4	O	x lost sight.			O					
	20	but add broth fairly promptly.									
A	5	~~No~~	~~Transplant into storage~~			✓	–	+	S	S	P1
A	4	O				O					
Control + reservoirs (11)						O					
	30										

(Note. all self-agglutinated except D5.)

∴ P1R1 may be paired, insignificantly, with P1. These expts. give little information or significance of pairing.

40 Also some time on yeast esci (W46) but could not break walls successfully. Suspensions too thick and numerous cells stick to surfaces of rather large capillaries needed. (Use dessicated ones?)

✓ D4. No lact. about 5 P1 : 1 R1

50

DATE: 1/17/54 (Sunday) PM. Pipettes for layering cells.

A4 ○ 2/18 ○
A3. Record? ✓ Probably mixup. Notes also refer to B4 pattern → A4, lost?

B 1 ○
2 ○
3 ○
4 ○

"A4 lost pass. in A4."

C 5/5 marked X. Probably several cells ✓ 3 others ○

D1 ○ 0.2. ○ Pipette too small! Carried at tip. Damaged? (was well shaped but too narrow for some cells. Passed others. Use only pipettes for filtrates??)

5/5

D2 } 1/ → ○
3 }

D4 1/ → ○ other damaged?

E2 } ○ ("sisters — may have
E3 } ○ switched?"
E4 ○

Initiate techniques of replacing fluid depths for replenishment and placement. Best arrangement:

	A	B	C	D	E	F
1	○	○	○			
2	○	○	○			
3						
4						
5						

Genes:

	Lac	Gal	S	T1
A3	− →	+	R	S
B1	−	+	R	S
2	−	+	R	S
3	−	+	R	S
4	−, ±	+	R	S R
C3	−	+	R	S
D2	±	−	S	R
3	"	−	S	R
E2	−	+	R	S
3	−	+	R	S

P1, P2 ?

extra fluid at A-0 etc.

~~Inoculate to measure~~ As control, check all squares and reservoirs and note extraordinary growth if ever.

O/N ✓

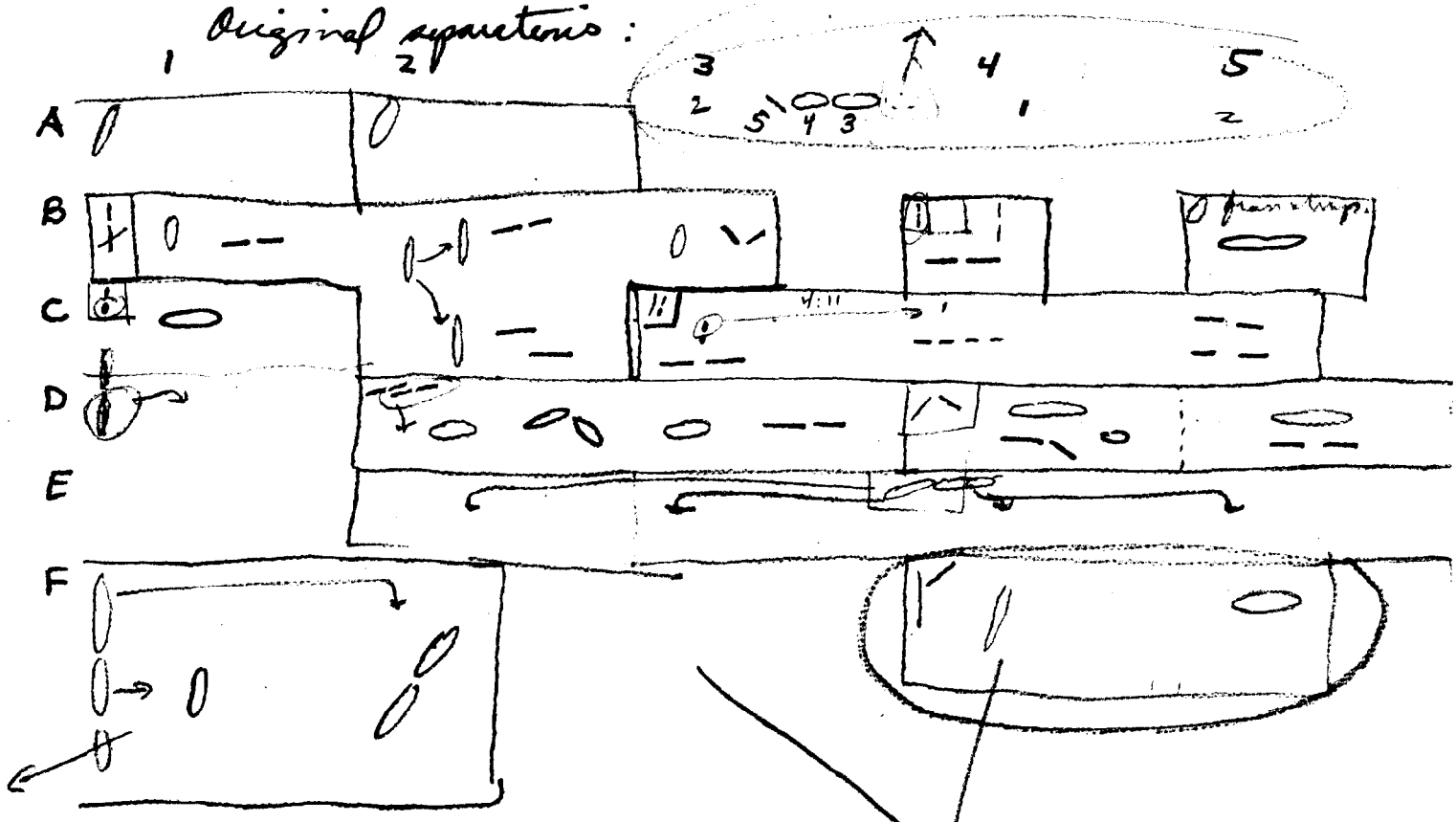
1/18/54.

10:1
P2:P1

$10^{20} - 1^{20}$ 1:1:5 in Pennsaw. Ochute 1:25 1:20 PM.

30 clones separated from about 2:30 - 5:30 PM (previous time setting up dishes, chambers, etc.) Noted that many cells had genus 2 - 4 cells. Refrigerate at 5:30 - 8:30 PM to permit further separations of selected cells.

Original separations:



Do all clones. wait for F4-5 if necessary

7 clones were transplanted, 9:15 - 11:30 PM to separate cover-glass squares, leaving one cell behind in situ. Thereafter plated directly on EMB lactose.

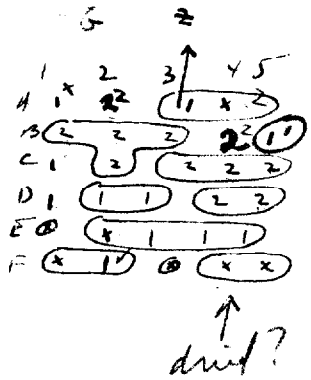
10^{43} empty.

Growth Type

Singles:

A1
A2
B4
B5 (franchimp)
C1
D1

G2 : P2
G1 : —
G5 P2
G4 ~~P1~~



G = plate on glass slide

Groups

A 3-4-5

Z 1-5 later.

died (dying?)

B 1-2-3-C2

B1 1
D3 ~~1~~ C2 ?
B2

C 3-4-5

5 1/3
1/4

D2-D3

E 3-4-5 large pair

4 5
2 3

D4-5 not stuck

B1 blank
F3 "

F 1-2
1
1

F 3-4 /

Deaths may have resulted from dry.... Were anticipated by 10 PM in failure of division of A4, F1, F4, F5. E1, E3 were blanks. E2 should have grown.

W1113 stock susp. was also plated on EMB Lac & Sor.
Counts difficult, possibly ~4 R1 : 32 P1 : ca 200-300 P2. on EMB Lac
On Lac-Sor, P1 : P1+R1 = 330 : 34 (very few R1 & P1)

P19. of P2, W1655, W2206 x P1. 10:1 ratio, Inoculate ~~Housed~~ Plate EMB Lac Sor.
W1655, W2206 No SR+ (> 300 -) each. P2 x gives 42 SR+ / 271 total (P1+P1R1)
(over) = 15.5%

Therefore the incidence of RI/PI isolations is now no better than chance!

Platings

DATE: 1/19/54. REF:

A) Direct platings of clones to EMB Lac agar.

			# cells recorded.	Lac: Found. 1/19 2:35PM.
possible leakage from glass!	1	(A2)	3	3 ±
	2	(A1)	3	0
	3	A5	3	6 ± (1 under glass)
	4	B5	2 (— —)	2 − Both Gal+
	5	B4	11 (some doubled?)	13 ± (1 under glass)
	6	B3	3 doubles	5 ±
	19	F2	3 (fuzz)	3 − (

to 11:10PM 1/18. Excellent recovery.

B) on cover glass. E1, E2, F1, 3, 4, 5 and A4 n.g. (as reported at 10^-3 also! as empty cover glass cell smear) all others gave, including 2 (extra from A3) 1-5.

		Lac	Gal	T1	SM.	Diagnosis	G↑:
2	1	—	+	S	R	P1	
	2	—	+	S	R	P1	
	3	—	+	S	R	P1	
	4	—	+	S	R	P1	
	5	—	+	S	R	P1	
A	3	—	+	S	R	P1	
A	5	±	−	R	S	P2	
	A1	±	−	R	S	P2	
	A2	±	−	R	S	P2	
	B1	±	−	R	S	P2	
	2	±	−	R	S	P2	
	3	±	−	R	S	P2	
	C2	±	−	R	S	P2	
	C1	−	+	S	R	P1	
	C3	±	−	R	S	P2	
	4	±	−	R	S	P2	
40	5	±	−	R	S	P2	
	D1	−	+	S	R	P1	
	D2	−	+	S	R	P1	
	3	−	+	S	R	P1	
	D4	±	−	R	S	P2	
	5	±	−	R	S	P2	
	E5	−	+	S	R	P1	
	4	−	+	S	R	P1	
	5	−	+	S	R	P1	
	F2	−	+	S	R	P1	

some pool

Gal+ colonies picked

	Lac	Gal	T1	S	
B4	±	−	R	S	Lac−
B5	−,+	+	S	R	Lac−

Inc. Lac+ Pap:

B5 ✓ :

∴ all parentals except B5!

High ratio of P2:P1 not necessarily efficient.

Perfect concordance with drop platings.

∴ of original cells, These were 7 groups of P1, 5 groups of P2, [redacted] 1 cell → P1 → P1+R1 and 1 group of P1+P2 → P1+R1

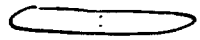
Cells: [redacted] P1 12 P2 1 P1+R1

✓ 2 Mixed and 5 dead

B5.

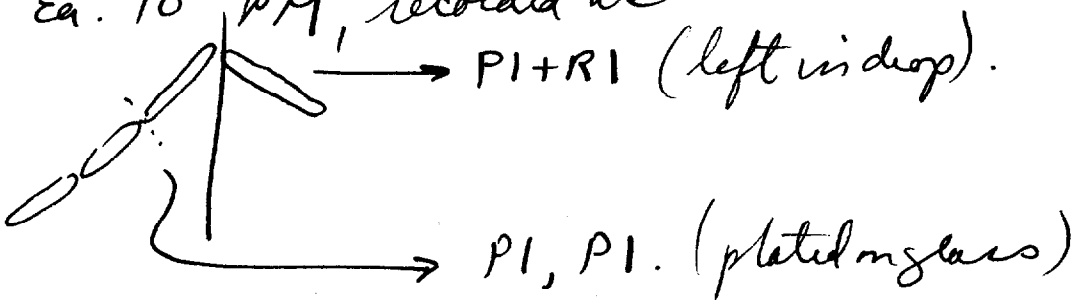
"Isolated as a single O cell from a clump."

at 5:12PM recorded only as



"Long"

At ca. 10^{30}PM, recorded as



see remarks over next page.

DATE: 1/21/54. REF:

11 – 1[30] P1×P2 1:1:8 Permassay.

Bil. (Intern. visitors ...)

at 5[10-30] 8[30]

X 1. large cells 4[10]

2. 4[14]

same at 5[10]

8+smaller

12+2 in one chain

A 3 } 4 } stayed together to 4:16 → → 5[10]

* same

5 } 6 } → 5 as unit 6 +2

4+4

0

separated

20 2 [not absolutely criteria but no other similar cells in neighborhood J.

X 6PM – 8[20]

9:9PM → A1

* 30 ** ***

Retransfer to B:

40 A1 → B1.

A2 makes → B2

A3

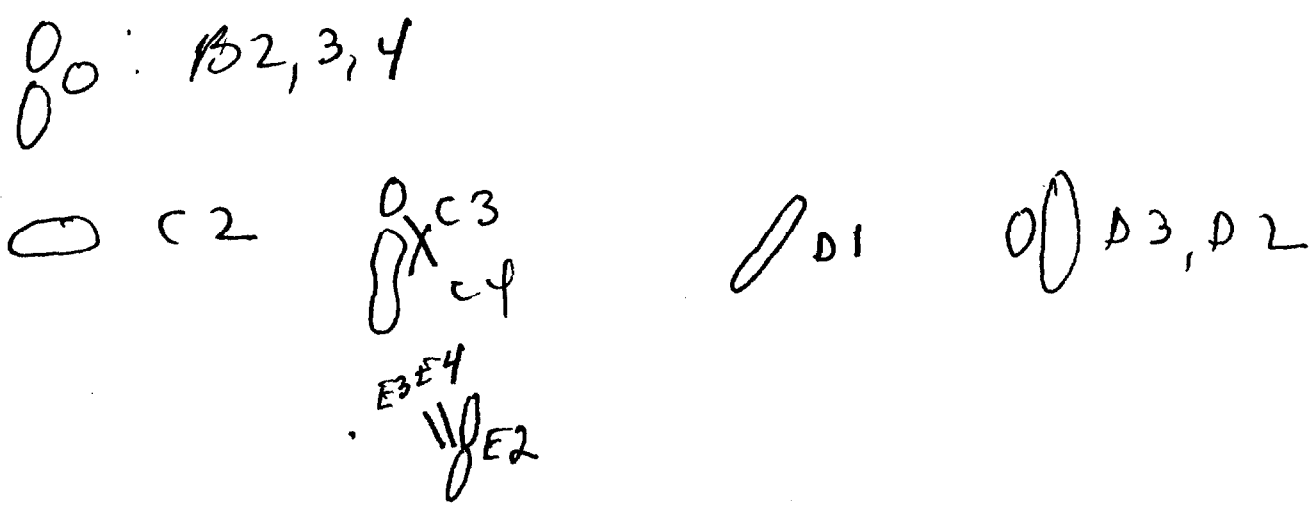
A4 → B3

5 A5 → A4

9:00PM A3 has:

(over)

1114C.



(all but E3, 4) = P1
= P2

1114
Series

EMBlac

A. 1 → B1 NG

2 ⇄ B2 1 P1 ← R1. ×1 SR V_1 S → P1+R1

3 4 P1 P1

4 → B3 NG; ↗ 0

5 → 10 P1 P1

6 6 P1 P1

B. 2 Lac+ SR Gal + V_1^S Pure Lac+ ! % A 2

C. B 2 Lac− SR Gal+ V_1^S P1

3 " " P1

4 " " P1

C 2 " " P1

3 " ✓ " P1

4 " " P1

D 1 " ✓ " P1

2 " ✓ " P1

3 " ✓ " P1

4 " ✓ " P1

E 2 " " P1

3 ± S^I Gal − V_1^R ✓ P2

4 ± S^S ✓ Gal − V_1^R P2

2 P2: 14 P1 :

A1 B1.  → A1, B1.

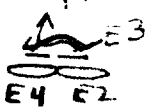
A3 B2 B3 B4 C1 C2 C3

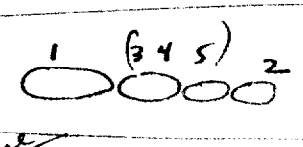
B2 B4

(B3, A3)

D1-2-3 D4-D5

E1 ?lost

E. 

F. 

	Growth	Lac (A25)	Gal			
A1	✓	−	R	+	S	P1
B1	✓	−	R	+	S	P1
A3	✓	−	R	+	S	P1
B2	✓	+	R	−	R	P2
B3	✓ SN → grew out A25 (P1 mosaic)	−	R	+	S	P1
B4	✓	+	S	−	R	P2
C1	✓	−	R	+	S	P1
C2	✓	−	R	+	S	P1
C3	✓	−	R	+	S	P1
D1	✓	−	R	+	S	P1
2	✓	−	R	+	S	P1
3	✓	−	R	+	S	P1
D4	×					
5	×					
E1	×					
2	✓	−	R	+	S	P1
3	✓	−	R	+	S	P1
4	×					
F1	✓	−	R	+	S	P1
2	✓	−	R	+	S	P1
3	✓	−	R	+	S	P1
4	✓	−	R	+	S	P1
5	✓	−	R	+	S	P1
G1, G2		− −	RR	++	SS	

all pure

(P1 2 P2

A25 controls OK.

λ^S ✓

Destruct appearance on EMB bac and gal? Gal possibly a minor mutant

last P2's { P1 No Ey; P1 Ey }

{ P1 }

1/23/54

1:1:3

4:25 - 3:05 at 33°

3:05 - 3:15 at RT to dil. for pour. - 5:05. Plating at 4:05 PM:

EMBlac sm. 3 P1·R1 per 167 (2%).

EMBlac: probable zygotes = 5/130 P2 / 113 P1.

In many 3/4 of 13 plates, colony appearance suggests following distribution: R1 P1 (P1P2 R1P1P2). Simple natural colonies P1+P2 not counted here.

111 ≠ HH 111

This may of course be inaccurate.

G1,2 = pour. D1, D2. " controls OK.

Grew P23 - P24 at R.T.

A1, 3, B1, 2 C1, 2, 3 D— E2, 3 (6?) F1, 2, 4, 5, 6.

∴ growth failed in (B3) [redacted]; D1, [redacted] 4, 5; E1, E4;

controls OK. Same may have dried out.

Morphology v Hg 28A except: B2 (motile, bacillary)

B3 has cells limited surface development. Hold!

large, wide elements

B4 grew in a small droplet. bacillary but not motile; - - , - -

D2 small drops. D3 small drops D4 — D5 drop (2 colonies?)

D6 drop

E1 — E4 — F3 small drop.

Induction.

1/25/54

W165J, W2338.

exp. cultures in Runnaway, 1:1:2 3^{20} at 37°.

3^{40} dilute 1:50. Try to find F+/F- pairs. But degression too dilute. However.

D4-5
E4-5 form poss. pairs, but both are morph. W2338 next day.

D1-2-3 from poss. { 10-2. D3 n.g.
10-1. D1,2- to EML.

contrib
OK.
A-E
1-5.

D3.

		EML F test (659)
D1	-	
D2	-	0/4
D3	0	0.
D4	+	8
D5	-	
E4	+	8
E5	-	

1/26/54.

P1 x P2 (old) 1:1:8 12¹⁵ – 2¹⁵.

(1) Plate on EMB Lac sm to rec V segregation (cf. V_1^S character of all SR+ so far!).
(B) ca 3PM. (B) zero rec

(2) Dilute 1:50 for single cell...
High ratio of minicells. This experiment messed rather [illegible] "glom cells" still possibly in stationary phase?
Drops empty overnight still sterile at N28. Colds —
Grew:

			EMB Lac	Gal	T1	lac	
1	B	3	—	+	S	—	S R
2		4	—				
3		6	—				
4	C	4	—				
5		5	—				P1
6	D	3	—				
7		4	—				
8		6	—				
9	E	4	—				
10		2	—				
11		3	—				
12		(4)	—				
13		6	— ; ++ Rev	[no lac I seen; T1: S, R.			
					S	—	R P1
14	F	1	NG (drop growth also peculiar)				
15		2	—	+	S		
16		3	—	+	S		
17		4	—	+	S		P1
18	G	1	—	+	S		
19		3	—	+	S		
20		5	±	–	R ±	S	P2
21		6	—	+	S		
22	H	1	—	+	S		P1
23		5	±	–	R ±	S	P2
24	I	1	—	±	S –	R	P1
25		2	—	±	S –	R	P1
26		3	±	–	R ±	S	P2
27		6	—	+	S –	R	P1

Plate

almost all P1

23 P1
1 P1, R1
3 P2
compare with high incidence on γ!

P1, R1 < Lac + Gal + S V_1
Lac – Gal + SR V

11 cols started → my Lac + V_1^S?

E 4 was murky. 5 [illegible] #5 n.g. But this was 150 minutes more.

1117B. Picks Lac+ from LMB Lac to classify proportions of types and to characterize Lac-V_1 segregations.

Columns 1, 2 6, 7 Brush 65 colonies on LMB Gal/TI (unpurs.) and indicate TI/Gal types Gal+V_1^S/Gal+V_1^R as easily typeable but some doubtful

Columns 3, 4, 8, 9 Lac types on streaks not to be reported

1117B: Initial (TC N.) 2×10^{-7} ml pumpkin.

LMB Lac	Lac+	40.6	Lac−	
$\bar{X}$:	Lac−	55.2		Lac− 48.8
	+/−	3.5	(SR+)	+/− 3.7
		99.3		

∴ SR+ = Lac+/−. Plates all showed Poisson of each type.

$$\% \text{ zygotes} = \frac{3.5}{99.3} = 3.5\%$$

$$= \frac{3.5}{58.7} \text{ of Pl}_{\text{Type}} = 5.96\%$$

...reported ca ~~[illegible]~~ 1.43 zygotes from 24 Pl- isolations.

Found 1 (see over): not different from chance!

Total χ^2 testing Poisson variance: $\chi^2 = 56.0$ d.f. = 50

Plate Jackalyne Newton Hetero 2-9 3-8

$\chi = 10.583 - 9.950 = .633$ $p = .6-.5$

1117

DATE: EMB Lac REF: EMB Lac

	1 Lac +	2 Lac −	3 het	4	5	6	7 Total	8 het	9 − het
1	48	51	4				55	4	51
2	44	48	4				56	1	55
3	31	67	3				53	7	46
4	52	61	3				62	9	53
5	28	49	4				42	3	39
			3					0	
Σx	203	276	4					3	$\Sigma x = 244$
Σx^2	10689	15516	4					7	$\Sigma x^2 = 12072$
$\bar{x}$	40.6	$\bar{x} = 55.2$	2					4	$\bar{x} = 48.8$
Σd^2	447	281	1					3	
χ^2_4	11.0	5.1	8					3	$\Sigma d^2 = 165$
p	.05-.02	.3-.2	3					2	$\chi^2 = 3.4$
			3					4	
			2					2	
			2					5	
			3					2	
			2					4	
			4					4	
			5						
			3						
			5						
			6						

Lac vs. Lac

$\chi^2 = \frac{32^2}{520} = 1.97$ $p = .2-.1$

Lac vs. Lac

$\chi^2 = \frac{73^2}{479} = 11.1$

$p < .01$

$\Sigma x = 78$

$\Sigma x^2 = 326$

$n = 22$

$\bar{x} = 3.545$

$\Sigma d^2 = 326 - 276.545 = 49.455$

$\chi^2 = \frac{49.455}{3.545} = 14$ $p = .4$

$\Sigma x = 67$

$\Sigma x^2 = 333$

$n = 18$

$\bar{x} = 3.722$

$\Sigma d^2 = 333 - 249.389 = 83.611$

$\chi^2 = 22.5$ $p = .2$

$F_{17,21} = \frac{4.918}{2.355} = 2.09$ $p > .05$

$s^2 = \frac{133.066}{38} = 3.502$

$\chi^2_{[17]} = \frac{9.9[.177]^2}{3.625} = .0856$ $p = .75$

$s^2 = \frac{145}{40} = 3.625$

∴ Het variance Poisson, means homogeneous.

	Col. 2,3	Col. 8, 7-8	
+	276	244	520
−	18	+ 24	42
	294	268	562

$\chi^2_{[1]} = \frac{(2232)^2 n}{\pi} = \frac{2.8 \times 10^9}{1.72 \times 10^9} = 1.6$ $p > .2$

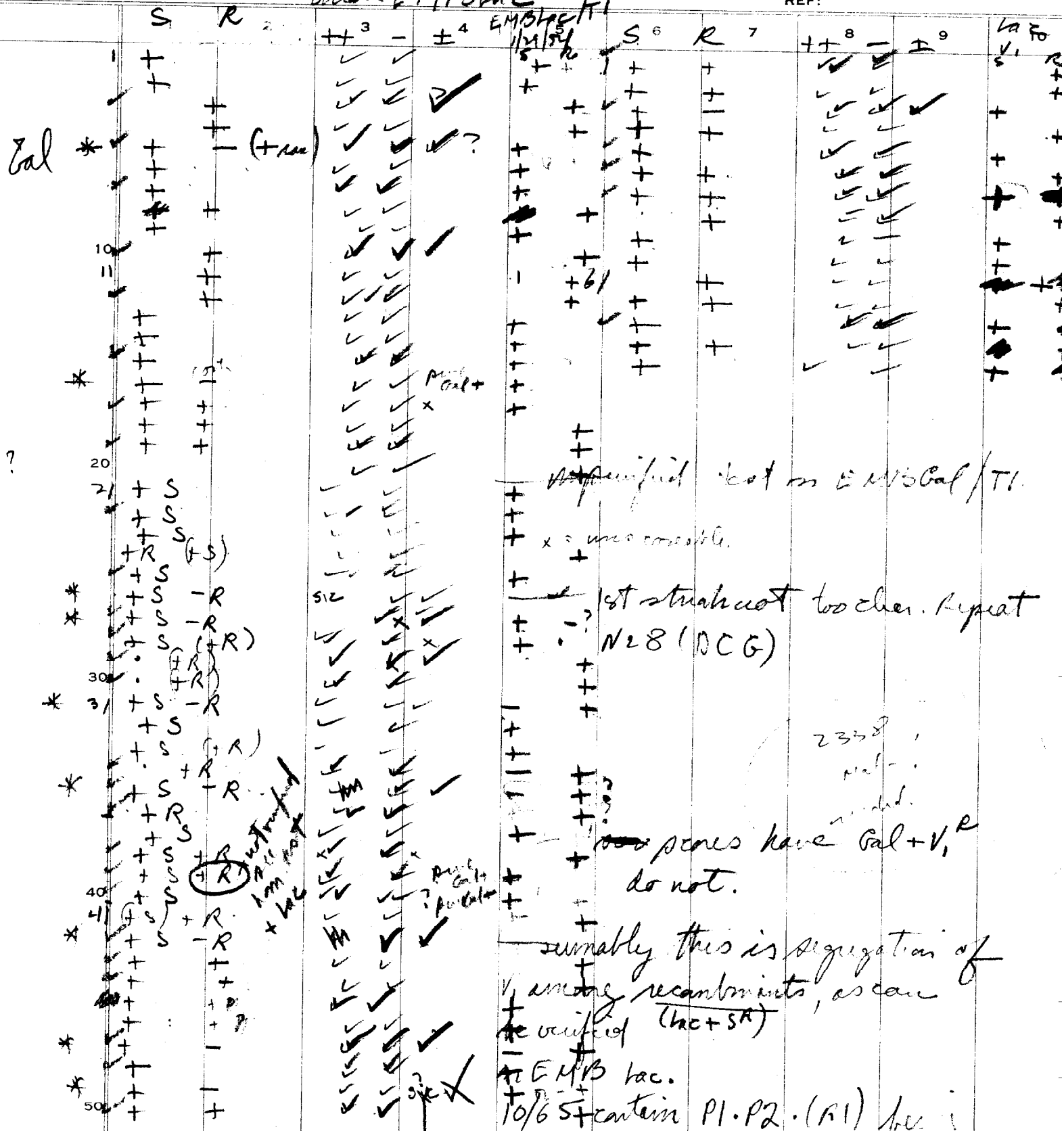
∴ Frequency of het. homogeneous in the two tiny het: lac.

11173
(1)

EMBGal 1/27/54

DATE: V1 Streak EMBGac

REF:



of 64 bytes relative to 1119B) scored,

31 lacked Val+V_1^R ($\pm$ Val-V_1^S), but had Val+V_1^S

32 had Val+V_1^R, at least 21 also Val+V_1^S (pseudoparalogs) and recombinants

Thus ~~no~~ clear signal for linkage S/R ~~if~~ the latter pure. Test for homogeneity of recombinants

---

① For this purpose, ignore pure + S strains

② Check ① Lac- four Lac+ from others for homogeneity.

~~Lac- V_1^S among pure+R~~ (true if exist in 1119).

Lac+ Lac/S segregate normally with 'al

Single most likely explanation definite

Rec ~ 27,57 cs

Very few Lac-V_1^R recomb.

These should have been detected if present.

In retrospect, all cases reverted Lac+ on V_1^S

Reread scores

Zygote chart: 1 R1
2 P1+R1+P2
3 P1+P2

V_1 chart (incl 3)

A pre-V_1^S Gal(+)

B V_1^R Gal+ present

• = V_1^S + notch

1173

(1)

also Lac- V_1^R A+

-HH

Final

~~2A~~ 25A

53-1 < ~~3B~~ 4A 26B

7-2 < 3B

5-3 <

+350 type 1

B type 2

+1 4 type 3

at 2½ hours

4 to be verified

Variety of V_1^R might be useful in separating type 2 and 3

2B = just the overcast stage (normal, confused c 3).

$R1^S + P2$

~~Totals 1,2 A 29~~

~~1,2 B 31~~

Notable discrepancies in Gal/Lac for #17, 28, 33, 45, 46, 57. were classified as 1B after confirmation, with 22 1A : 25 1B.

46 + S hea - R

A

x maybe 1A+ -R

2

3 x

49

In Class 2, which are det. tot. as Lac+V_1^S Lac−V_1^R ?

3 Bur
5 Ayer
10 Bur
7 B
3 A
3 A

17 A

∴ Totals:

1 < 25A, 26B — Lac+V_1^S / Lac−V_1^R — 2

2 < 4A, 3B.

$$\therefore \frac{\text{Lac+}V_1^S}{\text{Lac+}V_1^R} = \frac{25+2+4}{26+3} = \frac{31}{29}.$$

∴ no discrep. here — see 1117C

If retest in 1-cell isolation:

	Re-examine Lac/T1	Repeat Lac/T1
17	Clear plaques +	probably Lac+V_1^S
28	" " +	mixed: Lac+V_1^S/Lac−V_1^R Lac+V_1^R and score as 1 each.
33	" " +	Lac+V_1^S/Lac+V_1^R (P2) or remove from more.
45	" " ++	~~Lac+V_1^S/Lac+V_1^R~~
46	" " +++	~~Lac+V_1^S/Lac+V_1^R~~
57	" " ++	clearly Lac+V_1^S
	accidental Lac− only.	Lac−V_1^R

+ prob
++ certain
Lac+ V_1^R

17 28 33 may have −S, +R

45, 57 have −R+S

46? may have Cal−V_1^R

1 A

28
33
45
46

Homogeneity tests.

1117 S

1/29/54 (1117 L 4) II ~~Gal+ Lac+~~ Lac+ Gal+ of V_1^R

Pick Lac+ (prof. not ± or −) colonies and test on Gal/TT.

1/29	10:	Gal+	5R	1 S
4 per	26 3:		6	+ R
2	10 26 29		6	+ R

(v Lac) ~~Repeat Lac~~ Restreak R, S

Lac+ components (Lac− presumed V_1^S)

~~Clearance~~ / ~~Lac~~ homogeneous, ~~Lac~~.

1B	50	6	1 R
1B	51		+ R
1B	34		+ R
1B	~~32~~	6	+ S ! :: 1A Lac− both pure Lac+ (R same but weaker!)
	43	6	1 R
	44	6	+ R

~~Restreak~~ Restreak both types from these on Lac (−)

(Lac+ impurities)

Could this be a Lac− Gal+ by mistake? (= P1)

When streaked, S and R comparable

Some pure Lac+ / colonies, in 2B types

As ~~is~~ likely both P1 recombinant recombinants where there was a P1 or not! This is probable explanation. Restreak on EMB Lac ± soon for further analysis.

Conclusions:

① Most zygotes are homogeneous in yeast unless P2 still present. V_1 heterogeneity might be useful to discriminate primary + secondary recombination.

② V_1 S zygotes 1:1 of R/S

2/3 46: 10 Lac− are V_1^S 57: 9 Lac− : 2 V_1^S 7 V_1^R

1117B 57: 4 Lac+ S (V_1) 4: 4 Lac− S

screen: 4 Lac− S 11: 4 Lac− S

27: 4 Lac− S

30: 4 Lac− S

58: 2− R

2− S assoc. with Gal+ (Lac+?) V_1^R ~~and~~ any Lac+ V_1^S?

36 4− S

note, e.g. 58 Lac− also mixed R/S so

~~that~~ there is presumably Lac+R, Lac−R, Lac−S

Structure of V_1^R from 45: Gal+, mostly Lac− (some fuc+ papillae)

∴ 45 contains Gal+ { Lac+ V_1^S ~~Lac+ V_1^R~~ ~~Lac− V_1^S (PI)~~ Lac− V_1^R

Lac+ V_1^R? (new recomb? cf. 1107B5?)

46: Gal+, mostly Lac+ ~~(some −?)~~

∴ Lac− V_1^S presumed. (PI)

Lac+ V_1^R

Re ~ exceptions

1117B

2/2/54 stacy

① Restreak on EMB Lac, Gal:

17	pure Gal+ ;	lac+ seems weak.,	lac+/−
33	"	lac++	"
45	"	"	"
46	"	"	
57	"	"	

Types definitely seen.

1117 B3.

Gal+ unless qualified

. = tested for. by present test

□ = present

⊙ = " absent by present test.

	(Gal−)	Pl. lac−V_1^S	− R	+ S	+ R	tested
2A	3 ✓	□			✓	6
	4	□✓	.		□	4
3B	10 ✓	□		✓	✓	6
	11	□✓	.		□	4
	27	□ —	.			4
2B	29 ✓	□			□ —	6
	30	□✓	.			4
	34	□	.		□ ✓	
	36	□✓	.		□	
	39	□		✓	~~□~~ □	
	43	□		.	✓	
	44	□		.	✓	
	50	□		.	✓	
	51	□.		.	✓	
	58	□✓	✓		□	4

from original tab. as 1A!

but not all

∴ many (~~all~~) 1B types are plus V_1^R in lac+. Lac− not tested [difficult analysis to multimarker test] (and lac− V_1^S for "most" but of. 00).

	17	✓	R	✓	✓
	28	□	✓	□	□
	33	✓	✓	✓	✓
	45	✓	□R	✓	✓
	46	✓ ✓	· wk R)	✓	□ + [redacted] R
	57	✓ ✓	□	✓	⊙

of. 1107/35

∴ a fair proportion of colonies may have, in addition to −S (= Pl) 1, 2, or 3 addl. components. How many is not clear, as not many examples

R: weak + only.

If patient lac+ V_1^R were examined further. But note 39 above. V_1^R is therefore perhaps slightly overstated.

– Further colony analysis 2/3-4/54

Lec - 10.

17C1 → 4R 4S

2 → 4R

3 3R

4 8S OR.

(4 probably before C 1 ?
in earlier text)

Counted stats 2/4/54.

type 1A (presumed simple!) 25 (pure $V_1^S <^{+}_{-}$) − 25 + 5C

1B (given as simple but possibly containing +S also) 24 + 5C +1

C. Containing $^{+}V_1^R$ and $^{+}V_1^S$: (17, 28, 3 3, 45?, 46) 5.

D. (77) Containing +R, −S, −R. (1)

∴ Detected + R (parental) = 30

Screened + S (+R not eloquent) = 30.

= 1:1

? Possible [illegible] of a detected [illegible]

(1) Loc ± S^S [illegible] Loc+ S^R (Gal+).

2. Gal+ V_1^S [illegible] Gal+ V_1^R (Loc−)

i.e. pure V_1^S [illegible] " " S^R.

Left occ: Loc − S^S [illegible] Loc − S^R (probably best handled with additional pure numbers)

and ~~+ − V_1^S S~~ [illegible] Gal−

SR+ colonies picked & purification to EMBlac.
40+17. 1/31 Replica to EMBlac ± T1.

C1: Each streaked on EMBlac was lac+/lac−. With T1:

Unaltered recipients (i.e. lac+ V_1^R (lac− not verified)	19	6	25
pure sensitive lac+ V_1^S/lac− V_1^S	17	9	26
lac+ V_1^S/lac− V_1^R	3	2	5
[illegible]	1		1
	plate 1	plate 2.	

1-3 17C

no!

Or in sum: lac+ are 31 S: 26 R / 57 total.

Include at least 6 lac− V_1^R recombinants, possibly more.

1117 B: 31 S: 29 R.

	recomb. (S)	par (R)
B	31	26
C	31	29
	1	1

Totals:

Thus.

1082	26	25
1119	15	18

agreement is obvious
Recalso 1082. (25R: 26S)

Still queries on incidence of lac− V_1^R recombinants and association with lac+ V_1^R ($\underline{S}^R$). Are the lac− components of these pure?

Misc cells.

DATE: 1/29/54 REF:

1235 Overnight P1, P2 1:5 310 1:1.5 X. Obs at T
(1235 cross W2377 × W2341.) − 3:55

Cross with P8. EMB lac strains Gal, V, , S (Lac)

A 1 ✓ —
2 ✓ —
3 tiny dots 0 —
4 ✓ —
5 ✓ —

all nic Gal+ V_1^S S^R Lac− S =
except C2 = Gal− V_1^R S^R Lac
= P2
and E5 = P1, P2 (S^R +
V_1^S V_1^R papill

B 1 ✓ —
2 0
3 0
4 ✓ —
5 ✓ —

20

C 1 ✓ —
2 ✓ ± and 2 – 2 colonies (= contamination (also Gal+ V_1^R spath, not in nicked tube)
3 ✓ —
4 ✓ —
5 ✓ —

D 3 ✓ —
30 ✓ —
5 ✓ —

E 3 ✓ —
4 ✓ —
5 ✓ —
40

± and − in equal proportions P1, P2

F 4 ✓ —
5 ✓ — (Successive plating is unstable.)

{ N_0 S^R + (exc. at intersection).
Lac − = S^R on replica = Gal +
Lac + = S^S = Gal −

50

entes OK
A 3 OK
B 2, 3 n.g.

18 P1
1 P2
1 P1+P2

DATE: Jan 27, 1954 REF:

W2377 x W2341 12 35 – 50M.

A EMBlac

B EMBlac sm

C EMB lac T1. (for S-ratio)

D 10 = C ca 10X

C: ~~colonies~~ Lac–, Lac+ canp. (6 have only small papillae? multiple anything noted with stepton spot.)

19/165 tot.

11/122 } (patsquared)
5/170 }

35/357 +-/-. A: + ca = . Not acc. scorable for + vs ±, –.

B 30 +-/tot = 12/171
6/149
15/236
14/231
47/787

Satan (apparently) in B: 2+1?
C: 0

1/28. Pick B, C, D to EMBlac + other for later study.

This worsdoes pick up the also.

lateration of phage on or secondary crossing ?

Since virtually all of these colonies in B or in C are Lac+/Lac– sectored, no recombinants are here missed that are not otherwise picked up by only ca +/–.

Why discrepancy in rel. count? 10% in C
ca 6% in B

a) (contin) some 'papillate" V_1^R Lac+
b) on other hand, some S^R lac+ are V_1^S!

1119
B – C – D

DATE: 1/31/54. REF:

(B) Plates stored from 1/28 in frig.
Replica B to Lac, T1 to score the V_1 segr. ratio (cf. 1117B).
all colonies have Lac – V_1^R (P1 residue); score strokes for presence of Lac+ s/i T1.

2/1/54

	Lac/ch:	– T1	+ T1	Score type
1		+	(–) +	1
		+	+	1
		+	+	1
		+	+	1
		+ –	– +	1
		+	–	2
		+	–	2
		+	+	1
		+	+	1
10		+	–	2
		+	–	2
		+	–	2
		+	+ –	1
		+	–	2
		+	–	2
		+	–	2
		+	+	1
		+	+	1
		+	– +	2
20		+	+ –	1
		+	–	2
		+	+	1
		+	+	1
		+ –	– (+)	2
		+	+	1
		+	+	1
		+ –	– +	2
		+	–	2
30		+ –	+ –	1
		+ –	+ –	1
		+	– +	2
		+	+	1
		+	+	1

∴ 18 type 1 15 type 2.

Lac+	V_1^R	V_1^S
	(par)	(recomb.)

Totals now are ~~98~~ parental | ~~99~~
Lac / V_1 : ~~10~~ recomb. | 10^2
– among Lac+ S^R recombinants, neglecting V_1 reversal. $X_1^2 = \frac{12.5}{101.5} = .1$
$p = 0.8$

< 10 + all V_1^R

< 10 Lac+ Lac– all V_1^R ∴ type 1.

Restrict 1119 B ~~B~~ B 20 for components:
actual numbers Uniform type 1 : 19
2 : 14.
$X_1^2 = 0.7$

But p_{total} almost too high!

Scores: 1 : T1 no effect = + R / –.
2 Lac+ V_1^S / – R
[3 Lac+ V_1^S (+ V_1^R ?) / – R] probably ≐ 1, phage inadequate mixture possible but ignore here. They would not influence ratios ~~in some marked~~ 3. in any event.

DATE: REF:

Replace lac+V,R/−R to EMS lac + Sm.

2/1/54.

C1. 30. Lac+ equally prominent in EMS lac sm.

C2. ← C 10 / D 20. " " " " " "

D1. 23.

D2. 29.

Undetermined

112 all orthotype S^R among lac+V,R recombinants. Still possible that some S^S are present but obscured in mixture but no evidence for it. . . S behaves very differently from Lac, V_1. Other data show extreme rarity of Gal/S recombinants but this should be reviewed in multipoint tests.

Recheck in 1119 only to unify 13, 20.

2/5 See also 1076, just noted!!

Note also, in 928-94, W1895 × W1177 among lac+ S^R's, reported 4/S; 74R. (poss. of selection through linkage to TL?). Needs to be repeated.

Casual 1-cell cool. 1120

DATE: Jan 28, 1954. REF:

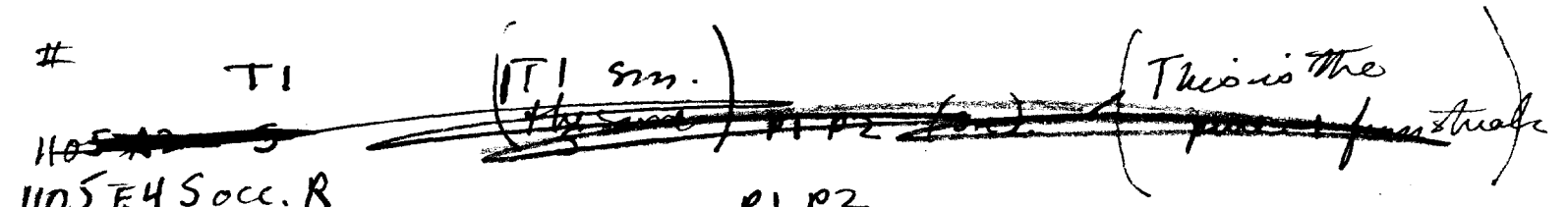
	1	2	3	4	5	6	7	8	9	10
Cross: old mouse P1×P2 1:1:8, 2:10PM – 3:30										
Setup 3:45. Morphol: (mostly ...) Bee degas 24 hours AFRT To										
Grow N29: N30 Gal T1 Lac S(A)30.										
A	1	∞	✓	not mot. (macro-)	+	S	–	R	P1	
	2	X								15 P1
	3	X	✓	1						5 P2
	4	∞	0	rec bacteria?	+	S	–	R	P1	
5. small cell from perhaps septa is septate, form group	5	0	✓	1	+	S	–	R	P1	1 P1, R1
	B1	∞	✓	1	+	S	–	R	P1	[redacted]
	B2	0	? 0	0						
	4	0	✓	1	+	S	–	R	P1	
	3	∞	✓	1	(+S)	(+R)	–, +	R	P1	R1 (V, R)
	5	0	0	0 rec bact?						
	C4 20	0			+	[redacted]	–	[redacted]		NG (with also unspiteful?)
	C1	∞	✓	1	+	S	–	R	P1	Previous tedious waste stock sent cahpsolate as well as cross back. Now omit the steak except when suspicious.
	2	∞	✓	1	+	S	–	R	P1	
	3	∞	0	0						
	4	v. Sup.		2 (+1?)						
	5	0	✓	2	(+S)	(–R)			P1	
D	3p	∞	✓	1	+	S	–	R	P1	
	2	∞	✓	1	+	S	–	R	P1	
	D3	D3	✓	1	+	S	–	R	P1	
	E2	2 3	✓	1	+	S	–	R	P1	
	E3		✓	1	+	S	–	R	P1	
	D4	∞	0	0						
	D5 40	0	✓	–	–	R	±	S	P2	
	E1	∞	0	0						
	E4	∞	✓	1	+	S	–	R	P1	
	F1	∞	✓	1	+	S	–	R	P1	
	F3	∞ (had one)	✓	2	–	R	±	S	P2	
	4		✓	2	–	R	±	S	P2	
dump	G3		✓	2	–	R	±	S	P2	
	(F3)									
	G4									
	G5									
	G6									

cultures O.K. Selection for type ... 1 P for medium

cells!

Review V, segregation in 1-cell reamb.

Cross bench against T1, T1 sm / Lac



1107 A3 S	P1 R1
1107 B5 S, R	P1 R1
1110 E5 R, S	P1 P2 ✓
1112 B4 R, S	P1 P2 ✓
1113 B5 S	P1 R1 ✓
1118 E5 S, R	P1 P2 ✓
1120 B3 R, S	P1 R1 ✓

now heavily mixed with R1 also.

New information:

1107 A3 R1 is S

1107 B5 ~~R1 is probably R~~

1105 E4 } stated as P1, P2 mid-stack.

11~~07~~ A7 }

misread label ("B52").

DATE: 2/1/54. REF:

P1, P2 Mix freshcells ~~1+2~~ 2:2:2 3rd (~9:30PM.)
look for clumps, pairs. Most resistance ca 4:30.
Note on motility. W2344 seemed much less motile in dilute growing cultures c 2338 than otherwise. Compare effect of growth phase, mixture.
See protocols A1, E1 ng. Controls D1 except
(1 very large clump = Lac+ Gal-) (Checking if possible accidents).

From clumps.

Lac S Gal V.
D

D
C inhibited on lactose.

A	B	C	D	E	F	D
x (large)	− 1 R	± 2 S	2 cells!	x	± 2 S	± S 2
− 1 R	+ S	− 2 R	− 2 R		− R	− R
+ S	− 1 R	± S	+ S	− 1 R	± 2 S	± S 2
− 2 S	+ S	− 2 R	− 2 R	+ S	− S	− R
− R	− S	− 2 R	− R	− 1 R	± 2 S	− R 1
± 2 S	− 1 R	± 2 S	± 1 S	+ S	− R	+ S
− R	+ S	− 2 R	± R	− 1 R	± 2 S	± S 2
				+ S	− R	− R 1
						+ S

P3

∴ No zygotes from these clumps.

P1 : 11

P2 : 17.

Note: clumps AB 1, 2
C all 2
D one 1, 2
E 1, 2
FD all 2.

Good agreement c morphol. predictions except for A4 which is drawn as smallest.

Use W2384 as P1 from here on. To date P1 has referred specifically to W2388

Misc. segr. 1122

DATE: 2/1/54. REF: Screen for evidence

A. W2381-6 × W2344

2:45 – 9:30 PM Plate 10^{-6}, 10^{-7} on EMB lac ±sm, EMB Mal sm.

[Usefulness of new Mal⁻ markers, esp. in assessing residual P2 carps.]

	EMB lac sm	Mal sm	Mal sm	EMB lac
✓ = SR+				
1	✓✓ – –	0	1 pinpoint: 3/1000	– –
2	– –	0	0 +/–	
2/2 3	– ✓	0	0	Segregating for M+l –
4	– ✓	0	0	
5				
6	– ✓	0	1	

Best these Mal⁻ evidently all/almost (nearly totally) the S^R segregators; and maybe equivalent. Use 2384 for future studies.

what A5?

B. 3.20 W2057 × P1, 2:45 – 9:30 PM

Plate 10^{-6} EMB lac sm. Streak lac+ S^R to confirm orthotypy for M+l, Mal among these recombinants in lines 1×28A.

EMB lac sm:

2/2 ✓✓ (Some pure lac+) Pool and restreak → ca 2% M+l – but analysis pure Mal+/several hundred Hi all lac+. from EMB lac.

Mal sm: pure Mal+. (Some colonies mottled very likely lac+/–.)

EMB lac: some lac+/–, often too crowded. ~~Picks as likely to EMB lac block Test~~

From EMB lac sm 10 lac ✓: 9 pure V_1^S 1 V_1^R (all M+l+) (different from line 1×28A in V_1 segregation!)

(V_1 orthotypy).

C. P1 – P2 (W2338 × W2344) plate EMB Cal T1 } to test for lac +/– ratio among V_1R^SR recombinants (cf 1082.)

(plate as from 1121. 9:30 PM) EMB lac sm T1 }

did not exist (over)

Freehand, pick EMB lac lac++ (A) and lac +/– (B) for Mal test.

10 · Mal – SR+ 3 Mal +/– Spath, SR+ (over)

Control:

P1 clear: heavy colonial background and partial survivors. Phage titer evidently inadequate.

Microscopy:

EMB Lac sm T1: Lac+ probably >> Lac− but many of former also nibbled! Microcolonies platings of cross (same T1) Lac− → Lac+!

EMB Lac T1 Fewer T1R and: not reliable!
Repeat & better phage stock!

Conclusions:

A. Mal− have all effectively linked to S, show orthotypy

B. W2057 has same elimination patterns for Mal, S, MR as W1895, but note that the V1 ratio is quite different!! (look for V_1^S in × W2377?) Have these V_1^R been tested with T5? This is same V_1^R as W1177 (= W−1 V_1^R); may be different loci.
Also earlier data with W1895 × W1177 where V1 ratio was strongly orthotypic!

C. No information: need fresh T1 for selections.

2/7/54

See 1175

Cont. of W2393 (2390 - 2393) = MtL - W2384.
Test for suitability in mating line. All showed very few MtL + SR compared to SR lac+, but W2393 chosen as having highest (inx P2) (abt 10%).

2/7: W2393 x W2344 on EMBMtL sup, Lac sens. [Cross all day.]

2/9. (1) Ratio of ~~lac~~ MtL+ to lac+: Pick SR+ from lac sens to EMBMtL.
Found: 2 MtL+ : 67 MtL-.

(2). $V_1^R/^S$ ratio in lac+ SR of W1895 x W1177. Test colonies smithy as classify as + S (A.) − R; + S (C) ....; and + R (B) .... lac T1.

1	A	A 1 B (lac+ sw. < ^S_R; - $^?_R$	31	A	But note: incidence of B suggests that most zygotes had already segregated, thus skewing trial.
2			32	B	
3	B			C	
4	A			A	
5	B			C	
6	A			B	
7	B			C	
8	A			C	
9	A			A	
10	C		40	C	
11	B			B	
12	C			B	
13	C			A	
14	B			B	
15	B			B	
16	B			A	
17	B			C	
18	A			B	
19	A			?	
20	C		50	C	
21	A				
22	B		51	A	
23	A		52	A	
24	C		53	C.	
25	A	17A + 1 A:B } 32 V_1^S			
26	C				
27	B	14C			
28	B				
29	—				
30	B	19B + 1A·B 20 V_1^R.			

2/3/54.

(A) 8:45

2384 x P2 1:1:2 3PM— Dilute ca 4PM,

~~[redacted]~~ Aerostain (G.D.) Also Plate 4:20:
i.e., ca 80 minute cross
(dense mix.)

EMB Lac + 2nn.

Gummy contaminant present (probably from W2384 broth and plant!) but ignore.

SR+ ca. 1%.

Pick Lac V (A: single sector B: others ± Lac++) to EMB Mal.
(Most had overt Lac+ type ~~colonies~~) → of ~~7A,2B~~ 7B, 2A, all but 1 had Mal+ (as suspected from colony appearance).

(B) W1177 x W2344. ca ~~8~~ hours in broth. Plate EMB Lac 2nn.
Pick SR+ directly vs/T1 on EMB Lac. Each of 29 Lac+ : V, R.
Review of V, R = orthotypy, cross should perhaps be reversed in re the V, R used. (Cf. 110735.... carrying $V^{R}_{1(234V)}$ F^{-}.

(C). Note W2384 stock culture contaminated with gummy ~~Pol~~ Lac+, possibly also phage. Recheck single colonies for agency.

(D). Of W2384 MH−, 1-7, all but #1 appear λ^{S}. #2 = λ^{R}?
Variable intensity of response to λ.

1-cells : W2393 ♂ × W2344 ♀.

1124

2/7/54.

see 1125A for crosses. Dilute at 1 hour.

Test isolates for MH, Mal, Lac, T1, S, Gal. Streptomycin Gal.

		Mal	MH	Gal	T1 n.g.	Lac	S	Gal Strep.
B	1	+	+	−	+	S	−	
	2	+	+	−	+	S	−	
	3	+	+	−	+	S	−	
D	1	−	−	+	−	R	+	
	2	−	−	+	−	R	+	
	3	+	+	−	+	S	−	
	4	+	+	−	+	S	−	
E	1	−	−	+	−	R	+	
	2	+	+	−	+	S	−	
F	1	+	+	−	+	S	−	
	2	+	+	·	(+)	?	−	

all parentals!

8P2
3P1

V_1 segregation in Hfr crosses.

2/6/54.

Recap. 1. In Hfr W2344 $lac^+ S^S V_1^R$ × line 28 $lac^- S^R$..., almost precisely 50% of the $lac^+ S^R$ recombinants are V_1^R. (1117B).

2. In line 1, W1895 × W1177, data are rather heterogeneous, but sum is 41S:74R. Should be recapitulated!

3. In W2057 × W2338 (line 1 Hfr $lac^+ V_1^R S^S$...), in one experiment only, found 1S:9R.

4. 1123B ~~W2057 × W~~ W1177 × W2344: No V_1^S recombinants. [see 1122]

2/7. A. W2393 × W2344 11:35 AM — PM. Plate on EMB lac sm and EMB Mtl sm. Test SR+ for lac (Mtl) and V_1 segregation.

1:1:5

[B. W1895 × W1177 ". see 1122.]

A). Mtl+S^R picked at 24 hours. Cf. isolated Mtl+ and original mass

	Isol lac	Isol	Mass lac	Mass V_1	
1	−	S	✓		includes lac+ papillae
2	−	S	✓		" " "
3	+	S	✓		" lac− V_1^R
4	−	S	✓		" lac+ V_1^S papillae
5	−	S	✓		pure − S
6	+	R	✓		includes, lac? S
7	−	R	✓		pure: same background of S.

lac+ (4 colonies each) is:

1	~~lac~~ Mtl − V_1^S
2	Mtl − V_1^R
4	Mtl − V_1^S
10	Mtl − V_1^R

(over)

∴ lac V_1

−	S	8
−	R	3
+	S	1
+	R	3
		15

Lpores. that some of these are Mtl+ recessors should be studied?

Also isolate the lac+ components: are they also Mtl+?

see 1129

∴ $V_1^R = 6/15$

Lac+ = 4/15

Exp. +R = 24/225 ≐ 1.5/15

but actually +R > +S!

8	−	S	✓
9	−	S	✓
10	−	R	no-S sm; lac+ pap.
11	−	S	✓
12	+	R	✓
13	+	R	✓
14	−	S	✓
15	−	R	✓

1 ? (over)

AA: Ross. [Hfr- lac+]

- S: 3

+ R 1

(+S-R) 1

- R 1

+S+S 1

1? S 1

Withhold conclusions at present.

Mtl+lac⁻ Sᴿ and Mtl⁺ lac⁻ Sᴿ

may occur in same colony!

Xyl – Lac segregations 1125CD

See 1129.

C. W2394 x P2 D. W2397 x P2 in SR+.

D showed many more Xyl+ SR / Lac+ SR than did C.

a: Test Lac+ SR by direct streak on Xyl.

b. Do. Xyl/Lac.

have Lac+

b) C : 1/1 lac+ D 4/8 Lac+

a) C: 3/28 Xyl+ D 1/25 Xyl+. Not conclusive. est to difference.

25E 2/12/54. Cross 2394, [redacted] x P2 on EMB Lac, Xyl sm.

No SR+ found on Xyl. See 1129 for repeat.

Numerous on Lac. (= 1126 cross)

Feb 10, 1954

W-2394 x W-2344 (P2) 1:1;5 6:30 – 8:00 PM. Then diluted 1:100 (Refr. for later renewal.

Note, in several previous manip, micropipette is used to ~~[illegible]~~ transfer cells to prepared larger drops. This allows possibility of contamination, key cells studied by ~~to~~ outside of pipette.

Set I (8:15-10:00) = ABCD Set II = EFG, H6 (original notation: ABC, 05)

Set A (Previous evening, same cross?) H (originally D).

			Gness	Lac	Gal/Tmal	Mal	Xyl	S
couple	A1 A2	1	✓	–	+ S	–	–	R
see D1		2	✓	±	– R +	+	+	S
	3× cont.	3	✓	±	– (+S) R +	+	+	R
	A4× Lot	4						
	B1-2 ×							
pair	B 3 4	4	✓	–	+S –	–	–	R
		5	✓	–	+S –	–	–	R
pair	C 1 2	6	✓	–	+S –	–	–	R
		7	✓	–	+S –	–	–	R
	3 4 5	8	✓	–	– R –	–	–	R
		9	0					
clump.	D 3 4 5	10	✓	±	– R +	+	+	S
		11	✓	±	– R +	+	+	S
		12	✓	±	– R +	+	+	S
			0					
see A2 (sib)	D1	13	✓	–	+S –	–	–	R
single	D2	14	✓	–	+S –	–	–	R
~~Lot E 1 5~~								
E	A 1 2	15	✓	–	+S –	–	–	R
		16	✓	–	+S –	–	–	R
0	3 4 5		0					
		17	✓	–	+S –	–	–	R
		18	✓	–	+S –	–	–	R
F	B 1		0					
8	2 3 4	19	✓	–	+S –	–	–	R
1	5	20	✓	±	– R +	+	+	S
		21	✓	±	– R +	+	+	S
G	C 1 2 3	22	✓	–	+S –	–	–	R
	DRY		0					
	DRY		0					
	5	23	✓	–	+S –	–	–	R
		24	✓	–	+S –	–	–	R
single	H6 G S C	25	✓	±	– R +	+	+	S
00	H 1-2	26	✓	–/+	(+S) –	–	–	R (Lac+,–)
10	H 3-4	27	✓	–	+S –	–	–	R
		28	✓	– ±	+S –+	–+	–+	R (all Lac–)

Is this sum and in future, leave small drops (observed) only, and explicit by micropipette in short interval.

(contam.?) or Gal-ucan.?)

OK.

∴ 25, 27 only non-parentals.

Recontacts

DATE: Feb. 11, 1954. REF:

H1-2. 1 = "volunteer" ♀ = P2.

H2 = "large pair" ♂♀ = P1 + K1. (V_1^R)

H3-4-5 = couple.

H4: P1

H5: P1+P2.

[In retrospect, I would not rely absolutely on purity of H4 isolate in view of remote possibility of contamination from outside of pipette. In any event, the coupling is trivial.]

AH Recircle H2, H5.

H2 = Lac − V_1^S } 4 each testis.
Lac + V_1^R }

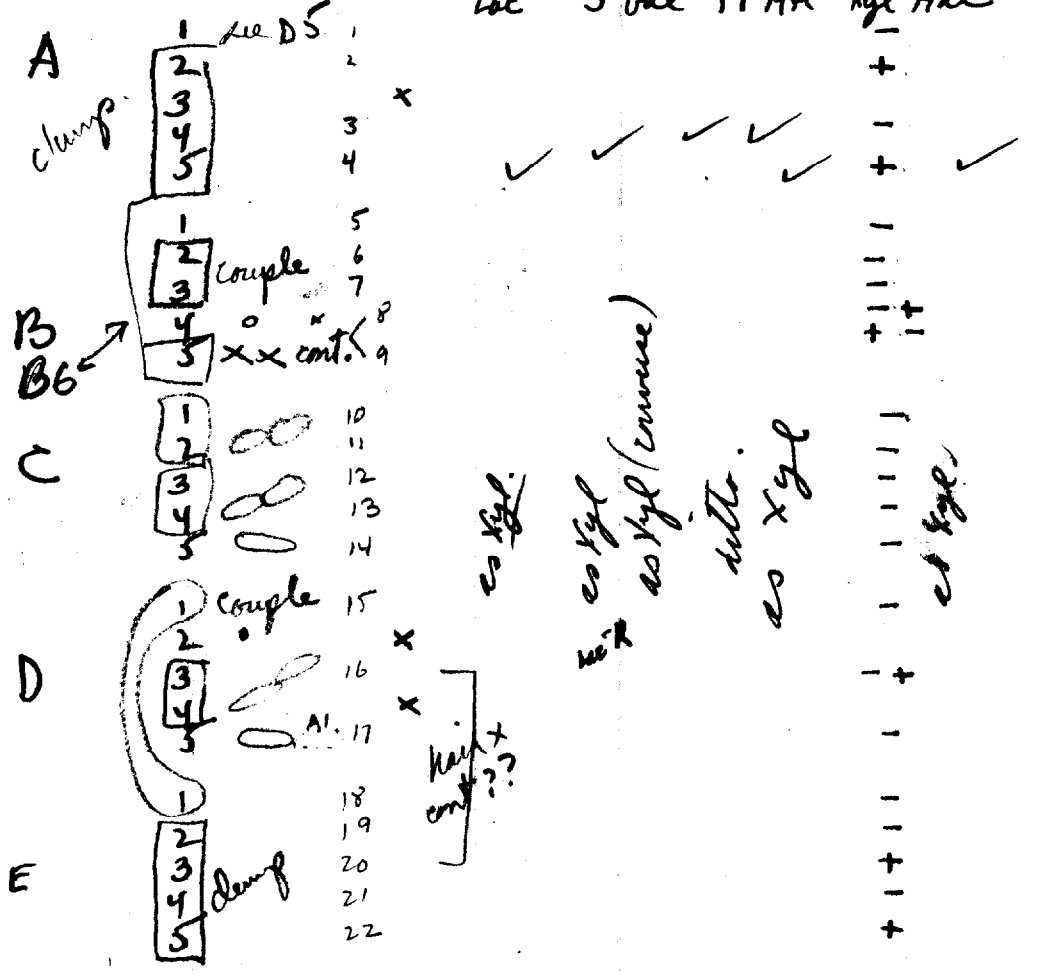
H5 = parentals.

Feb 11, 1954

W-2397 x W-2344

1:1:4 3:35–4:25. Dilute 1:20 (Kyf. also)

Also plate 6:20



∴ all parentals except D3. B5 was doubly inoculated with P1, P2 as recorded in notes. When picked, this droplet had two separate clones, and was moderately well separated.

Phage reaction of D3 not clear enough to analyse total content, though only Gal+, V's are seen. Cf. S14 which showed only Lac–S^R, no Lac^+.

D3: P1–P2? ✓ Lac–S^R { Xyl Mtl Mal – / + }

Remaining are P1 16

P2 5

1-cell. 1128

DATE: 2/12/54 REF:

	1	2	3	4	5	6	7	8	9	10

Fresh cells (ca. ½ grown) 1:1:1 3⁰⁰ — 3⁵⁵ deliv.
W2397 x W2344 ca. 1:10.
Many coal clumps noted. Some interruptions. Separations
began at ca 4:15 PM.

				Gal	TS	Mal,
A1	large	1		+	S	
10						MH,
E1		11		+	S	Xyl,
2		12		+	S	
3		13		+	S	S
4		14		+	S	Lac
C1		2		+	S	Gal.
2		3		+	S	
20 3		4		+	S	T1.
4		5		+	S	
5		6		−	R	
D 1		7		−	R	
2		8		+	S	
3		9		−	R	
4		x				
5		10		+	S	
E 30		15		+	S	

all others as from single clump

equivalent, P1 or P2 except for.
8,10 = Lac − SR
Lac + SR
MH −
Xyl −

#1 has peculiar Gal characters (Lac)

∴ Repeat Gal/TS for 8,10 and streak out #1.

8,10 both V, S, Gal + pure.
#1 shows peculiar growth, as if Glu − ? (Pur − type?)

40

50

Xyl, lac segregation: random?

1129

DATE: 2/13/54. REF: 1125 CD.

A W2394 } × W2344 1:1:5 12 hours overnight.
B W2397 }

C. W2394 SM- mutants from EML × W2344 2:45 PM —

A, B — Yields of Xyl+ S^R about same in both (≈ 10% of Lac+ S^R).
Picked to EMB lac. (most staff Xyl-).

[C: Crossed overnight. Plate out N14. See EML. Choose #16 = W2480 of list.]

A, B. Picked to EMB lac. A5, A6, B3, B5, B6, B15 had lac+.

Tested directly to EMB MM, T5.
MASS ↓ Lac, V. of MM (or Xyl+) comps

	lac	T5 (MM)	MM [⊕ = papillate]	
A.	1	S.	⊕ — S	MM+, - all lac - V, S
	2	S	— — S	X + L - Vs, - - S, R
	3	S, R(-)	⊕ — S	as A 1 MM - V, R must also be pap.
	4	S, R(+)	⊕ — R	MM + Lac - V, R; MM - Lac - V, S
	5 •	S, R(+-, -+)	+ — S	Xyl + Lac - V, S; Xyl - Lac - V, S; Xyl - Lac + V, R.
	6 •	S, R(+)	⊕ + R	X + L + R; X - Lac + S; X - Lac - S
	7		— — S	X+, - S - S
	8	S, R	— (but → +.)	MM+, MM - } L - S.
			— S	
B	1	S	— — S	Xyl+, - S - S
	2	S, R	— — S	~~No MM+~~ " and - R (Xyl+) purified
—	3 •	R	⊕ + R	X + L + R; X - L + R
	4	S	— ~~—~~ - S	X+, - S - S " and X -, - R ~~rec~~ and proved,
	5 :	R	— + S	X + L + S; X - L + R
	6 :	R.	⊕ — S	+-S, ~~No MM+~~ + S12 + R 17 - S 8
	7	S	— — S	+-S, - - S No MM+ 2 - R 3
	8	S	+, - — S	MM+, MM - } - S 1 + S 1
	9	S	but ⊕ + — S	No.
	10	S	⊕ — S	+-S, - - S No MM+ 2 + R 3
	11	S	— + S	"
	12	S	⊕ — S	MM+ L - R, M - L - S
	13	S, R(+,-)	⊕ ⊕ - R	
	14	S	— — S	
	15 .	S, R(-+)	⊕ — S	+ ref. 1125 - - S
	50			11 - , 12 ⊕.

6+, 17-! 12 S 11 R (S) but suspicious: Xyl+ not always recovered.
Xyl - MM correlation? at least partial • half lac+

1- cell.

~~#30.~~
Labelled 1129

DATE: 2/13/54. REF:

n993 W2397 W2394

Fresh cells. Do not mix before manipulation. Q. clumping alone and together. Ca 1:5 dilutions of the cells

Set up 2:55 PM – ca 5 PM.

		Gal	T1	Lac	Lac replicas: Gal	Gal T5	Mal	Xyl	Mtl	Stl	Sm
A	1A2	+	S	–							
	A3	+	S	–							
	A4	–	R	±							
clump.	B1	+	S	–	✓	✓	✓	✓	✓	✓	✓
	B2	+	S	–							
	B3	+	S	–		accordant.					
	B5	–	R	±							
	2C1	–	R	±							

∴ all parentals SP1 SP2 NoR.

2/17 Improved media to avoid clumping. Test P2 x W2400 (3 hours) in ① Nutrient broth 1% Glucose 0.2% ② = ① + Gelatine 1% ③ Nutrient broth, no salt. Clumping (macro) is largely avoided. Streak out Lac ++:

① < 1% ② ditto ③ 2-3% O/K. Use NB for crosses if less macroscopic clumping than Penassay.

~~1129~~
1130

W2401-2 × P2. Pick from Ara sex; test: Lac TS
Infected components: Lac, V_1

% Lac+ among Ara+
17/19 :
21/29 :

(1).

	Lac+	Lac−	+	−
1	S	R	R	
2	R		S	
3	R		S	R
4		S	S	S
5		R	S	R
6	S		R	R
7	R	S	R	R
8	R	S ?	R	S
9	R	S	R	S
10	R	S	20	

Lac+ < R 12 // 12 / S 5 // 4 (R)

Lac− (when pure) < R 1 // 5 / S 1 // 3

(2)

	+		−	+	−	+	−	+	−
1			S	R		R		R	
2	R	S		R			R	S	
3			R		R		S	R	S
4	S		R	R	S		R	R	S
5	R			R	S		S	R	S
6	S		R	S	S	R		R	S
7	R			R		R		R	S
8	R				R	R	S	R	S
9	R			R		R	S	R	S
10	R					R	S	R	S

What are Ara's scores under this heading?

Ara almost certainly linked to Lac. Confusion by Ara ratio among Lac+ S R:

(Ara, TS): par = Ara − V_1 S, + R

		pooled
− R	7	− R
− S	18	− S
+ R	4	+ R
+ S		+ S
+ R, − S	8	+ R
− S, − R	3	− R
+ S, − S	1	+ S

∴ 14/37 have Ara+ (< ½).
definite linkage to V_1 is apparent.

	Ara+ V_1 S	Ara− V_1 R
	1	13
	18	5
	Ara− V_1 S	Ara− V_1 R

Not clear whether Ara/Lac linked. Use V_1/S crosses.

31

Pending:

complementing linkage tests

of Lac - V_1 - Ara.

The independence of Lac - V_1 - Ara

has not been settled, partly on account

of short supply of acridine (phage stocks).

Probably not substantiated

11305

2/15/54

~~W1422 x W1895 3-9PM. E 1915 Laz Sm. (for V_1^S/R)~~

3/8/54. W2407 x W1895. For Cal $^+V_1^R$ recomb.
possibly Hfr!

all 10± Cal+ isolated probable reversions (see DCC notes)

DATE: Apr 27, 1954 REF:

Control platings 2 and 3 parents.

A. W2324 + W1177 — young cultures 1 ml each per 10 ml/2 : 10 PM. Plate at 4:50 PM. EMB Lac

B. " Lac+Gal−S^B Mal+Hfr " Lac−Mal−S^R + W1394 Lac+Mal+S^R F−

(better to use 1895; 2324/Lac−Gal−!)

C. W2324 + W2401 (for Mal+S^R selection) — Incr moderately ± sm.

4/28/54.

D. W1895 × W1177 — Young cells. 1:30 PM —

E. " × " × W1394. X too smeared.

4/28: (3) Pick sectored colonies. Test Mal/S. Mal+S^R indicates F−/F−
14/11 plates × 150 colonies scoreable Mal+S^S " Hfr/F−
But Gal−Lp^S character of Hfr parent Mal−S^R = F− parent.
may prejudice results. However at this time,
the Hfr was probably not strictly distinct from W1394. (judging from plate + sm)
Est 3-4% recomb in A/sm

A29: 5 only had Mal+. 6 were pure Mal−. 3 clearly Mal+S^S/Mal−S^R.
of remaining 2, one may be pure Mal+S^R, other is Mal+S^R/Mal−S^R.
(4) (5)
Restreak to characterize components.

1152 B1-2 : checked by DCG next page

1152

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

B1 and B2 streaked out from spots on B mal.
B1 → mal + and mal − ; six of each picked
B2 → 2 types mal +, one dark, one light

	ed. picked as	mal	gal	lac	mtl	S all resistant	Vs
10	B1 + (1)	+	+	+	+		S
	2	+	+	+	+		S
	3	+	+	+	+		S
	4	+	+	+	+		S
	5	+	+	+	+		S
	6	+	+	+	+		S
	B1 − 2 (1)	−	−	−	−		R
	3 (2)	−	sl	+	−		R
	4	−	sl	+	−		R
20	5	−	sl	+	−		R
	6	−	−	−	−		R
		−	sl	+	−		R
	B2+ (dark) 1	+	+	+	+		S
	2	+	+	+	+		S
	3	(+)	(+)	+	+		R
	4	+	+	+	+		S
	5	+	+	+	+		S
	6	+	+	+	+		R
30	B2+ (light) 1	(+)	(+)	+	+		R
	2	(+)	(+)	+	+		R
	3	(+)	(+)	+	+		R
	4	(+)	(+)	+	+		R
	5	(+)	(+)	+	+		R
	6	(+)	(+)	+	+		R

40 (+) = light +

Osmear 1152 B1-2-3

Try W895 lac⁻ + W1177 + ~~W1394~~ Y10
Hfr lac⁻ SR lac⁺ S^S

∴ B1 = pure S^R.
Mal⁻ : W1177 plus recomb!
Mal⁺ : W1394

B2 - pure S^R also but pure W1394, does not contain W1177. Might be 2344 x W1394.

∴ Either a 3-coincidence or Hfr parent potentiates crossing after!!

Lp^S exercises

4/27/54 ff Was given irradiated plates of W478 and Beal, E (M−) suspected sensitives marked.

(1) Picked possible sensitives, streaked one to O; spotted in order on complete → replicated to D(milk)

→ 31 Lp^S ; 2 auxotrophic; all salt+

(2) Several plates replicated directly to D(milk) to pick up non-Lp^S auxos (5 plates, ca 150 col/plate)

→ 3 possible distinct auxos.

478-4
Both sensitive & resistant on parents
original col
auxotrophic
Trypt −
W2475

478-5
Sensitive
4 auxotrophic
Trypt −
W2476

478-1
Spread in D(milk)
discarded

478-2
resistant
AA2−

478-3
resistant
Trypt −

5/6/54 ff Streaked E irradiated plates of W478 - before 16 Lp^S allowed; nutrition checked by E ML

5/11/54 ff 478 UV(8). Procedure as before. 29 Lp^S allowed; nutrition checked by E ML

Notes

1152A

DATE: May 8, 1954 REF: 1152

1152 B suggested W1177 × W1941 sibs/sons of Hfr (W2364) repeat design.

1. Y10 × W1177 × W1941
   F⁻ lac⁺ S^S F⁻ lac⁻ S^R Hfr lac⁻ S^S
   Grow together 1:1:10 in 1230 PM - 5 PM.
2. Y10 × W1177 (both F⁻)
3. W1941 × W1177 (both lac⁻) Then streak out on EMB lac sm. or plate
4. W1941 × Y10. (both S^S)

5/10:

1. 5 plates (> 200 each) all lac⁻
2. 1 all -
3. 1 all -
4. 1. N. G. -

parents checked for lac, S : o/k

Hfr x F⁻ (xF⁻) & motile 1153.

DATE: April 28, 1954. REF:

Desiderata for zygote isolations:
① 10x both parent motile
② 3 component mixtures as technical control.

10:30 – 3:30 Mix

A W2341 mot (TCN) x W1177 Hfr.
B " " x W1394
C " x 2401.

128 or ? 40s. cell isol.

8:30 A 30. Dilute ca 1:50 to prepare for isolations.
Defr. 10:30

~~D Fresh cultures W2401 x W2344 Mot (TCN) 12:30 – 4:30~~
M1 OK for plating (i.e. test of s.c. of 2344 mot,)

5/1/54 D. 9A1 Older cultures as inoculum. — N.1. Defr. [to 3:25. Delete due to setup: 4:10].
W2401 x W2344 Mot 1:1:20

[Should study rate of crossing at low cell density & motile and immotile parents, perhaps in viscous medium.]
Could be done in a competition experiment! Cf. W1895, W2334 mot + immot.
No – need some Lac-linked marker (V6, V1 ?)].

1153 B (128). Random single isolates. 40 placed. F1-G1-H1 flowed together. No Lac++ (as indicative of Lac+ Gal+ recombinants).
5 N.G. (A3,4; B2; D2; G5) agree with drops.
Described as actively motile at n=2⁵: C1, C5, D3A D5, These and G2, H4 appear to be v. slow Lac+, others Lac⁻. Have DC G streak out on EMB Lac, M+L; repl to Lac sus., Mal

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
	Aerosin isolates									
		Lac	SM	Mal						
A 1										
2										
5										
B 1										
3										
4										
10										
C 1		±	S	+						
2										
3										
4										
6		±	S	+						
D 1										
3		±	S	+						
2 B		±	S	+						
4										
5		±	S	+						
E 1										
2										
3										
4										
5										
F 30										
2										
3										
4										
5		±	S	+						
G										
2		±	S	+						
3										
4										
H 40										
2										
3										
4		±	S	+						
5										
50										

not recombinants
resistant (strong Lac+)
(not W1394). pneu.

DATE: REF:

		1	Progress	3	Scores	5	6	7	8	9	10
1153D		[129]			Lac	Mal	Gal	SM	MM.		
1	A 1	pair !	28		−	−	+	R	−		
2	B 1	pair !	28		− +	−	+	R (Lac+,−)	−		
3	E [2		28		−	−	+	R	−		
4	[3	mut?	28		+ −	−	+	R	−		
	4		1		+	+	−	S	+		
	F 1	clump	28		−	−	+	R	−		
	[2		1		+	+	−	S	+		
	3]		1		+	+	−	S	+		
	4		1		+	+	−	S	+		
	5		1		+	+	−	S	+		
5	G [1	pair !	1		+	+	−	S	+		
6	2]	pair 28 ✓	28		−	−	+	R	−		
	A [2		1		+	+	−	S	+		
	5]		1		+	+	−	S	+		

no parent F

∴ definite correlation of pairs with recombination. Note disappearance of the Hfr cell!

∴ 4 pairs have given ♀ 2 zygotes! B, E the Hfr parent reappeared, but gave 3. A1: no recomb. detected so far. G: both survived, no zyg. These pairs are almost certainly significant: Hunt them further. Save 1153D-1-6 for later complete survey of markers.

E. coli : motility observations and isolations. and other notes on strains

1154

DATE: REF:

4/28. E. coli C - 4th passage (see 1158) gave entirely motile culture, still coccoid. Continue passage at R.T. (1157B4)
(This culture started from micro-isol of motile cell → semi-motile clone. Then to agar + gelatin at 30°, then to motility agar at 37°.)

Cf. W2401: No motile cells seen at all. No motility in gelatin or agar at 3 days at 37, 30, R.T., or 20°. Remincubate. [do. 52C sent.

W1895 - sluggish, occ. motile. [Are unselected motile cells F⁻?]

W2284 - [Hfr mot → F⁻]. Highly motile.

Select by pas[illegible] motility tubes. [illegible] + Hfr strains, isolate [illegible] each stage; F⁻ stable

1st passage W1895, W[illegible] still Hfr (reduced [illegible] not. DC6 is checkin[illegible]

[illegible] 5/8

1895 : [illegible] ≅ 1817 than ≅ 1177. [illegible] Hfr lac +.

2344 : about equal no. of prototrophs ≅ 1177 + 1817; also all lac +

Isolates / are

May 2 Mon Ecol

a) + gelatin 30°

b) + gelatin 37. → M2B

others rem[illegible]

Streaked out 1895 M2 + 2344 M2. Will single col. of each + repeat cross, use fresh tester cultures. Note clones of the 1895 & 2344 cultures used. Results of these crosses:

	0	1177	1817
1895	0	lac -	65+, 7-
2344	1-	0	11+, 11-

5/23. Note: in recent weeks

♂ = W2344/M1 Hfs$_2$ ♀ = W2401 F$^-$
wg 1. wg 28A

Insertion of λ reactions

W2401 proves $\lambda_2^R \lambda_2^R$.

cf. W2284 = λ_2^S.

isolated i Mal$^-$ as before? Streak out overgrowth of W2284/λ_2.

DATE: MAY 3, 1954 REF:

Save united rufig. cross as 1155., the first dilution left at room temperature ca. 11:30AM - 5PM 5/3/54, as 1155 A.

9:05AM Make up control ($F^- \times F^-$) W2331M1 × W2401 1:1:20

B. (Penicam); B1 (NB) (overn.) (36 h.) MAY 4 1954

132 + Gutexp. n.g., throw out

C. W2334M1 × W2401 in NB. 1:1:20 9AM 37°.

1155 A has grown to ca 5×10^7 (density) by this point.

Redilute for set up = A2. A.T. Also fresh dilution from 1155 = A3 1:100 dec 37°

See next page.

Most pairs give obvs. mixed clones not worth picking. Some of these were however picked apart quite early. Viz.

- A2-B5 +/- — (A2 not unicell) No test
- × D1-D4 D5 all bac⁻ Presumably was not a pair.
- D2-D3 ±; +/- c5 x, ⇒ —
- F1-F2 ±; +— ⇒ ✓
- E3 F5 G5 ±(—⁺)— ⇒ ✓
- G4 H5 — +-

∴ every pair thus separated evidenced zygote formation!

H corrected as dessicated pair

Could pairs come from fission of Zygote?

Isolate couples Hfr x F⁻.

1155 A

DATE: May 3, 1954. REF: 1153D!

	1	3	4	5	6	7	8	9	10
	9:30 AM Freeze cultures (not reproven)								
	W2344M1 x W2401. 1:1:10 9:20 – 11:30 37°. (Refr. for later stock)								
A).	dilute 1:100 for isolations. Keep this at room temperature.								
	130 But most of these pairs give mixed "clones". F.								
	1153D!	8PM	10AM	Pick	1155A	lac	Lac'ase	Mal	Mtl	Gal
A 10	1-4PM									
1			2 p.							
2	P	+ –		A2	A2 B5	± –	R– (rare R+)	+ –	+	– +
3	P	+ ; –	; 0			–	R	–	–	+
4	28 fragl.									
5		– +			D1	–	R	–	–	+
B 1	P	+			D2	+	S	+	+	–
2	P	+ –			D3	+ –	R+,–	–	–	+
3	P	~ ++	0		D4	–	R	–	–	+
4	A 3m	++			D5	–	R	–	–	+
20	A2 28.	28	28	B5						
C 1					E3	±	S	+	+	–
2	P →	++			F1	±	S	+	+	–
3	2m?	++			F2	+ –	R+–	–	–	+
4	28 e2	0	0		F5	– +	R–	–	–	+
D 1	P? Reclas ⊖	–		D1	G4	–	R	–	–	+
2	P	+		2	G5	–	R	–	–	+
3	D2	–		3	H5	+ –	R+–	–	–	+
4	D1	–		4						
30	D1	–		5						
E 1	P	+ –								
2	P 28	+ –								
3	P 28	+ ;		E3						
4	P		+ –							
5	P → 2+	ju ; +	0, +							
F 1	P in	++		F1						
2	P 28	– –		F2						
3	3m	++								
4	E1	+ –								
45	P E3			F5						
G 1	P	++ w								
2		++								
3	P 2+	++ ; + –								
4	P?			G4						
5	E3	–		G5						
H 1	X									
2	P 2+	– +								
3	E4	– +								
4	—									
50	G4	—		H5						

On restreak, D2, E3, F1 are pure lac+ (cal⁻); 5, 8 are pure lac–; 2, 4, 7, 9 are lac– and lac+ cal+ (recombinants) 4 had + only in center spot.

Save initial cross times is tabs for later analyses

Did any pairs here give pure 28? D1 G4. Not certainly pairs from above.

Total P isolated: 19.

Jl. gen. expt

Zygotes

1156

DATE: May 5, 1954

REF:

	1	2	3	4	5	6	7	8	9	10

Parent cultures overnight. W 134 MI × W 701. Hw 1:1:20 in Permassay 10.15 AM 37°. plate MAY 6 1954

134 EMBlac S MR; Mal Gal CLASS

Growth pattern A7

10	A	1	1	+		+	−	♂
		2	0 (28)					
		3	1	+		+	−	♂
		4	0 28					
		5	28	−	R−	−	+	♀
	B	1	1	+		+	−	♂
		2	28	+	Rales+ R	−	+	●
		3	0 28					
		4	1	+		+	−	
20		5	28	++ −	R −+	(−+)	+	● Malsic! MR−
	C	1	28	++ −	R −+	−	+	●. ♀
		2	1	+		+	−	♂
		3	28	++ −	R −+	−	+	● ♀
		4	28	−	R−	−	+	
		5	28	+	Rabble contamination	+		♀
	D	1	28	−++	R −+	−	+	● ♀
		2	1	+		+	−	♂
		3	0 28					
		4	0 1					
30		5	0 1					
	E	1	0 28					
		2	1	+		+	−	♂
		3	1	+		+	−	♂
		4	28	−	R−	−	+	♀ V_1^S
		5	1	+		+	−	♂ B2 thus lacks ♂ but appeared odd at first.
	F	1	0 1					
		2	0 28					
		3	28	−++	R −+	−	+	● ♀ ~~B5 may have ♂~~
		4	78.1 d					
40		5	1 died	+		+	−	♂ C1, D1, F3, F5 lac+ S^R
	G	1	0 28					
		2	1	+		+	−	♂ E4 only pair not yet
		3	1	+		+	−	♂ showing odd recomb.
		4	0					
		5	28	++ −	R −+	−	+	● ♀
	H	1	0					
		2	28	−	R−	−	+	♀ B5 has ♀, 2 recomb
		3	28	−	R−	−	+	♀
		4	0 28					
50		5	1	+		+	−	♂ B2 ♀, recomb
	I	1	1	+		+	−	♂
		2	1	+		+	−	♂
		3	1	+	Thus 5.	+	−	♂

1 − H3
2 − D1
3 + C1

I-1 I-2 I-3

DATE: REF:

B2, B5. — both non motile in direct test.
DC 0 also tested C1 D1 E4 F3 G5, non swarm
& C5 - swarm (as expected).

E4 } pure Gal+ by streak.
B5 }

E4: V_1^S

B2 originally showed weak lac+ reaction and some lac+ S^R.
It appears now pure Gal+ by first streak, but some lac+ S^R indicated.
Maybe simply lac+ S^R recombinants in low numbers with ♀ excess.

B5: shows lac (φ) S^R ; Lac+ S^R but also some late Mal^+ $(\sigma?)$. Not evident on early streaking.

E4 though from a pair is not obvious recombinant. (v arabinose).

Check single colonies:

			Mal	Xyl	
B2	lac+ ①	Gal,S	lac +	+	Xyl+ lac+ recombinant
	2		+	+	
	3		+	+	
	4	all+R	+	+	all Mtl−
	lac− 1		−	−	
	(2)		−	−	♀ parent
	3		−	−	
	4		−	−	
B5	Mal+ ①		+	+	Xyl+ lac+ Mal+ recombinant
	2		+	+	
	3		+	+	
	4	all+R	+	+	
	− 1		+	−	Xyl− lac+ Mal− recombinant
	(2)		+	−	
	(3)		−	−	♀ parent.
	4		+	−	all Mtl−

all carry the orthotypes markers Gal+ S^R and ~~probably~~ Mtl−.

10/6-7/54. Also found, in B5, a Mal+ Lac− recombinant among

1158B35 - 4 = Mal+ Lac- MH- Cal+ Ara- S^R Xyl+

---

all S^R MH- Ara- Cal+

∴

Lac+ { Mal+ Xyl+

Lac- { Mal- Xyl-

Fresh Zygotes

1157

DATE: May 6, 1954 REF:

W2401, W2344M1 grown overnight.
Regrow 1:10 9:05 - 10:05 #14. Dilute & place in separate drops for fresh isolations 135

Early mixture.
Pairs not well defined

A 1-2-5 proved all nodule
A 3-4 ditto
B1 ∅ ?
B3 ∅
C1 ∞
C4 ∞

	#	Proved
A1	1	♂
2	2	♂
5	⊙	
3	3	♂
4	4	♂
B1	11	pure ♀
3	5	pure ♀
C1	12	pure ♀
4	13	pure ♀

∴ These pairs ~~are~~ are all ~~illegitimate~~ illegitimate. Were recorded as indecisive.

Hfr x W1177.

Conjugal pairs.

~~#58~~ 1157.

DATE: MAY 8 1954 REF:

W2344M1 x W1177 fresh cultures. Mix 2PM. Redilute and (not) not? examine 3PM. (1 hour mixture)

doo 135.

	Lac	S	Gal	Mal	
1	+		−	+	
2	+		−	+	
3	+		−	+	
4	+		−	±	
5	−	R	++	−	
#6	−	R	++	−	
12	−	R	++	−	
13	−	R	++	−	
14	+	D2	−	+	♂
15	+	D5	−	+	♂
21	+		−	+	♂
22	−	R	±	−	♀
23	+	E4	±	+	♂
24	+	E5	−	+	♂
30	−	R	±	−	♀
31	+		−	+	♂
32	−	R	±	−	♀
33	−	R	±	−	♀
34	+	H2	−	+	♂

1157

E1 E2 ~~E6~~ ~~E8~~

G-1 2 3

all pairs eliminate all depressed as selected

Ø

. No recombinants here.

2 surviving pairs are legitimate. Note lethality of remainder (see protocols). of 5 other pairs, 1 had no survivor, 3 had ♂ only; ~~1~~ 1 ♀ only.

Lac + + others S.

Details:

E1: + → E2 (less not) → ♀

E1, E5 ♀, ♀ !

Assoc. ca 1 hour! but no recomb detected.

♂ → 21, more not ♂

G2, G3. ♀ ♀.

Ø

Assoc ca 15m. but no recomb detected.

~~Trace bubble passed~~!

= 1158 ~~21-25~~ ~~31-33~~

Gal ++ = 2101
Gal ± = 1177
Gal − = 2344

Hfr x W2401

1158

DATE: ~~MAY 11 P.M.~~ MAY 10 P.M. MAY 10 1954

REF: 136

Fresh cultures X: 2:15-3:40 PM.

			Comment	Cell Type	5/11 lost	EMBlac	Gal	SM	Mal	Mtl
	A	1	1hour, many sep.	1		+		S		
		2		28		−		R		
		3	plate 01	28	1	−		R		
		4	7 hours.	−						
		5		0	28	0				
*	B	1	1hour together	28		− ++	+	R	−	−
		2		1		+ +		S		
		3	s.p	1		+ −		S		
		4		28		−		R		
		5		−						
	C	1	s.p	1		+		S		
		2		28		−		R		
		3	s.p	1		+		S		
		4		0	28	0				
		5		28		−		R		
	D	1	> 1hour	1, 2	1	+		S		
		2		28		−		R		
		3	s. buy 28	0	1	0				
		4		0	28	0				
		5	PAIRED & 1R	0	28	0				
	E	1	s.p	1		+		S		
		2		28		−		R		
25		3	s.p	1d		+		S		
		4		0	28	0				
		5		28		−		R		
	F	1	s.p	28		−		R		
38		2		1	—	+	−	S	11	11
		3	s.d	1		+		S		
		4		28		−		R		
		5		28						
	G	1	s.p	[redacted]	028	0	u			
		2		[redacted]	01	0				
		3	s.p 0	28		−		R		
		4	−	[redacted]	028	0				
		5		[redacted]	028					
	H	1	s. 1hour	0	1	0				
*		2		28		− ++	+	R	−	−
		3		[redacted]	d	[redacted]				
		4	s.p	28		−		R		
		5		−						

MAY 11 A.M.

MAY 12 A.M.

s.p = s. in pipette

Note: most pairs were s.p. of 10 pairs, both survived in 7, both lost in 2, 28 only lost in 1; 1 only lost in rest of other pairs

(see over)

1 was "separated by surface tension" (during): both lost.

of the 5 persistent pairs, 4 were "separated" spontaneously

B1-2 → ♂ ✓ ♀ and ⊗ ✓

H1-2 → ♂ died ♀ " ✓ don't count D4.

A3-5 A' (!!!) died A3 (!) → ♀ only.

D1-2-5 ♂ → 1 ↓ ♂ ; ● ↓ ♀ ; ⊗ ↓ both died ← count as "viable pair"

~~B1~~2 ♂ = W2344M1

2B-♀ = W2401.

A1-2

1 was manipulated at 1 hour

○ → ♀, ♂ only.

Save: A1-2, 3 ; B1-2; D1-5; H2; F1-2-5

F2 simply looked peculiar under microscope as if motile type 2B but pure bal⁻ lac⁺ indicated (♂) save for further comparison. Abandon ~~B~~ 147 as these show no particular heterogeneity (among survivors). probably old

F2 was plated out on EMB Lac.

Zygote isolations

MAY 8 1954

1156. 15 pairs isolated. 28 failed in 8. 6/7 of
131
remainder, solely from line 28 parent. 1 prob. ♂+♀+R.

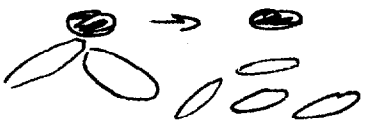
pair, ═══ ♂ lysidat pipette.

1155 Most pairs not separated (19). 4/4 from l28 parent
130

53D 3/4 partly separated.
129

Total to date (W2344/11 × W2401): 13/16

Queries:

1. These are all fairly late pairs. Earlier should be studied to ensure that zygotes result from pairs, not conc[illegible], and to seek dikaryon cells such as 1156 B2. Notes should be consulted in event of doubt on purity of this cell. None indicated: 

2. Perhaps fuller pedigrees.

3. Review possibilities of line-1 crosses (of 1158. Mich Fla 1177 or 1895!

4. Cytology.

5. Find previous resumé!

6. Problem of undetected recombinants: why not more?

1 hour fresh zygotes

MAY 11 1954

1159.

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
A	12:30 - 1:45	♀ × ♂		V. young cultures. Divide ca. 2:50.						
B	10:30 - 11:30	"		Refr. — 3:10						

B) — 3 - 3:40 PM Few or no pairs (Disjunction already?) after refrigeration. X. use 145 for ♂ unless indicated

		10	146	pairing 145	Current	CELLTYPE 146	♂	Loc 145	146 EMBLoc	SM	Mal	Mtl	Cal	Sperm 145
A	1	)	♀	A5	SIP	0	♂	+		R				
	2		♀			0								
	3	)	♀	A4	SL. MANIP.	28	♂	+	+	Loc+				
	4		♀		lysed?	28			−					
	5		♀			28			−					
B	1	)	♀	A3	long conjunction	0	♂	+						
	2			C1		0	♂d	0						
	2	3	♀	A1-2		28		d	−					
	4	3	♀			28			−					
	5	)	♂			1			±	S	+	+		
C	1	)	♀	C3; C5		28	♂	+ +	−					
	2		♀			28			++					
	3		Several ♀	♀♂	CLUMP	28..			− +	3	Loc+ Loc+			
	4		♀	; C4	lysed?	0	♀	−						
	5		♀			28			−					
D	3b	)		SD5 ♂ NOT SEP	: ONE			0						
	2					0								
	3			CLUMP NOT SEP'D - MIXED CLONE NOT PICKED										
	4													
	5		♀			28			++ −	4	Loc+ −			
E	1		♀	E5	SIP	0	♂	+						
	2													
	3			E4 ♂	NOT SEP'D →	MIXED CLONE ♂	NOT PICKED							
	4													
	4	5												
F	1		♀	F4		02.	♂	+						
	2													
	3		♀	F5		28	♂	0	−					
	4	3	♀ ♀			28 0			−					
	5		♀			28			−					
G	1		♀	G5		2.	♂	+						
	2		♀			28			−					
	5	3	♀	G4		0	♂	+						
	4		♀			28			−					
	5		♀			28			−					
H	1		♂	H5	SIP	28	♂	+	−					
	3		♂ dance		3h. interval	1			+	S	+	+	−	
	4					28			−	R				

all OK 65? ♂ all OK. +

all three lines

146 heat for all parental lines except

Short Pedigrees

1160.

DATE: May 12 1954

REF:

A. Start to isolate numerous pairs, but this was interrupted. Rather late culture.
144 A1–D3 are isolatable. But only A1–5 were separated (65) quite late). B1–D3 give only viability data: from the untouched pairs, both ♂ and ♀ grew in 9; ♂ only in 3 and both died in ①. This suggests that inviability maybe connected with separation but other differences are possible.

In A1–4 ♀ ♂ copulation separated early (watch ALR!); A5 at 30+30 min. sequence in A1 is not clear from notes. (was it copulation?).

B. [143] Pick 5 pairs (rather clumsily) for pedigree analyses.

A1: 1-A, B, ♂ ♀ 1 cell — B 1 cell

2A: ♀ C — A2 ♀ B2 ♂ A2 D2 F2 C2 ! E2 — all grew.

3A: A ♀ ♂ BL BK ♂ C ♂ D ♀ ! E ♀ 1 cell — all but E grew

4A: 144 E1-4 A1-4 — B ♀ A ♂ C ♀ D ♀ ! E4 — B 1 cell only

5A: — A ♂ B ♀ — both grew.

chance to correlate growth delay & zygotes.

Note fair survival of these, too.

DATE: MAY 14 1954

REF: 143 - 144

	1	2	3	5	6	7	8	9	10
	143	EMB Lac	Lac	Gal	Mal M4 Vgl	S			
1	A 1	+	+	—	+ -	S			
	B 0								
	C 0								
2	A 28	— +	— +	+	—	R -+			
	B 11	+ +	+ +	— —	+ +	SS			
	C 28	—	—	+	—	K			
	D 28	—	—	+	—	R			
	E 28	—	—	+	—	R			
	F 28	—	—	+	—				
3	A 28	—	—	+	—	R			
	B 11	+ +	+ +	— —	+ +	SS			
	C 28	—	—	+	—	R			
	D 28	—	—	+	—	R			
	E 0								
	F 28	—	—	+	—	R			
	G 0								
4	A 1	+	+	—	+	S			
	B 0								
	C (144E)	—	—	+	— — —	R			
	D (144F)	—	—	+	— — —	R			
	E 0								
	F -								
5	A 1	+	+	—	+	S			
	B d.								

6/5/54 Lact component died out
all parental ran true; true.
Concordant.

DATE: MAY 14 1954 REF:

	1	2	3	4	5	6	7	8	9	10
	144	EMS/Lac		Lac	Mal Mtl	Xyl	Gal	S		
A. 1	·?	−		−	−		+	R		
2 a	1(28); [redacted]	+		− − +	− +		+ − +	R $R^{Lac^{+/-}}$		not separated
b	[redacted], 28	−								
3 a	1-28	+, −		+ −	+ −		+ +	$R^{Lac^{+/-}}$ $R^{Lac^{-}}$		
b	28	−								
4	28	−		−	−		+	R		
5	28	−		−	−		+	R		
E 5	28	−+		−+	−		+	R Lac+-		

E1-4F1-4 see 143

not clear zygotes.

all parentals Lac+

E5 from A1, but long contact!

A2-3 eff. unseparated

A4 clear test?

$B^- \rightarrow \cdot\cdot B^0_0$ + (1111)

saved

no zygote.

Survival not clear.

A5 Long contact, still −!

saved

9 A.M.

1160

MAY 14 A.M.

MAY 1954

9AM 1431

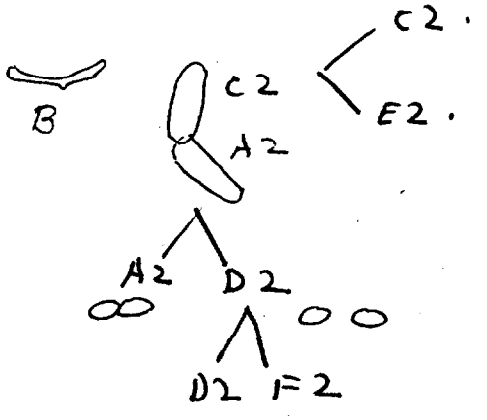
1. ♀ died B ♂ A ♂.

2. A2 zygote ♀ save as 1160-2-1

B ♂

C D E F ♀

80 flapping



3 A - C - D - F ♀

E - G ○

B ♂

4 A ♂

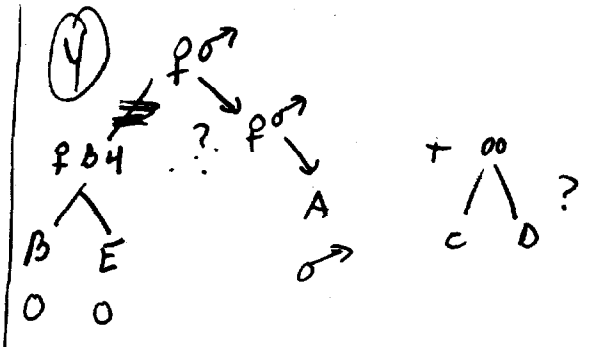
B E ○

C D ♀

5 A ♂

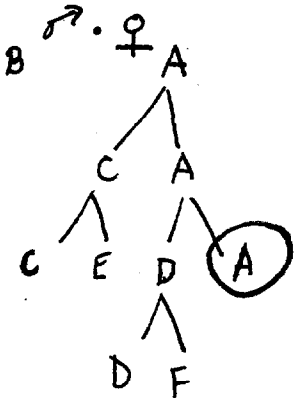
B ♂.

(4)

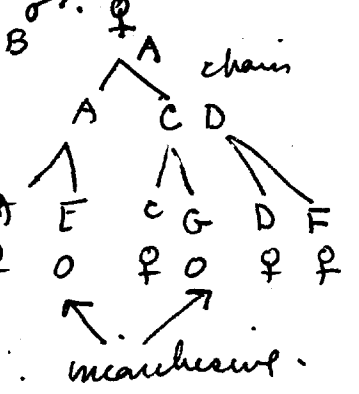


poss. inconclusive also.

(2).



(3)



∴ inconclusive.

DATE: May 13 1954. REF: 142 - 141

142

Overnight, x 10^{30} - 11^{55} 37° 1:1:10 Penassay.

A1
A2 picked ca 130.

Freshcross x 12^{30} - 3PM sic.

Not separated: ~~Reg~~ Refrigerated overnight + separations to 142, 141, A14.

		EMB	Lac	Gal	Mal	Mtl	Xyl	S
141	A4		−	+	−			S
	A5		−	+	−			R
	C1		+	−	+			R
	3		−	+	−	✓	✓	S
	4		++ −	+	−			R (Lac+/−)
	5		+	−	+			S
	D 1		+	−	+			S
	2		−	+	−			R
	4		−	+	−			
	5		−	+	−			
	E 4		−	+	−			
	5							
	G 1		− [redacted]	+	−	✓	✓	
	H 1		− [redacted]	+	−			
	2		− [redacted]	+	−			
	3		− [redacted]	+	−			
	4		− [redacted]	+	−			
	5		− [redacted]	+	−			

all controls Encodmt.

1161 is a Mal+ yielder not showing a Mal+ Lac− recomb but exhibiting a Mal− Lac+ recomb. also

142	G1	++ −	(Pair of ø)	R (Lac?!)	Mal	Mtl	Xyl	
	2	+		S				
	3	+		S				
	5	+		S				
	H 4	0						
	5	−		R				

	Lac	SM	Mal	Mtl	Xyl	Gal
G1	++ −	R (Lac)	(−+)	−	(−+)	+
2	+	S	+	+	+	−
3	+	S	+	+	+	−
5	+	S	+	+	+	−
H 5	−	R	−	−	−	+

Gal among Mal or Xyl+ needs to be confirmed.

Re − 10/54. Pure Gal+.

Found a first: A (Sm)
- Lac− Mtl− S^R Mal−Xyl+
- Ara− Lac+ C Mtl+ S^S + +
- Ara− Lac+ D Mtl− S^S − −

No Lac+ S^R, Mal+ S^R (Reexamine for ① Lac+ S^R No ② Lac− Mal+ No (+40 more colonies tested))

1/62

DATE: May 14, 1954. REF: 137

		1	2	3	4	5	6	7	8	9	10
			CMBlac					X 1:1:20 (fresh) 12:30 - 2:10			
A	1		✱ 0	♂♀d.				(set up T 2:40).			
	2		+	♂ ♂, ♀d							
	3		+	♂							
	4		±	♂ ♀d							
B	1		—	N.S. ♂d.				Trapping technique, worked v. well but pipette maybe too narrow.			
	3		+	♀d.							
D	3		—	♀							
E	5		+	♀d.							
H	5		++ —								

(G5 ♂) not picked.

Three viable pairs only: A3-D3 ♂·♀ no ⊗

(G5)-H5 ♂·♀ ⊗

B1 (n.s.) ♀ (susp appearance — restock CMBlac.

See protocols for other isolates. V. Poor Viability !!

Poor incidence = 1/3 pairs zygotic

		loc	Xyl-Mal-	S	Cal		
A	1	0					
	2	+	+	S	—	♂♂	all concordant
	3	+	+	S	—		
	4	+	+	S	—	♂	
B	1	—	—	R	+	♀	
	3	+	+		—	♂	
D	3	—	—	R	±	♀	
E	5	+	+	S	—	♂	
H	5	++ —	—	R	+	♀	

Paris. Tay class transmigration trap Salmonella. 1163.

DATE: May 19, 1954. REF: 150.

(1) Hfr culture (W2346M1) seems sluggish. Resolute motile cells = A1-4. A1 n.g. (A3) probably best to be picked. From this serial #, use 6 x 8 coverglass mountings. Lettering in sequence A B C D E F G H.

(2) Set up parents in adjacent degs: ♂ ♀ ○ next to trap deg, noted that ♂ have to swim through ♀ to reach trap. May have worked moderately well, but setting of ♀ suggests better to allow intimate mixture before trapping. Many very early pairs seem to separate very readily indeed, precluding any isolation. But no systematic data!

(3) Conventional mixture 1:1:6 2¹⁰ - 3¹⁵ deobtained from ca. 3⁴⁰ - 4:40, assay trap deg

B 1 2 3 4 5 6
B 5 6
C 1 2 3 4 5 6
D 1 2 3 4 5 6
E 1 2 3 4 5 6
F 1 2 3 4 5 6
G 1 2 3 4 5 6
H 1 2

Comment: survival fairly good.

Note: both manips ♀♀ died but also 2 others. Would predict several ♂♂. but may be some post clone deaths not detected.

Pick sequence:

A 3	D 1
B 1	4
4	5
5	6
6	E 1
	E 2
C 1	3
2	
3	E 4
4	5
5	F 4
6	5
	G 4
	5

all clones to date have agreed & recorded type of cell unmated. Note will be made of any discrep.

please turn page

DATE: 5/20/54. 5/21/54 5/22 REF:

Ag	Stat		History	Dep type	3	EMB lac	5 Lac	Gal	Mal	S 8 (Lac)	Save	10
B		1	ss.	♂		+		−	+	S		
		2	>1h.	●								
		4	s. doused	♂ ♂		+ +		− −	++	S		
		5	late pair	♀		−	1	+	+−	R −		
		6		♀		−		+	−	R −		A_2+
C		1	ss	♂		+		−		S		
		10	2h.	♀		− +	(2)	+ −	−+? +?	R −+		
		3		♂		+		−	+	S		
		4	s.i.d.	♂		+		−	+	S		
		5		♀		−	3	+	−	R −		
		6		♀		−		+	−	R −		
D		1	late pair	♂		+		−	+	S		
		2	Manip.	●								
		20	late pair	♂ ♂		+ +		− −	++	S		
		3	s.s.	♀		−	(4)	+	−	R −		
		6	2h.	♀		−++		+	−	R −+		
E		1	oil bubble	♂		+		−	+	S		
		2		♀		−	(5)	+	−	R −		
		3		♀		−		+	−	R −		
		4	s.s.	♂		+		−	+	S		
		5		♀		−	6	+	−	R −		
		30										
F		4	>2h.	♂		+		−	+	S		
		5		♀		●						
G		4		♂ R.		+		−	+	S		
		5	2h.	♀		− ++	(7)	+	−	R −+		
		6		♂ ●								
G		1	Manip 2 stuck tip	♂		+		−	+	S		
H		40		◎								
		2		◎								

all exc.

at footground reading party 5/7

These C2 may be a dibranyon.

Streaked out on EMB lac, Gal. Both lac+ and − are present.

a) spot some of these on plate for 1164 tests

b) check motility!

(ROC)

S.S. = ss. + 1 post

s.i.d. = s.i.d. after pipette

record on C2 was:

3:54 f0

st. ca 5:15

6:25 $\frac{0^2}{3\ 1}$ presumably separated by slight manipulation.

No comment on behavior at isolation. No basis for question on the isolation - possibility of contamination at isolation is not inherently excluded.

In view of rarity of this event even now (and reduced incidence per zygote for fem pairs, the matter must be strenuously questioned.

No remark is noted of motile cells in the drop.

Why is proportion now so low if true? See next page →

Tests on C2: Streak out on EMB Gal. Gal+ > Gal− colonies.

Pick and stroke: 5 Gal+ 6 Gal−. all are Lac− Mtl− Mal− Xyl− S^R and are thus simply a Gal−/ prototype recombinant! Streaked on EMB Lac, no Lac+ are noted, but some colonies are pink (Lac− Gal− ?). Where crowded with some pink "+" reactions.

{ None motile in motility agar 10/54 }

a) Check Hfr, Lp, mutation. b) Might be Lac+ Gal− Xyl− .... (needed by Gal+ − x ?)

c) Presumably not Lac+ and Hfr would give Lac+ Gal+ recombinants. d) Check motility, Mal+?

1163
C2.

DATE: May 23, 1954. REF:

1 2 3 4 5 6 7 8 9 10

① Already identified as carrying F and Lac⁻? Gal⁻ Mtl⁻ Mal⁻ Xyl⁻ S^R. On initial spot and especially on first replica to Lac, definite + reaction was indicated, suggested possibility of modified Lac⁺ Gal⁻ Xyl⁻ etc. Also doubtful possibility of presence of Mal⁺.

11A23. 10 Repicks from original spot to ~~stab~~ and 1163 C2

a) mobility – more on succ. from agar #C2, C2', C2'' etc.; no in agar.

b) streak EMB Lac again

c) Mal, Mtl

d) λ, x – Gal+

20

e) Complementarity: AM × 1895M2; ♂: P25 / M26

				P25	M26
1	C2A	1895M2		0	Slac
2		2206		+	all –
3	C2B	1895M2	+++	++++	+? – ?
4		2206.		++	+? –

also plan check for Lp, Hfr status, Lp, may intrude & former. nutritions. Should certainly be crossable & Y10 line.

f. Nutrition: both M-H – orthotypy: C2A = Gal⁺; C2B = Gal⁻ colonies.

30 presumably Hfr.

Brought in Pervasive than A.

P24 ↓ Pure Mal⁻ Mtl⁻ Lac⁻ & free⁺ reaction in background. Recombinate.

Rare reaction comes from interaction of Gal⁺ Lac⁻ / Gal⁻ Lac⁺ clones (W2502)

attempt Gal⁺ reversion of W2502

40 This recomb. could very readily be missed unnoticed! Ar⁻.

Note exaggerated orthotypy of e2 & e3

50

Segregation of motility, etc. We must put this in notes! (post-it)

[redacted]

no. degree of motility

DATE:

	1 num motile	2 per motile	3 highest motile
lac + gal + mal + suc +	1	6	15
+ + + −	0	1	1
+ + − −	0	1	4
− + + +	0	0	3
− + − + 10	0	0	0
− + − −	0	0	1
− + + −	0	0	0
Total	1	8	24

No - this rose is

DATE: MAY 20 P.M. REF: 151.

stat 1205 – 130 .1 ♂ : 1 ♀ : 10 Broth.

MAY 21 A.M.

		History	Cell Type	5½22 EMBlac	Mal Mtl Xyl Gal	S⁻ (lac)
A	1		♂	+ ✓	– – – +	S
cf. standard	2	sld	dense cuticle ♂?	– 1 ✓	+ + + –	R –
	3		♀ dense cut.	– ✓		
	4	ss	♂?	0	etc.	
	5		♀ Fr.	– ✓		
	6		♀ Fr.	– 2 ✓		
B	1	loose at first.	♂ v. active	+ ✓		
20	2		●	10		
	3	ss.	♀ (reinoculated)	– ++ ✓		R – +
	4		● ♂	+ ✓		
	5	V. loose then tight.	♀	– ✓		
	6		♀	– 3 ✓		
C	1	slp	♂	+ ✓		
	2		●	● ✓		
	3		♀	– ✓		
30						
	4	slp.	♂	+ ✓		
	5		♀	– ✓		
	6		♀	++ – 4 ✓		R – +
D	1	long.	♂ ♂	+ ✓		
	2		♀	– ✓		
	3	sl. menip.	♀	– + (++) ? 5 ✓	Some Mal etc + ? +	R – +
D	4		♂	+ ✓		
40	5	ff first then dumped	gh. Resort	– 7 ✓		
	6		♀	– ✓		

(1-3)(4-6) ♀

C3E3 ♀ ♂

Pick P21.

50

D3: no motility seen in direct scrap. None in cage.

D3: all Gal+ but Lac Mal ±

4 pot acid colonies on EMBlac.

all Gal+ reinoculate

D3 includes
Mal+ Lac− MH+ S^S } test of these!
Mal+ Lac+ MH+ S^S

Mal− Lac+ MH− S^R (1)

perhaps should be screened even more thoroughly.

Mal+ purified all S^S; same organ. give Mal+ S^R "contam" or mutants?

Recombs: (1.s.c.i) Sec 10/1/54

1 − Lac Mal− H-M(−) Ara−V1^S
2 Lac+ Mal− H-M− Ara+V1^R
3 Lac− Mal+ H− Ara−V1^S
4 Lac+ Mal+ H-M− Ara+V1^R

Sec 1183. In routine check, H− segregants found among 11603. Among isolates 1–4, #3 was H−M+ (others presumably H−M− as no growth on M agar). To try to find other types @ streak growth of mixture through D(H) on EMB Mal, found ca 1% Mal− mass (all Mal−). (2) streak on EMB Mal and test

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
			MAY 21 A.M.							
						concordant, no recombinants.				
E	1	sl. manip.	Cell Type	lac		Gal	Mal NH4Yyl		S (lac)	
10	2		♂ ♂	+ +		− −			S	
	3		○							
			d ?							
	4		♂ ♂	+ +		− −	+ + +			
	5		♀	−	● 8	+	− − −		R	−
	6		d (green)	w						
F	1	lh.	D				etc.			
	2	ss	◉							
20										
	4		♂	+		−				
	5 :	lh.	♀	− 6		+				
	6 :	ss	♀ (F. Gbus.)	−		+				
G	1		♂ ♂	+		−				
	2	long.	● gh.		● 8					
	3 :		♀	−		+				
	4		♂	+		−				
30	5	lh.	d.							
	6 of F6.									
H	1	ss	♂							
	2	rarely	● gh.							
	4 :		♂	+		−				
	5 :	ss.	♀	−	● 9	+				
	6		d.							
40										

Note losses/scars during lethality. Try saturating oil & water.

Counting only complete pairs: only 2 = 1/6. ; add incomplete pairs: ~~4~~ 2/4

Complete: 1-6: Incomplete 7-10

2/6. 1/3 3. Total 3/9 only.

#8 is certainly not in lethality (fortuitous d. of E6)

& probably 9 also.

1165

Lab

DATE: MAY 21 1954 REF: 152

	1	2	3	4	5	6	7	8	9	10

X (old), 1♂ 1.0♀ 5 ml broth 37° 9 - 10[30] AM. Trap.

(B) Comparable cd233/M2 Examined at same time: no parus.

Try 234/M2 (F-). see 1166.

			Disp	Lac	Lac	Mal MH (A25)	Gal	S (lac)		
A	1	X	○							
	2	manip.	♂							
10	3	.	♀	—	✓		+	R (-+)		
B4 -	4	.	♂	+	✓	Mal, MH	—			
	5	X loose	○							
	6	.	♀	—	✓		+	R		
B	4	.	♂	+ +	✓		— —			
	5	. ss early +K3μ.	♂	—	(1) ✓	uncer.	+	R		
	6	. .	♀ ♀	+ +	✓		+ +	R R -+		
C	1	. last disp	♂	+	✓		—			
20	2		[illegible]		(2)					
	3		♀	—	✓		+	R		
	4	.	♂ ♂	+ +	✓		— —			
	5	Aug. ss	♀	—	(3) ✓		+	R		
	6	.	♀ ♀	— —	✓		+ +	R R		
D	1	.	♂ ♂	+ +	✓		— —			
	2	. ss	♀ ♀	+ +	(4) ✓	[illegible] -*	+ +	R R -+		* some + ?
30	3	.	♀	—	✓		+	R [illegible]		
E	1	.	♂	+ [illegible]	✓	+	—			
	2	. sl. mon.	♀	—	(5) ✓	—	+			
	3	.	♀	— + +	✓	—	+		+	
	4	.	♂	+	✓	+ [illegible]	—			
	5	X distorted	○			+ one		as Mal		
F	1	JX manip.	♂	+	✓	+	—			
40	2	.	○							
	3	.	♀	—	✓	—	[illegible]			
	4	.	♂		6 ✓					
	5	. ss	○							
	6	.	♀	—	✓	—	+			
			♀	—	✓	—	+			

50

A1 B1 all lightly manip.
D4 " " manip

D2B: Mal - and seeming mucoid Mal +/- See over

D2A. Lac +/−, pure Mal −.
some +, −

D2B. Lac +/− ; Mal − and Mal "v".

D2B1.

↳ 4 of these plated out. Prove to be mucoid, no distinct indication of segregation +/−.

3 Lac + muc. D2B2

1 Lac − muc. D2B3

	Mal	Lac	S
1	−	+	R
2	+	+	S
3	+	−	S

becoming less mucoid. then all auxotrophic.
not segregating.

D2A has ♀, Lac + Mal −

D2B has ♀, Lac + Mal −, Lac + Mal +, Lac − Mal + all Gal +.

presumably failure of Mal elimination & crossingover / S/Mal.

of 1161D3! In both cases, only ones which show Mal +, all 4 combinations are seen. Is Lac + Mal + #for giving reciprocal recombinants?

5/23/54.

					EMBlac	lac	Mal Mtlz Xyl.	Gal	S (lac)
G	1	10 :	ss	♂	+	✓	+	−	
	2			♀	−	1 ✓	−	+	
	3	30 :		♀	−	✓	−	+	
	4	11 :		♂	+	✓	+	−	
	5	00 :	ss	♀	−	8 ✓	−	+	sMal
	6	00 :		♀	−	✓	−	+	
H	1	1111 :		♂	+	✓	+	−	
	2	0 :	ss	♀	−	(9) ✓	−	+	
	3	0 :		♀	−++	✓	−	+	R −+
	4	11		♂	+	✓	+	−	
	5	0	ss	0					
	6	0		0					
J	1	0 :	ss	♀	−	−	− −	+	R
	2	0 :		♀	−	−	− −	+	R

all deaps concordant ie the mutants.

a pair complete, ♀ zygote! Note segregation in 54 pedigree.

♯ 4: zyg. ss; ss, sl. mes; ss

5: non zyg (def.) last deap; s.p; ss, ss; ss.

Competative pairing

W-2401, W-2344M1; W-1895M2
F- Hfr F-

DATE: May 22, 1954 REF: 153

Yesterday's observations suggest that motile F- (cf. also Salmonella) does not pair with W-2401. This can be properly confirmed only by a competitive pairing experiment. Suggest: menage a trois with Hfr mot; F- mot; F- ~~non~~ non-mot. Pick pairs and diagnose. For simple diagnosis, it should not be essential to seprate out the pairs, but would be useful if most of these, as expected, will be bisexual. Similar expt. possible with F+. also permitting "F-duction" test.

Cf. DCG notes and 1154. W-1895 used here is second passage motility, and second colony ~~reisolate~~ reisolate showing F- behavior (with peculiar segregation ratios). (before reisolation).

Ovenight cultures, to 10 ml. Penassay, 37° 9:25 AM

A. W-2344M1 (.1) + W-2401 (1)

B. do. + W-1895M2 (.1)

C. W-1895 M2 + W-2401.

Previously concerned about lyp's of W2401 but EML finds it pos. to λ. (possibly Mal-Lyp 2?).

W2338, W2384 being incubated.

11:40 - 12:40 Isolate pairs. Leave at R.T. Some probable motile - motile pairs seen also.

In controls, no pairing was seen in C as compared to A.
Control for B: streak out on EMB Lac, Test Lac+ on EMB Gal to verify ratios of Hfr / F⁻.

N23: A, B show SR+, not C. ca 1% on Lac sm.

EMB Gal: Lac.

A ca. = +, - (sic! indicates growth diff.?) + almost = -.

B + > - + = -

C all +. + almost = -.

Sample Lac+ to EMB Gal. (Pick very clear + available to avoid pairs on slight diffuseness of appearance.) : 23 Gal+ : 1 Gal-
(F-) (Hfr)

Competitive pairing.

153

A 25

			Inoc.	Dup A23	BLac	Lac	Mal	MH	Gal	S (lac)	
A	1	♀	①	♀							
	2	♂	00000000	♀	−	−	✓	−	+	R	
	3		40	♂							
	4		11	♂	+	+	✓	+	−		
	5		11	♂	+	+	✓	+	−		
	6		00	♀	−	−		−	+	R	
B	1	♂	5	♀	−	−	✓	−	+	R	
	2		50	♀	−++	−++	✓	−	+	R	−+
	3		1+	♂	+±	+±	✓	+ +	+	S	
	4		·	−							
	5		1111	♂	+	+	✓	+	−		
	6		60	♂ O	0	0					
C	1	♀	1111	♀	−++	−++	✓	−	+	R	−+
	2	♂	than 0	♂	+	+	✓	+	−		
	3		60	♀	−++	−++	✓	−	+	R	−+
	4		0	♀	+−	+−	✓	−?	+	R	−+
	5		1[10]	♂	+	+	✓	+	−		
	6		0	♂	·	·					
D	1		11111	♂	+	+	✓	+	−		
	2		0000	♂	·	·					
	3		111 111	♂	+	+	✓	+	−		
	4		00000000	♀	−	−	✓	−	+	R	
	5		111 1111	♂	+	+	✓	+	−		
	6		0000	♀	−	−	✓	−	+	R	

SIC 1895

Start

Date of ♂ pick already?

why are Ab ppts gone? Recover later.

lac presumed Gal−

all rare exc. B3

c 4-n studies pure Mal

		Gal	disp	Blac	Lac	Mal	Xyl	S(lac)	Cal		
E	1		○		○						
	2		◎ fat mut		○						
	3	·	~	♂	+	5	+	+		−	
	4	·	○	♀	−		−	−	R	+	
	5	·	+	♂	+	6	+	+		−	
	6	·	○○	♀	−		−	−	R	+	
F	1	·	−	♂	+	7	+	+		−	
	2	·	○○○○	♀	−		−	−	R	+	
	3		○		○						
	4		○○○○ mut!	♂	+		+	+		−	
	5		⊕	♂	+		+	+		−	
	6		○○○		○						
? G	1	·	○○ [redacted] ○○	(♂)?	+		+	+		−	V_1^R · V_1^S } parental
	2	·	− [redacted] //	♂	(++)	(9)	++	+		+	
	3	·	1+	♂	+	8	+	+		−	
	4	·	○○○○	♀	−		−	−	R	+	
	5			−			[redacted]				
	6			−			[redacted]				
H	1		○		○						
	2		◎○○ m.	♂	+		+	+		−	
	3		○○○○	♀	−		−	−	R	+	
	4		ⓘ		○						
	5		ⓘⓘⓘ	♂	++		++	+		+	
	6		✱○	♂	++		++	+		+	

cancer

∴ all pairs were either Hfr/F^-_{28} or (G1-2) and (H5-6) which carry [redacted] not F- alone or ♀ Hfr.

Following distribution noted: (complete pairs only):

pairs	singles
8 Hfr/F^-_{28}	7 Hfr_1
1 Hfr/F^-_1	[redacted]
0 F^-_1/F^-_{28}	23 F^-_1

Need replication 2/8 zygotic

Could we detect recombination between G1/G2? Only crosses are Gal+/V_1? Test /75.

DATE: May 25 1954 REF:

A. (old cultures) W2502 + W~~45~~ 1895 M2 (W2503)

1 : .1 ; 10 10:45 AM

B. W2332 + W2401 (F deuteron).

No pairs but possible mixups & inadvertent use of T2 both.

MAY 24 P.M.

9[5] Some EMBaabmore tests on lac⁻ recipients.

1163 B5 B6 C5 C6 E2 E5 E3

1164 A2-3 A5-6 B5-6 C3 D5-6 F5-6 H5

52 [1165] A6 C3 C5-6ab F356 G23 G56 J1-2

53 [1166] D4 D6 E4 E6 F2 G4 H3

1163

all are Ara⁻ except ? (B6)

63C2A,B	64D3
Ara⁻	Mal⁺lac⁺ — Ar⁺
	Mal⁺lac Ar⁻

Thus lac⁻/Ar⁺ recombinants would not add appreciably to tal.

1164D3:

lac⁺ M+l⁻ Mal⁻ S^R (Ar⁺) all Gal+

lac⁻ M+l⁺ Mal⁺ S^S S.I.C. (Ar⁻)

recheck, might be mixed.

Lac⁺ M+l+ Mal⁺ S^S ?SR " " " " (Ar⁺)

No Lac⁻ Mal⁻ picked

M+-Mal⁻ recorded on all but 1 record.

15 3

A6 - Lac Gal⁺ Mal⁻ M+⁻ S^R

F - ductus ; TZ

1167

DATE: 5/24/54. REF: del 1113. (139).

W2206 is recorded as very futile F+. Use for F- ductus in chance of also detecting recombinants from pairs.

A. ♂ ♂ ♀ TZ .1:1:10 ~~is~~ necessary 8:25 min 37°

B ♀ + ♂ TZ 1:.1:10

C W2206 + ♀ TZ .1:1:10

Repeat 2:30- + fresher mix.

Conc. (1) W2206 insuff. motile (2) TZ at these levels inhibits motility in these strains (of previous observations?).

Pass W2206 again - Recover later if necessary.

T.O. isolates.

DATE: May 25 & 26, 1954. REF: 140

A 10^{30} – w2332, 1 ; ♀ 1 : 6 — 12 N. overnight cultures
B 12:10 2502, 2503 1:1:5 Freshly inoculated – not full growth.
C " 2332, ♀ 1:1:5 — 1:30 PM. (" ").
to 2PM.

what date on F⁺ F⁻ pairs?

B) T. O. first batch etc.

5/25 140 H-1-2 s.i.p. F1 — < shaped cell.
few if any pairs were noted.

5/26 12 N. (C) (F⁺ ; ♀)

A1) 5 but not
3 2) ♂ not
B 1 2) sip.
C 1 ♀? (transip pr.)
D1 Y cell
B 2 D 3) b : — c+
D4 ♀ trans not rec.

prob. not.

(B) (28 AF⁻ x " not Hfr)

A4 not smaked
B3 " "
G3 sip -> 3 cells
G2 ↖
F2 3) —○ n, l.
F4 5) —○
H 3 4) ↖○

1168 ~~69~~

stab

DATE: 5/28/54. P27 A28. REF:

140

	1	Exp.	Inoc type	Hog type	Blac	Disposition	8	9	10
A	1	68C	not?	↷	−	False pairs.			
	2)	"	not	↷	−				
	3	68B	snake	○					
	4.	C	not?	↷	−	"			
	5								
B	1	" C							
	2)	" C	s.p not	↷	−	"			
10									
C	1.	"	♀	○		"			
C	3.	B	not snake	○					
	4	C		↷	−	"			
D	1.	C	> not	↷	−				
	2)	C	not	↷	−				
	3)	C		↷	−				
	4.	C	♀	○					
20	1.	B_0	> not	↷	+				
F	2)	B		↷	+				
	3	B	○ ○	♀	−	~~test~~ test for recomb.			
	4	B		↷	+				
	5)	B	○.	♀	−	"			
G	2	B		○					
	3	B	...	↷	× . +				
H	1)	B_0	s.p ♀		−	"			
30	2)	"			+				
	3)	B			+				
	4)	B.	○		−				

Many initially cool pairs evidently iso ("false") & still usually visible.

Impression that W250 & mono induces clumping of F⁻ cells. of 1170 also. (mostly mono)

Try stimulus of silky. mating?

No valid F⁺/F⁻ pro. vis this set. possible 2002 × 2003 pairs to score and check reactions of motile side: F 2-3, 4-5, H 1, 2 H 3, 4. In particular, check out Lac⁺ for presence of ~~Lac⁻~~.

Cause: 1168. — all pure Lac⁺

1169A.

F Kemp.

DATE: May 27, 1934. REF:

	1	2	3	4	5	6	7	8	9	10

9[30]

A. W2206 M1 .1. ♀ 1. : 10 broth – 11[30] Numerous pairs! (140)

~~B W2332 .1 ♀ 1 : "~~ leave out in view of A success. 11[30]-12[30].

C W2502 1 : W2503 .1 : 10 " CCC, D. 6-8 PM.

ce, AA 12[45] AA – 2[10]. C showed very few pairs.

D = ♂ × ♀ D. Y10 X 2502. 6-8 PM. 140 F1-2 F1 X. F2X.

late PM: numerous clumps & pairs noted but not now picked.

1169A. (F+/♀)

		140–154	Inoculum.	deoptype A28	Glac Acq	# disp.	VF
B3			♂	–	0		
~~4~~	b1		♂	~~pd~~	+		
	2		♀	pd	0		
20	3		♀...	28d	0		
~~B4~~						1169-	
B4			♂	1	+	A1	
separated p27.	b4		♀ 7.)	28	0		
	b5		♀ 1.	28	–	B1	
B5			4♂	1.	+	A2	
	a1		4♀	d	0		
30							
C2			♂	d.	0		
	a2		4♀	pd ♀	–	B2	
	a3		8♀	pd 28	–	B3	
C5			8♂	1? pd	0		
see	a4		4♀ not cult.	(1) 0.	+	~~A4~~	
D5			8♂	1.	+	~~A5~~	
40	c1		16♀	d.	–	B4	
	c2		16♀	28.	–	B5	
E2			6♂	1.	+	~~A6~~	
	c3		8♀	28	–	B6	
	c4		not?	1.	+	A6	
	c5		"	1.	+	A10	
E3			8♂	1.	+	A6	
	d1		8♀	28	–	B7	
50							
E4			♂	1.	+	A8	
	d2		16♀	28	–	B8	
E5			8♂	1.	+	A9	
	d3		12♀	28	–	B9	

1169A.

DATE 4/0 – 15/4 REF:

1	2	Lig type A28	4/a c	5	6	7	8	9	10
G5	6 ♂	pd	~~O~~	~~[redacted]~~					
d4	5 ♀	28	—	B10					
5	20 ♂?	1	+	A22					
H5	all ♂? (same mating).	1.	+						

1410.

Precedent.

Note: if W2206 is still infective, these pairs should be examined. The only way is to compare plating of ~~W~~ 1169AA ~~with~~ for F deletion. This culture W2206M1 is mass culture of first passage of the stab stocks of W2206. Also plate out single colonies, etc., for comparison of fertility (W2206 was recorded as highly fertile).

1169AA also plated on EMB/lac sm. No SRF noted, no special point now in looking for recombinants among 1-cell progeny.

Respot semis A, B.

1169C = plating of 1169AA (infecting day) as control. Spot single colonies on EMB.

To EML A2 for F test. A = ♂ component } 8/10 F+ EML.
B = ♀ " }
C 4/5 F+ EML.

1169
AA.

lac A29

138

11:45 - 2:10 PM. F+/♀. broculum dig.

					Dispersive F✓
A 1	]		0		
2			0		
4		♀	28	−	
3		♀	28	−	B1B
B 1		♀	28	−	B12
2		♂	0		A11
B 3	]	♂	0		
4		♂	0 (sm.)		
5		♀	28	−	B13
C 1		♂	1. 28 1.	+ +	A1213
2		♀	28	−	B14
5		♀	28	−	B15
c 3		v.s.	0		
4		♂	1	+	A11
D 1		♂	1	+	A14
2		♀	28	−	B16
D 3	]	♀	28	−	B17
E 1		♂	1	+	A15
2		♀	28	−	B18
E 3		♀	28	−	B19
4		♂	1 pd	+	A16
5		♀	28	−	B20
F 1		♂	1	+	A17
2		♀	28	−	B21
F 3		♂	1	+	A18
4		♀	28	−	B22
5		♀	28	−	B23
G 1		♂	1	+	A19
2		♀	28	−	B24
3		♂	1	+	A20
4		♀	28	−	B25
5		♀	28	−	B26
H 1		♀ ♀	28, 28	− −	B27,28
2		♂	1	+	A21
3		♀	0		
H 4		♂	0		
5		♀	0		

why such less dying than 140? all inert.

DATE: May 28, 1954. REF: [155]

Y10M1 + W2502 .1 : 1 : 10 Per essay 37° 100 – 245 ...
(A B C 1 D E 1). 2:30 – 3:45 min NB (C 4 E 4 F G H).

Three 16 "pairs" isolated, but many proved inviable. On early inspection, 8 PM, record as mixed only:
A1, C1, H1 and these were separated & later proved correct.

However A1 B1 E1 may still have some wg28 type cells.

∴ Pick A1-2, C1-2, H1-3 and B1, E1. in this sequence.

W2502 though nonmotile is not morphologically quite so destructive from line 1 as is W2101. Should compare directly.

		dsps	Lac P30	same streak out
A 1, 2 (mixed cell)		1, 28? 28	+ –	→ Lac+, few – (and Lac-S^R).
C 1, 2		1 28	+ –	→ pure Lac+
H 1		1	+	→ pure +
2	(2.28)	1	+	→ pure +
3		28	–	
B1		1-28?	–+	→ Lac+, –
E1		1-28?	+–	→ "

evidently not!

B1, E1.

① Streak out A1, C1, H1-2 on EMB Lac for lac⁻ recomb. (Gal⁺ or S^S)

② Malto- ~~as Epistac~~ /mm.

A1 is presumably a mixture of parents only as lac⁻ = S^R. Others show no lac⁻ in single cell progeny of F⁻. ~~but table should in B1, E1~~ For more efficient tests see ff. ∴ Lac⁺/V, R.

Mal +/− prototrophs. 1171

DATE: May 30 1954 REF: 1163-64.

A) Estlin crossed W2574 x W2118 on EMS Mal. Picks Mal+ to EMS Mal) (mod. crowded), replica to EMS Mal, 1 sun. 45 Mal+. Of these, 9 also had Mal+ S^R. DC & is checking 8 of these for concurrence of other classes.

9 also had Mal− S^R thus Mal− S^R/Mal+ S^S. (presumably "twins"). check Lac concurrence.

1-8, by DCG: #3 also had Mal− S^R #6,8 also had Mal+ S^S. Not clear cut whether twins

B) Remainder, look for twins (non crossover) 11-21. Streaked on EMS Mal. (Number not clear) 10 had Mal+/−

Lac	Mal	
+	+	3
	−	2
−	+	2
	−	0
−(+,−)		4

No indication of significant twinning. Results altogether inconclusive.

of zygote data (not prototrophs)

DCG 1171.

1171 A

1171A 1-8 (known to contain mal + S^R) ; Streaked out on Smal; picked 10 col. from each streak. Spotted each on Sgal; replicated to Smal and Smal SM. Results:

1: all mal + S^R

2: " " "

3: 5 mal + S^R; 5 mal - S^R

4: all mal + S^R

5: " " "

6: 9 mal + S^R; 1 mal + S^S

7: all mal + S^R

8: 1 mal + S^R; 9 mal + S^S

1171A 11-21 Streaked out on Smal to isolate mal +. In most of these streaks no mal - appeared. Spotted mal + and mal - (mal - ? depicting Smal SM plate) on Slac.

	mal +	mal -		mal +	mal -
11	lac +	lac +	16	lac -	lac +
12	+	-	17	-	+
13	+	-	18	+	+
14	-	+ and -	19	+	+
15	-	+ and -	20	-	+ and -
			21	-	+ and -

(these two were different)

1171 B

Picked ~~single~~ mal + col. to Slac; replicated to Smal and Smal SM.

Only 3 contained SR components
#8 & #15 contained mal – SR
#14 mal + SR

8 & 15; Spotted mal + and mal – on Slac;

	mal +	mal –
8	lac –	lac –
15	lac –	lac + and –

14; Streaked ant on Smal (→ no mal –); picked 16 colonies to Slac; replicated to Smal and Smal SM. All 16 were mal + SR

Misc crossing tests 1172

5/31/54.

		D(o)	Lac:
1.	W2206M1 × ♀	0	
2	1165D2B2 × Y10M1	few ?	
3	1164D3— × "	+	
4	♂ × ♀	see 1171B +	
5	W2581 × Y10M1		1+/>100 —. pure+ col (orthot.)
6	W2583 × W1177MY		ca 1–2% +/— colonies
7	W2583 × W2407.		Probable lac+/— but notched + (ortho is only —).

M ↓ 3-4 h., plate is washing on D(o), or dilute on EMS Lac T5.
EMS Had

∴ W2502 is verified as Hfr + orthotypy pattern seems similar.

6) should be most profitable for detection of zygotes. also repeat plating on EMS Lac dilut.

♂ nonmotile × ♀ motile
Uni 28A Uni 1

DATE: July 2, 1954. REF: 158 - 159

Back to Lab!

W2583 × W2639.
♂ ♀ W894 Mal+ motF

1135 — begun.

ratio 2:1:0

	Final pairs	cell sol.	Drop	EMB/Lac	Mal	Mtl	Gal	Sor
Same cone	A 2	1	1	(+ −) (2)	+		−	S motile.
	3	1	1		→ pure V, S			
	4	1	0					
10	5	28	28	0				
	6	28	0 pd					
B	3	28		+		S		
	4	1						
	5	lost		0				
C	2	1		+				
B	6 SS	28		+				
C	20 3	28		+				
	4	1						
B	2	1		+				
D	1	28		+				
C	5 2	1		+				
D	3	large 1	0					
D	30 5 Sup	28	0					
	6	1	0					
E	3	1	0					
	4	28	0					
E	1	1	0	−				
	2	28	0					
	5	28	0					
	6	1	0					
	40							
F	1	1	n-g	−				
	2	28		+				
	3	1	? 0					
F	3	28	0					
	4	1	? 0					
G	1	28	0					
	50							
G	4	? 1	0					
	5	28	0					
H	3,4	1 28	0 0					
H	5,6	28 1	0 0					

Final pairs: all Lac− SS, Lac+ SP
Mal+ Mal−
Gal− Gal+
no other recombinants.

parental

Sup.

Why This half

Inconclusive: 0 = 0 growth / not tested / only?

(2) was from long duration pair set., unfortunately others inviable.

thumbback

1179B

DATE: July 3, 1954. REF: 179

		2	3	4	5	6	7	8	9	10
		Cellobiose	Arbutin	Lactose	Maltose	Galactose	Methyl	Sorbitol		
A 2	manip	28	28	+						
3		1	1	−						
4		28	28	+						
5	sip	1	0							
B 1	sip.	28	0							
2		1	1	−						
10										
B 4		28	28	+						
5			28	+						
6	manip	(28)	0							
A 6		1	1	−						
C 1	SS	28	28	+						
2		1	~							
B 3		1	1	−						
20										
C 3	sip	1	1	−						
4		28	28	+						
C 5	sap	28	28	+						
6		1	1	−						
D 1		1	1	−						
2	SS	28	28	+						
3		1	1	−						
30										
D 4	SS	28	28	+						
5		1	1	−						
6	(tumour)	1	1	−						
E 1		28 ↔	1	−						
2	slm.	1	1	−						
3		1	0							
E 4		28	28	+						
5	SS	1	1	−						
40		28	28	+						
F 2	SS,	1	1	−						
3		1	0							
F 4		28	28	+						
5		1	1	−						
6	manip	1	0							
G 1		28	28	+						
2	s.p	1	1	−						
G 3		1	0							
4	manip	28	28	+						
50		1	0							
6		28	28	+						
H 1		1	0							
2	sap	28	28	+						
3		1	0							
HH, 5	SS	1 (28)	28 0	HH +						

(1PM − 3PM × 10 dilute 3 − 4/10)
x-ratio 1:10
(separate 5 − 5 3PM)

all Lac− are Mal+ Mtl+ S^{S} Gal+
Lac+ are
all Lac+ are Mal− S^{R} Gal+ !

No recombinants
many s-p or
invisible 0 should be
OK but not
recomb.

all coincident ; Woz !
non viability again.

DATE: July 5, 1954. REF: 160

W2583 x W894 Mal+ H3+
= W2639

V_1^R Gal+ Lac+ UV5 D2

Crossed 10:1 12 30 to 2 30 37°

1 - all lac -

28 - all lac+

except D5 = lac ± /-.

probably not spread

H4 formed mixed. Record — 0.

28,1 28

Cross 0 - fams 1175

DATE: July 4, 1954 REF:

10 ml pinassay, ½ ml ivg-X immediately ± ½ ml W2801 (ivg 2½ hr 37°)
7/5. Over motility tubes.
Examine cultures carefully P6.

	ivg.	X: motility	control: motility	
1175: 1	3	0		
	4	0		
2	9	0		
3	10	0		
4	11	+	+	(rare cells)
	17	0		
5	18	0		
6	27	+	+	(rare)
	31	0		
7	51	Rough +	+	as known
	53	++	++	
	54	0		
8				

Preliminary tests in motility agar, same result. No Tor S. 48h. 30° same.
Remoculate into Pinassay at 25° for pos. temperature effect.
(also try 34, 38, 40, 41, 49, 50 : preliminary controls: non-motile). (see over)

DCG is repeating extensively.

[redacted] of WS cultures

	tube (48h.)	motil
22	Swarm throughout tube	motili present
32	slow	motili present
34	non motile	no motili seen
38	" "	" " "
40	" "	" " "
42	flare	no motili seen
47	Swarm throughout tube	motili present
49	non motili	no motili seen
50	" "	" " "
52	Swarm throughout tube	motili present
55	" "	large proportion motili

[redacted]

DATE: July 7, 1984 REF: V61

	1	2	3	4	5	6	7	8	9	10

W25 83 x 639 .5 : .5 : 10 12:15 PM – ca 2:30 PM

(So many pairs became stuck there is no advantage to using recess of non-motile parent!)

		cell	drop	taz
A	1 4	1	1	
	2 2	28	28	
	3 1	1	1	
A	4 x	B	1 1	- Lac -
B	1	00.	28	28 - Lac + except E5.
	2	1	0	
	3	1	1	taz ±
B	4	0.	28	
	5	1	1	
C	1	00.	28	
	2	1	1	
	3	1	1	
C	4 5 6	①① 00	28	
D	1	①	1	
	2 3	⊃	X	
	3	①	1	
D	4 5	①① 4/(28)	1 28 ..	
	6	①	1	
E	1	1.	1	
E	2 3 4 5	1. 0000	28 (1)	not scored
F	1	00	28	
	2	1.	1	
	3	1.	1	
F	4 5 6	0.	28	
	6	1	1	
G	1 2 3 0	00	28	
	3	1	1	
G	4 5	00	28	
	6	+ 1	1	
H	1	⊙000	28	
	2	① lost?	⊙	
H	4 5 6 0	0.	28	
		00 ⊠?	1	

very bad

♂ × ♀

1177

162

July 8, 1934

~~10~~ 105 — 250 +

(Richenberg demonstr.) ♂ × ♀ 1:10. Ster. v. high incidence of pairs.

		cell	disp.	Lac
A1	♂	0	28	—
2		(11)	1	+
3		0.	1	
4		0. (1)		+
5		0	0	
6		0	0	
B1			0	
2	♂ ♀ firm	0 (1)	28	—
3		0		
B 4		0 (1)	1	+
5	✓	(1) 0.	28	—
6		0.	28	—
C 1		?	1	+
2		(1)	1	+
C 4	✓	(1)	1	+
5		0.	28	—
D 1	✓	(1) (1)	1	+
2			28	—
D 3		(1)	(1) (28) → D3	+ —
5		0.	—	
6		0.	—	
E1		(1) (1)	0	
2		0.	0	
E 3			0	
4			1	+
5			—	
6			0	
F 1		(1)	0	
2		0.	28	—
3		0.	0	
D3RL		0. 0.	•	•
G F 1		(1) 0.	1	+
2	✓	0.	0	
3		0.	28	—
G 4		0	1	+
5		0.	0	
H 1		(1)	1	+
2		0.	28	—
3		0.	0	
H 4		(1)	1	+
5		0.	28	—
6		0.	0	

but see sec after 1179
sec. divisions not worked

B4L not spec. Cortical pr presumably absent! (Leighbody?)

Many invable. Complete marked — No (2)!

F "Clumps"

1178
(1177)

July 10, 1934.

164

W2640 : W2639 1:50. 1^{05} - 3^{20} pairs ~~off~~ unfragmented
Letter plated at ca 3^{30} while I isolated.

			disp	#	interest	
A	1	○	28	39		
	2	!	0			
	3	!)	1			
A	4	∞	28	40	P	F+ ..... ✓
	5	!	1	2	P	
	6	!	1	3	P	
B	1	✱ —)	1	4	P ? .	
	2		1	5	P	
B	4		1	6		
	3		0			
	5	all notule	1	7	IP	
	6		1	8		
sip C	1	✱	0	41		
	2	①	28 1	9	P	
	4	∞]	0			
M.	5	-	0			
	6	-]	1	10	P'	
D	2	✕	0			
	3	-	1	12	✕	

15 del. random. 0 F+
7 del pairs 2 F+

D 4-6, E 4-6, F 1-6, G 1-6 are random isolates of notules (atend)
13-15 18-20 21-26 27-32

H 1
2 all notule not pair. 33 34 35 } IP F+ }
3

H 4 ∞ ① ...] 1-28 36 ✕ (28+1).
5 ∞ ①] 1 37 P. F+ ✓
6 ① 1 38 P' F+

E 1 ○ 28 42
2 !
3 !) 1 16 17 P P

∴ 1-18 and 37, 38 should be checked as pair F+ progeny
39-42 are pair P+
6-8 and 35-55 may be illegitimate pairs

to ΣHL (pulled to 1nd prossary PII)

OXF, random isolates

DATE: July 12, 1954 REF: [158] [165]

1 2 3 4 5 6 7 8 9 10

X 2 - 2+ hour intervals essential pre-pair experiments. Crosses might have been confused?

Note fairly numerous colonies type 28 lac+ and -

---

lac stripes (10/8) O Recorded as 28 days

[158]

E4	-	A1	-	B5	-	D6	+	G4	-
E1	-	2	-	6	-	E2	-	5	-
E2	-	3	-	C1	+	3	-	6	-
G2	-	4	-	2	-	4	+	H2	-
H1	+	5	?1	3	-	5	-	3	-
		6	+	5	-	F1	-	4	-
		B1	-	D1	-	4	-	5	-
		2	-	2	-	5	-	6	-
		3	-	3	-	6	-		
		4	-	4	+	G3	-		

10/8 what is this expt?

[165]

A1	-	A3	-	D4	-	F5	-	
2	-	5	-	5	-	G1	+	
4	-	6	-	6	-	2	+	
B5	-	B1	+	E1	-	4	?	
C1	-	2		3	-	6	0	
C2	-	4		4	-	H1	-	
C3	-	6		5	-	2	+	
C6	-	C4	-	F1	-	6	-	
D2	-	5	-	2	?			
E6	-	D1	-	4	+			
H3	-							

11/8

Where are products. presumably occurring of ⊕ among myc and 8 isolates.

(1+ should be doubled for width)

DATE: 7/18/54

REF:

162	mal	2 lae	3 gal	4 mill	5 sy	165 6	7	8	9	10
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	+	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
	−	−	+	−	R	−	−	+	−	R
10	−	−	+	−	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
20	−	−	+	+	R	−	−	+	−	R
	+	−	−	−	R	−	−	+	−	R
	−	−	+	+	R	−	−	+	−	R
	+	−	−	−	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	−	−	+	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
	+	−	−	+	R	−	−	+	−	R
30										
		prob. miscarried by virtue of Bal.								
40										
50										

DATE: 7/18/54 REF:

156 mal	lac	gal	mlt	SM	160 mal	lac	gal	mlt	SM
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
-	-	+	-	R	+	-	-	+	S
+	+ ± -	+ ± -	slow?	R	+	-	-	+	S
+	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	sl?	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	sl?	-	+	S
-	-	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	-	sl	sl?	-	R
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
-	-	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
+	sl?	-	+	S	+	-	-	+	S
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	+ ± -	R
+	sl?	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S	-	sl	+	-	R
-	-	-	+	S					
-	-	-	+	S					
-	-	-	+	S					

Sept. 20, 1954

At my request for "the aerogenes strain used in the Baskett-Hinshelwood expts. (PRS 139:58-73, 1951) received a culture labelled simply "Aerogenes aerobacter" 19.7.54 This is stated to have a lag of about 5-6 days in synthetic-arabinose medium.

Initially it was streaked on EMB-L-arabinose and found positive.

Alack Bernstein received culture and stored it as W-2654. For first experiments, slant from single colony on L-arabinose was used. Subsequently, used slant directly from Hinshelwoods vial.

9/20. PM. Inoculate D(m) (citrate!) and D(0) for inocula. Latter grew well in 24h; former shows slight initial growth.

p21: From D(0) above, streak out EMB-Darabinose (Dar) and inoculate: (.1ml /10)

	A22	P22	A23	P24	P25
D(m)	±	✓	+	+	
D(0)	+++	✓	✓		
D(m,Dar)	±	± ··+ ?	+	+	
(to avoid citrate until D(A)= D(m) s/citrate mix is made up) T(m)	÷		±	±	
T(glu)	++				
T(Dar)	÷		±	+	

÷ is faint turbidity scarcely more than inoculum. Should try smaller.

A1. EMB-Dar plate all negative. (tamit pinks by afternoon)

A2. P22. Streak out from D(Dar) above which showed some growth previously? all negative. No papillae seen.

B1 Restreak original W2654 (Blac) for single colony for initiation. Prepare current slant and D(0) inoculum tube from this.

P24. Streak out ① from T(Dar) ② sole papilla on 1 colony of A2 ③

C: sole papilla on 1 colony of A1. to EMB Dar. In future expts., minimal medium D(Ar) is based on salts & citrate.

D. Incr fresh D(0) culture from B1 to D±Ar for mear selection.
1= .1ml 2= .01ml

P24
E. TAr. to D'Ar, D'(0) P25: +, +++. Rerun. TAr is +++ (E0)
N26 E-1 is +++

P25 N26 A27 P28

D2: D(o) +++ ✓
D(-) — ✓ ∓
D(Ar) ± ÷ ✓ ✓

D1: D(o) +++; +++ ✓
D(-) ± ± (Poor?) ∓
D(Ar) ± ± ✓ ✓ ✓

P25 end.
C1-2-3 no + but some diffuse in shading

more papillae now seen on A1-A2: C5 ~~C6~~ C7

P25
F1 = EO on EMB-Dar.

N26: C1 } mostly slow +
(48h.) 2 }
3 mostly −, 1 colony "+"

(24h.) F1: } mostly (heterogeneous) weak +
C5: }
C6
C7. distinctly two colony types − and +

no stray +.

New plating: N26: E1 is still slower on arabinose than on glucose. Wait on the D series for definitive series; meanwhile sub. E series to look for fast arabinose. Note: C3 is a + from first stage papilla of A1. Replate C3, C7 +/− and ✓ on D(o), D(Ar.).

EMB

A27: C3A − −
C3B ± centres
C7A. + and ++ }
C7B − }

1 ml/10 dia. P28 C7A'

~~D(o)~~ ~~D(Ar)~~ P28

D(o)	D(Ar)
+++	− −
+++	± +
+++	+ +++
+++	− −

D(o), D(Ar)
E1 A +++ + ++

DATE: REF:

N3. Since yesterday, 1 tube of D1 has begun to grow. (other D1 and D2 still negative).

C7A" seems now to be as rapid on glucose and arabinose. on EMB agar, still weak +.

Plan (1) Restreak D1A, C7A on EMB, DAr and inoc. 1 ml. D(Ar) for comparison.

P3-P4 (24 hours) — C7A" has formed colonies, often two are semi-, pinpoints., D1A' has scarcely begun.

P4: 48 hrs/hours, C7A" has grown optimally (lag = glucose)

Plate inoc 9/30, D(Ar). shows C7A' forming good sized colonies (two sizes). W2654 forms numerous pinpoints & definite stimulation from Ara+.

Conclusions (1). EMB probably not a good indicator for this problem. However papillae on EMB suggest a non-homogeneous response (contra Hinshelwood). (2) possibility of trying indirect selection on DAr.

Take C7A" single colony to NSA as 1181A.

P4: Note original 9/30 DAr plate shows no papillation yet for W2654 stock.

P7 picks + and - to D(o) for check.

P6: on D(Ar), D1A' shows about 1% dense colonies

P6: D2A is now ++.

P7: D2A ++
D1A (++)
D1B now +.3

P11: 1 all v.s.
2 mostly +, few v.s.
3 " "

strains as G1-3 not adapted?

still heterogeneous

A12 A14

P#7 D1 +
D1 − colony to D(0).
W2604

H	3	+ by P11	++	+++
	2	−	−	−
	1	−	−	−

∴ these are destruct.

Now D(0). all +++ in 4th.	D(4th)

P14

1. + and − colonies. ○ ○ about =
2. ++ and a few − ○ ○. any +?
3. " "

∴ heterogenity is still obvious.

None of these grows nearly as well as a D(blu). What was Dean's finding? Write H?

10/15/54 Conclusions.

① Initial strain, Ara⁻ grows v. slowly on gar, gives v. small flat colonies which appear to decay slowly as original.

Mutants not observed on O(Ar) (except possibly after 3-4 cycles) but heavy inocula have not been tested

② On O(Ar) liquid, Ara⁻ grows initially to ca 10^{6}-10^{7}/ml. Then stationary turbidity for 4-5 days, then slow growth.

③ Platings of these first cultures show mixture of Ara⁻ and Ara± (denser, faster colonies) on O(Ar) agar. Only trace for conversion of colonies with subsequent lag time.

④ These cultures still have long lags on Ara, but successive transfers are gradually shorter. After 4-5 transfers, fully adapted cultures are found.

⑤ No critical experiments on deadaptation except from 1st stage.

⑥ Platings on O(Ar) and EMB Ar at various stages suggest several mutational steps.

A. Cultures adapting in Ara liquid are not homogeneous.
B. Not proven whether induced or selected.
C: Needed: ① Platings of dense suspensions & hope of identifying

the first step mutants ⊘ lac$^+$/lac$^-$ markers for confirming heterogeneity of response.

Interactions on phenotypy "F⁻".

1182

DATE: Sept. 22, 954. REF:

i Luca.

P21 Anor/10 ml Penassay: i W6, W1177, W2207, W1305, W2437
M-F+ TL⁻F- M-F+ MTL⁻F+ MTL⁻F⁻.

.5 ml per.

10:20 A 22 Anor/10 ml Penassay i and s aeration.

	Aer.	
W1305	−	A
"	+	B
W2437	−	C
"	+	D.
W6	−	1
"	+	2
W2207	−	3
"	+	4

Plan: set up all combinations (A,B,C,D)(1,2,3,4) and wash, cross mixtures to W1177 (mass.) to test interactions. Plate comparable aliquots on D(0) agar

3:45 mix 1.5 ml each culture + 7 ml Penassay. - 4:45 sporulation

5:30 Resuspend in 1 ml. water each. 20 ml culture (mix above i aeration) W1177 to 5 ml.

Plate W1177 each pyplate.
.1 ml other

		1 W-6 −	2 W-6 +	3 W2207 −	4 W2207 +
A	F+ W1305	22	17, 17	30, 84	153,
B	F+ " Aer.	3	7, 2	2, 5	18,
C	F- W2437	60	15, 6	0 0	0
D	F- " Aer.	29	10, 10	0, 0	0
E	Penassay.	131	22 25	0, 0	1, 0

Controls
E2-
E3- 0 A × 0
E1- 0 C × 0
C- 0
A - 0

This exp. uncontaminated
continuation by Luca.

Pay Preliminaries.

1182 revertants

1) Recovery of aerated cells in Penassay.

2) Effect of 1 hour aeration on unaerated cells.

3) Effect of aerated ~~E~~ 1305$^-$ on ~~W~~ Waerated.

Comparisons

① E2: either aeration was ineffective in recovery in Penassay.

②. 4 series: a. W1305 reverted. W1305 aerated still reverted but qualified by ①

3 series: ~~W~~ W2207 aerated also reverted, less effectively ~~E~~ by aerated W1305.

③ 2 series: see ①. However B2/E2 suggests that 1305F$^+$ aer. inhibits recovery.

④ 1 series of A1/B1/E1: W1305 aerated may transmit mutagenic to W6 unaerated.

W1305F^a ~~/~~ × W6F$^+$?

F$^+$ —× F^a

Isolate pairs W2344/M1 (♂) × W2401 ♀ 1183.

DATE: Sept. 27, 1954 REF: 173-174

Obj: Begin to look for pedegrees on the ♀ side. Do not try to obtain ♂ pedegrees but record viability and isolate pools.

9:50 AM. Mix overnight cultures 1 ml ♀ : .1 ml ♂ : 10 ml Penassay 37°.
9/28 (PM) Plate dops. See protocols for picks schedule and pedegree details.
10 Plate to EMB Lac. Score P 29 and A 30.

A 30: all parental on Lac except A 2 and E 16. Pending further tests, the results may be summarized:

save A 2, 4, 5, 6

A: 100/100 → 10 → → 10 and ♂ ♂ ♂ ♀ ♀ ♀ (Ara+)
20 ♂ ♂ ♂ ♀ (2) ♂ Lac! ≠ still segregating. Might represent n=0, n=2 or n=4.

B: 10 all died

C: 10 → ♂ parental. [1/4 ♀ died]
♀

30 D: 10 → ♂ ♀ died.
♀

E: 10 → 1♂ → ♀
save E 16 and pools representing E 11, 13, 14 D 6, E 30
♀ [still paired; several generations].
♀ lost (2)

all other isolates concordant (♀ par.) on Lac, Lacsm, Mal, Mtl, Xyl, Ara, Gal.
~~(Not tested on ~~ ~~).~~

∴ n ≠ 2, still segreg. [Note 2/4 sibs lost].

F. 10 ⇄ parental. Note: [3/4 ♀ died] [several gen from 1/4].
try Hfr × M1 for heterozygosity

G. 01 → ♂ ♀ died.

H 01 → ♂ ♀ (only "1/8" survived).
parental.
F4W1553?

∴ 2 (2) from maximum of 5 possibilities. Considerable mortality in the latter.

Summary re pairs.

9/23/54

presumably no losses on ♀

(1184)

DATE page REF:

	1	2	3 Dol.	4 Int.	5 ♀	Zygotes 7	8	9 Zyg.	♀ with third
Expt.	1153D	129	4	1	4	2 ✓	1	0	
	55A	130	4	3	4	4 ✓	3	3	
	56	131	16	5	9	7 ✓	11	5	2 dead pairs
	[57	W1177X	7	2	3	0	5	0]	
10	58	10	8	8	8	0	8	0	3
		5	2	4	6	2 ✓	3	1	0
	59		14	4	8	3 ✓	12	2+1?	1
	60		5	2	3	1 ✓	5	1	
	61	142	17	—	8	2 ✓	14	1	2
	~~64~~	~~160~~	~~16~~	~~10~~	~~12~~	~~1~~			
20									
	~~76~~	~~161~~	~~14~~	~~9~~	~~11~~	~~1~~			
	77	162	15	5	10	0			
	66	153	8	8	8	2 ✓	8	8	( )
	62	137	16	2	6	1 ✓	8	1	5
	63	150	12	6	7	3 ✓	11	3	1
30	64	151	~~15~~	5	~~10~~	3 ✓	14	3	0
	65	152	~~18~~	8	9	4 ✓	12	4	3
Σ.		corrected	166 ✓ 158 166	62	98 101	34			
					101 93	~~34~~ 32			
40	68B	140	✓	4	4	0			
Reverse.	73A	158	13	2	6	1			
	B	159	16	9	13	0			
	74	160	16	10	12	1			
	76	161	16	9	11	1			
50			63	34	46	3.			
			229	96	144	37			

do not tabulate comp. pairs

see further for 107 details 25

19. 21 1164 151 B3 +  Lac+

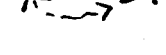
28 C6 + Lac+

24 D3 +  4 types: [Ara, Lac]$^{+}_{-}$ [Mal, MH, S]$^{+S}_{-R}$ MH$^{?}$

23 30 1165 152 A3 +  Lac+

31 A B6. Lac+

32 B V.i.

23 33 D2 +  A: Lac+ (Mal+)

34 B. 4 types [Lac$^{+}_{-}$] [Mal, S]$^{-R}_{+S}$ MH−? MH−?

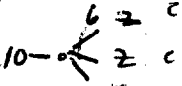
all V, S+Ar−

24. 35 E3 +  Lac+

25. 36 H3 +  Lac+

26. 1166 153 C4 +  Lac+

37

27 38 C3 +  Lac+

39 Lac+

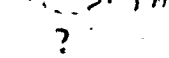
28 19 1159 1176 C5 − pair to C1 imp. Lac+

29. 9 1158 C1 +  Lac+

30 10 131 C3 − Lac+

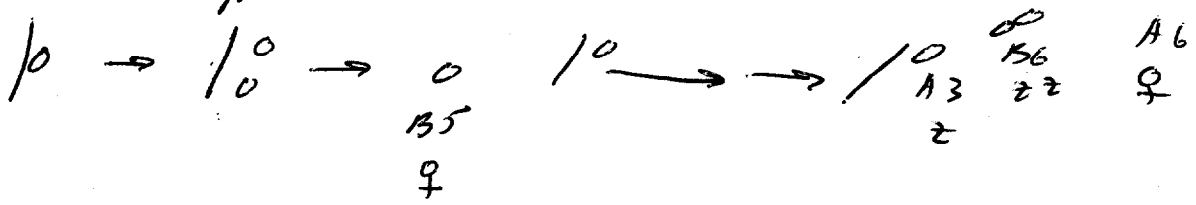
31 11 D1 +  Lac+

32 12 F3 − Lac+

33 13 B5 +  Lac+

?

1165 A3-B6 (Hyp. reasoning)

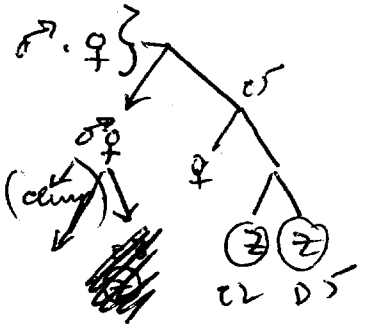


Enumerate zygote pairs. 9/24/54.

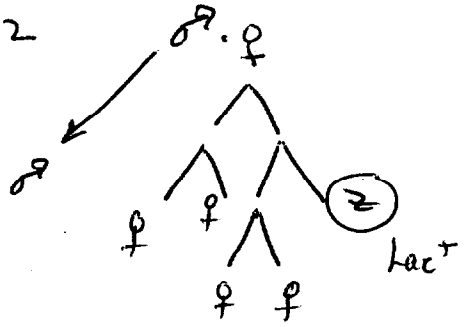
F- parent plus
[orthotype re Gal, Mal, Xyl, Mtl, S unless noted]

#	♂ surv?	Pedigree notes (✓ if interesting)	Recomb notes
1	153 D ~~2~~ [129]	–	~~Hfr~~ –·–	Lac+ ....
2. 2	" D ~~4~~ "	–	→·< ♀ z	Lac+
3. 3	5 T A [130] D3	+	8-·< (4) z / (1) x	Lac+
4. 4	" " F2	+	–·–	Lac+
5. 5	" " F5	+	4–· (2) ♀ / (3) z	Lac+
6. 6	H5	no rec.	· z ♀ / z z	Lac+
7. 7	56 [131] ✓B2	+	–·–	Lac+ Xyl+ Mtl– Mal– Gal+
8. 8	B5	+	z·< x / z	{ Lac+ Xyl+ Mal+ Mtl– S^R } Lac– Mal+ ; { Lac+ Xyl– Mal– Mtl– S^R } Lac– Mal– S^R
			↖ F^+ [Lac] [Mal, Xyl] F^- all Ara– S^R Mtl– Gal+
9. 14/58	[136] B1	+	–·–	Lac+
	~~33~~) 15) H2	–	–·–	Lac+
10	16 59. [146] A 3	+	–·< ♀ z	Lac+
11	17 { C2 / ~~C4~~ D5	+	v.i. ✓ (most of descent)	Lac+ "
~~12~~	48			
220	60 (1160-2) 143 · A2 (1160-2A)	+	v.i. ~~Hfr~~ z, dil/xgz. after 2 dil.	Lac+
324	61 [M2] C4	+	– ·< x / ♀ / z ✓	Lac+
14	22 1161 142 G1	–	–·–	"Lac+/– Gal+ Mal, Xyl–/+, S^R/(Lac–)!" prime. No Mal+ S^R mucoid clicks
15	73 1162 137 H5	+	–·–	Lac+
16	24 1163 150 E2	+	>·–	Lac– Gal+ Ar– Mal. T+/r ... W2502 (~~Hfr~~
17.	25 1 D6 (1161)	+	>·< z ✓	Lac+
~~18~~	26 G5	+	–·< x / z	Lac+

1159 - C2



1160 - 2



but > 34 zygotes!

Notes on summary:

(1) all zygotes must to be tested for segregation of V_1, A_r. (cf. notes on colony segregation).

(2) of 38 zygotes, σ survived in 26. Probably as high as controls.

(3) Pairings after division analyzable in 8, 7 are completed. (Exc. 56 65)

(4) Pedigrees 2 or more generations in 7. all still segregating!

Following in $\frac{\#}{\text{total}}$: 11592 calc. 2/8 (sibs)
1160-2 " 1/4
61-64 1/4 (1x)
65-#3, 136. 3/8 (sibs)

6502 2/8
H3 1/4
27C3 ± 2/8

(5) Review distributing of Mal. (#16 uncertain). 3 cases of Mal+ all segregating! B5 should be examined for S^s also Say 2 cases of S^s and both show 4 phenotypes!
(Recall test for recurrent recombination).

(6) of 34 zygotes. 28 are Lac$^+$...

Retests on 3yr (w)

11184

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
A										
B										
C										

A All review for numbers (1-39). # 40, 41 = 11183 A2, E6 resp.

B Rev specific isolates

C Replicate 1-41 on D(o), D(M), D(H). (4 types!)

10 # 29 grew on hist/mic 11640 3 - were on D(o).

19 " " methionine 11593 Lact⁻...

20

30

40

50

OC6 tests.

1184

DATE: REF:

1 2 3 4 5 6 7 8 9 10

all zygotes presumably contained some Lac - V_1^R Ara − . Additions, formed (for Lac+):

	Lac+ : V_1 ::	Ara	Notes:
	S	−	
	S	−	
	R	+	
	S	−	
	R	−	any -S?
	R	+	
	R	+	
	S	+	
	R	−	
10	R	−	-R?
	S	−	and -R!
	S	−	
	R	+	
	S	−	
	S	−	
	S	−	
(clump)	S	−	
	S	−	
	R	+	-R?
20	S	+	
	S	−	
	R	+	
	S	+	
Lac−	S	−	(6352 ?)
	S	−	
	R	−	
	R	−	
	S	−	
	R	+	
30	S	−	
!	S	+	
	R	−	
	S	−	
	S	−	
	S	− +	any Ar+?
	R	+	
	R	+	
40	R	+	-R :
	S	−	

Between sessions: 6/12/52

over: ignoring complications & assuming that recombinants are all Lac+, plating tells:

DATE: REF:

among lac+ :

	Ara+	Ara−	
V_1^R	(12)	5	(17)
V_1^S	3	20	23
	(15)	25	40

Ara and V_1 are clearly linked to each other. Are they linked to lac? The parentals in coupling with lac+ are tricky. There is a definite excess of recombinants, possibly significant (no!) However, incidence of couplings may be exaggerated by admixture.

~~Data~~ Table above is uncorrected for a few sib zygotes. Note especially 1165 B6A, B (31, 32).
30

Pedigree Hfr x F⁻ 1185

DATE: Sept. 30, 1954 REF: 175–177

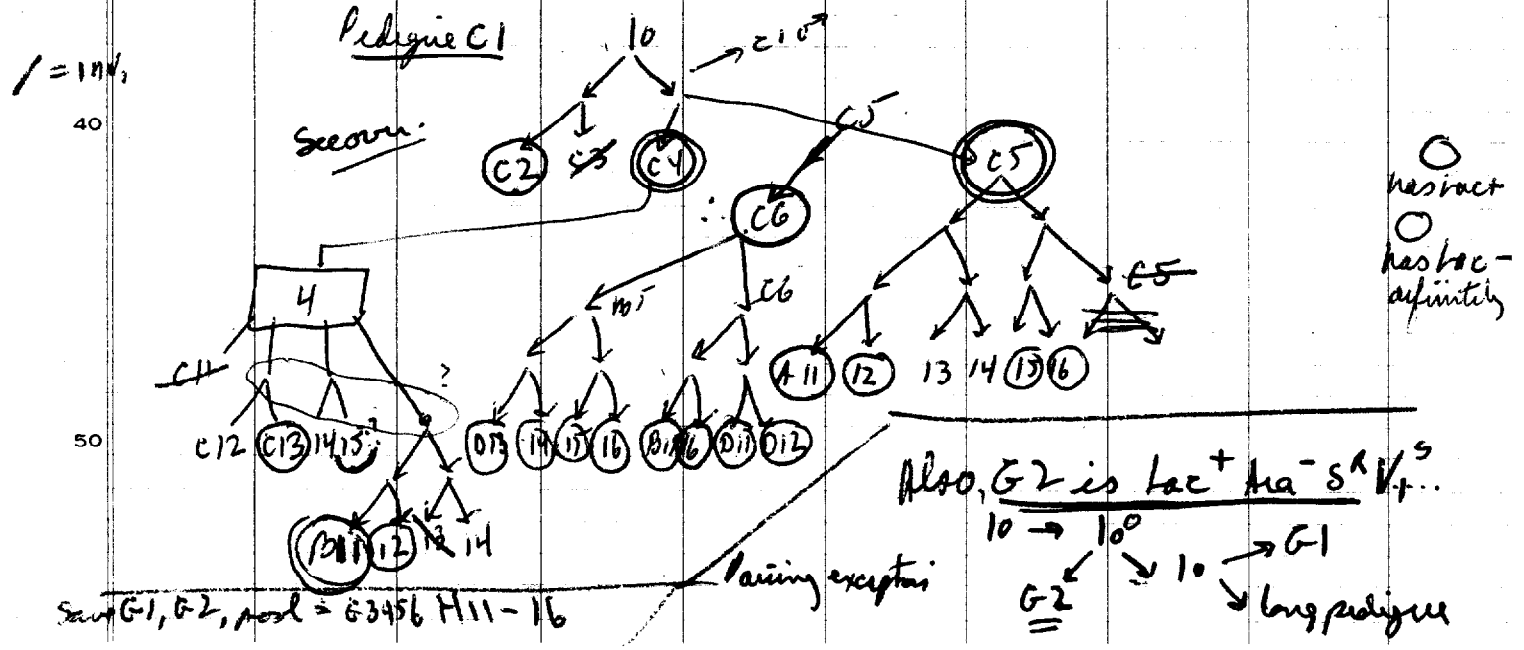
Second sum. overnight cultures, 1♀ : .1♂ : 7 ml broth 32°
10²⁰ – 11⁴⁰ to set up. 8 pairs isolated initially, 12:20–12:40 PM.

B1 was initially suspicious and proved illegitimate. No viable ♀ from A1; (Second) paired exconjugant from B1 also inviable. Other pedigrees to 4–6 generations.

All protocols for pairing schedule on sheet V – II – VII / VI – VIII (lac – or lac + parents)

			lac	lac streak	Mal	Mtl	Xyl	Gal	Str	Ara	V_1
III	A	11	+	+ (pure)	–	–	–	++	R	+	V_1 R
		12	+	+	"				R	+	V_1 R
		13	–	–	"				R	–	V_1 S
		14	–	–	"				R	–	V_1 S
		15	+	+					R	+	V_1 R
		16	+	+					R	+	V_1 R
	B	11	+–	–, +					R	–+	+V_1R – V_1S
		12	+	+, –					R	+–	+V_1R – V_1S
		14	–	–					R	–	V_1S
		15	+	+					R	+	
		16	+	+					R	+	
IV	C	12	–	–	–	–	–	++	R	–	V_1S
		13	+	+, –					–+	+	± V_1R, V_1S
		14	–	–					–	–	V_1S
		15	– +?	–, +				"	–+	+	+ V_1R – V_1S
	D	11	+	+					+	+	V_1R
		12	+	+					+	+	
		13	+	+					+	+	
pure?		14	+	+				"	+	+	
		15	+	+					+	+	
		16	+	+					+	+	

Pedigree C1



except for this pedigree, other exhibits are concordant with the segregation indicated

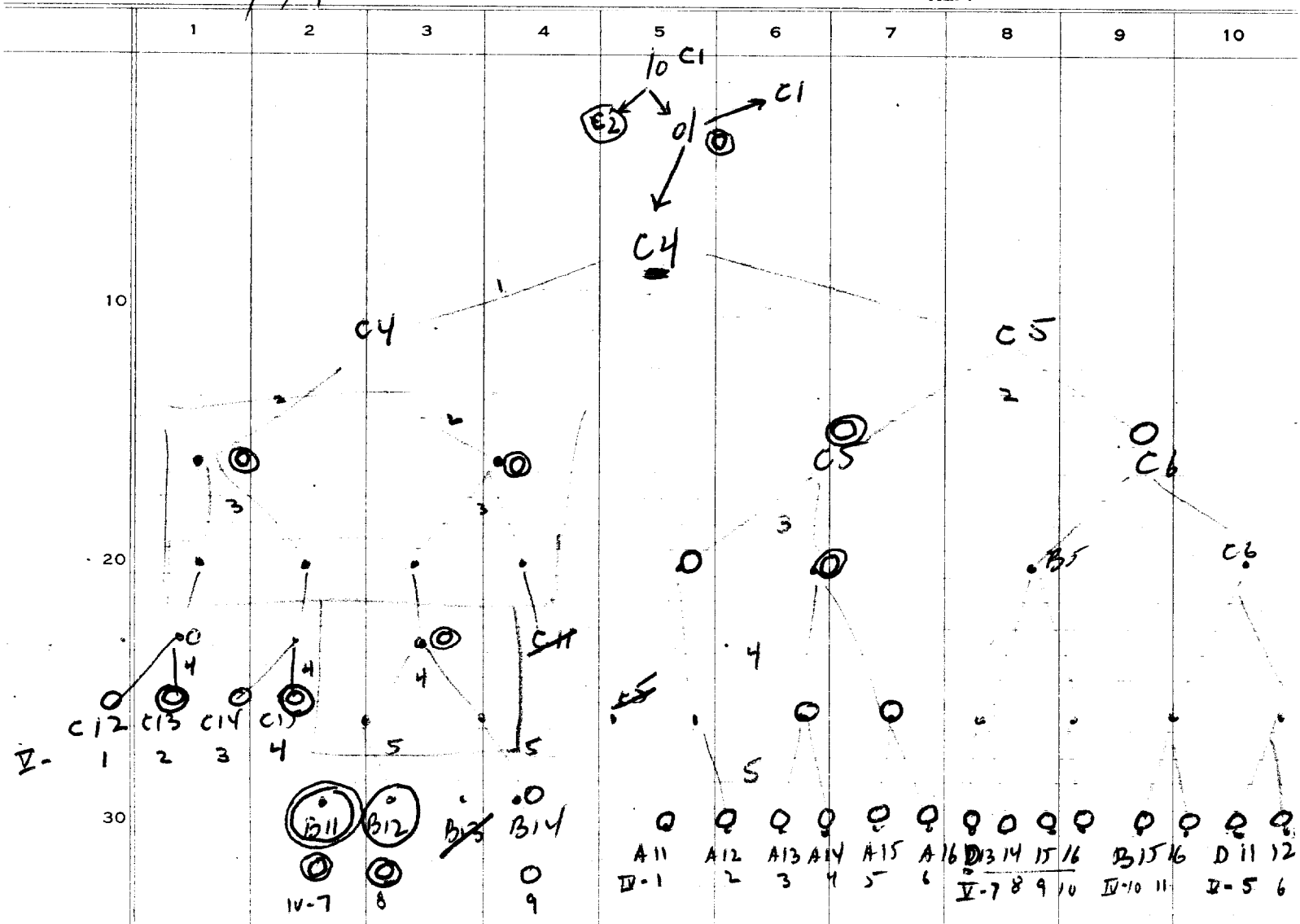
---

In all likelihood, a single recombinant is represented, though of the most common type:

$$\left(\frac{\text{Lac+ Ara+ } V_1^R}{\text{Lac- Ara- } V_1^S \cdots}\right)$$

(absence of other recombinants argues against double meiosis)

DATE: 10/2/54. REF:



○ Recomb

○ ♀ parent

∴ B11 shows two lines still segregating after the 5th generation, while the C6 clone seems to have segregated at the 2d. A13-14/A15-16 probably at the 4th. Pedigrees generally should probably be carried to 4 generations.

Note: both C4, C5 are 3n, ♀. Time of fertilization?

1186

	♂	♀	⊕	
A1	×	✓	✓	Lac+... ; Ara+...
A4	aband			
B4	✓	×		
C1	✓	✓	✓	motile.
C4	× without	✓	✓	lac+ara+
E1	✓	✓		sip
E4	✓	×		
F1	✓	✓ put		
F4	✓	✓	✓	lac+..., Mal+...
G4	✓	×		
~~B~~ 1	✓	×		

G4 aband. maybe sibly. ♂ rec. no ♀

D1	✓	✓	✓	lac+...
G1	✓	✓	✓	
H4	✓	✓	✓	[illegible]

lac+ara+
lac−Ara+
no lac+ara−

DATE: Oct 5 1954 REF: 178 -179 -180

Cross #10: Hfr W2401 : W2344/14. ca 8[45] A.M. Cross is therefore somewhat old when picked (10[30] - 11[15]) = 1:45 - 2:15 hours. 16 pairs were picked initially. Results:

A1 — ♂ → ♀ — ♀ → ♂ died → ♀ B12

A2. Ara+

B12: Lac+ Ara− ../♀

A2: Lac− Ara+ ./♀

A4. — ☆ abandoned as too complex (B1) ?

B4 — ♀ sip ♀ died

save C56 C5 A56

C1 complex ☆ = ! notable What is C3 - originally tested as notable. no!

C4 — ♀ → 100

C11: made withered away.

Note C12 exception.

C12: Lac+ Ara+ ♀ only

♀ → ♀ C12 ♀ , ♀ C11.

E1 — ♀ sip both survived → E1 ♂ , E2 ♀

E4 ♂ → ♂ ♀ died ♂ → E4

F1 ♂ ⋯→ cluster ♂ ♂ ♀ → F1 ♂ , F3 ♀

F4 ♂ → ♂ → F4 ♂ , F6 ♀

F5 ♂

Note pairing combination

F6: A ♀ Lac+ Mal+ Xyl+ SR , C Lac+ Mal− Xyl+ SR , D ♂ } Ara− Xyl−

no SS . (23 + 5) Mal+ tested no Lac+ Mal+ found

DATE: REF:

D4 ♂ ..... all ♀ exc. eventually died, not before dividing

H1 ♂ → H1 ♂
↘ all 6 ♀ died! (fully sporul sep.)

G4. conferred ♂' → G4 both ♂ ♀ died?
↘ G5

For fuller pedigrees see below.

D1 ♂1 → ♂ ♂ → D1 ♂♂
X
♂ → D11
D12 ×
D11 D13 ... 7 progeny all ♀: D13, 16, E4, 25
B13, 23
C16
6 progeny all segregants?
1 inviable

Presume conidiation
D11
D21-22-23-26 } Lac+ ...
D26

see pedigree
G1. ♂ → ♂ G1
♀ ♀ ♀ G12

see pedigree
H4 ♂ → ♂ H4
F11 ♀ ♀ ♀ F12 ... n=4
Mal+
F2 F16
Q E14
E13 E12 E12
B16 B24 n=8
A23 A24 F25 B23
+ + +

This has mutations.

see later.

still segr.

Zygote summary:
viable exconjugant in
A1, C1, C4, E1 (+,p), F1, F4, D1, G1, H4.

♀ in ♂ = 1/9
pairings seen in C4, F4, D1,
and probably conidial in 2/3.

1186

DATE: REF:

		#	Lac *	Ara	Mal	Xyl	Mgl	Gal	S/Lac	D(M) 9	D(M) 10	Note
	1	A 2	−	+−	−	−						
	2	5	−	−	−	−						
I	3	6	−	−	−	−						
	4	3	−	−	−	−						
A.	5	B 2	−	−	−	−						
	6	C 5	−	−	−	−	all other −	all other +				
	7	6	−	−	−	−						
	8	E 2	−	−	−	−						
	9	F 3	−	−	−	−						
	10	5	−	−	−	−						
	11	6	−+•	−	−+	−			−+ ←	all on the Mgl space a 2nd rep.	{−− −+ +−; no ++	
	1	G 4	±	+	+"	+	+	−	S +			
II	2	B 12	+−•	−	−	−			+			
	3	13	−	−	−	−			−			
	4	C 11	[illegible]	−	−	−			− [illegible]			
	5	12	+•✓	+−	−	−			+			++/−− only
	6	D 11	+✓	−	−	−			+			
	7	13	−	−	−	−						
	8	16	−	−	−	−						
	9	E 14	−	−	−	−						
	10	15	−	−	−	−						
	11	16	−	−	−	−						
		C 16	−	same for latter check				all other +				
	1	F 11	−	−	+•	−	✓		Mal − only			
	2	'	−	−	✓	−						
III	3	'	−	−	✓	−						
	4	'	−	−	−	−						
	5	'	−	−	−	−						
weak Lac+	6	16	−+•	−	−	+✓			−+			
	7	G 11	−+	+	−	−	✓		✓			
	8	12	+−	+−	−	−			+			++/−− only
	9	14	−	−+	−	−						
	10	15	−	+	−	−						
	11	16	−	+−	−	−						
	49	A 21	−	−	−	−						
IV	2	'	−	−	−	−						
	3	'	−	−	+•	−			Mal − only			
	4	'	−+•	−	+✓	−			S!			
	5	'	−	−	−	−						
	6	26	−	−	−	−						
	7	B 23	−	−	−	−	✓					
	8	'	+✓	−	−	+✓						
	9	'	+[illegible]	−	+−	+			(Mlgt+ Mal − only)			
wk. Lac+	50 10	26	−	−	−	−						

* if streaked, ✓ = pure; mixture as indicated

all R exc. as noted

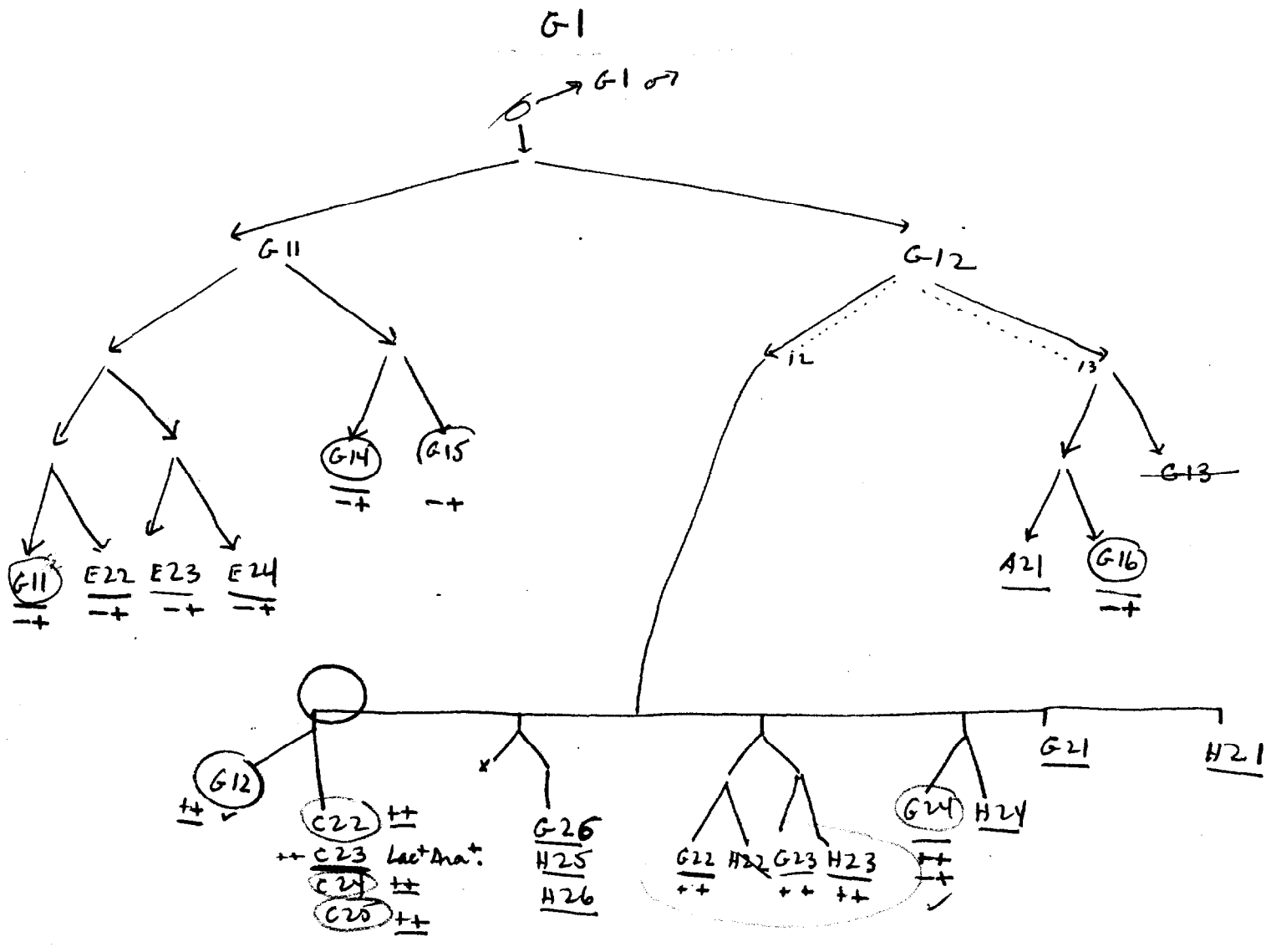
_ singles bac colony.

c 2 2(A) - pure Cal+
lac±. save

c3: non motile. 1 colony sayes, pure Cal+. ∴ typ. ♀.

DATE: REF: D(n) D(H)

	#	Lac	Ara	Mal	Xyl	MPl	Gal	S/Rac	9	10
V	1 C21	+-	—							
	2	+- ✓	+						48 negative colony A	
	3	+	+							
	4	+ -	+							
	5	+ -	+							
	6 26	—	—	all -	all -	all -	all +			
B.	7 D 21	+ ✓	—							
	8 P	+ ✓	—							
	9	+	—							
P	10	—	—							
	11	—	—							
	12 26	+ ✓	—							
VI	1 E 21	—	—	-				no Lac		
	2	—	+	-						
	3	—	+	-						
	4 24	—	+	-	all -					
EMP	5 F 21	—	—	-						
	6	—	—	-						
FDP	7	—	—	-						
	8	—	—	-						
	9 25	—	—	+ ✓	—	—	+	S v. sig.		
V	1 G 21	—	—	-						
	2 22	+ ✓	+	-			all +			
G22P	3 23	+ ✓?	+	-						
	4 24	+ -	+	-		all -				
	5 26	—	—	-						
G21P	30 H 21	—	—	-	all -					
G22P	7 23	+ ✓	+	-						
	8	—	—	-						
GKP	9	—	—	-						
	10 26	—	—	-						
VI	1 B4	±	+	+	+	+	—	S		
	2 C1	±	+	+	+	+	-	S		
87	3 C3	—	—	±	+	-	+	R	M-H -	~~NOTICE!~~ + mut. yes!
	4 D1	±	+	+	+	+	±	S		
	5 D6						-	S		
	6 E1									
	7 E4									
	8 F1									
	9 F4									
VII	11 G1									
	12 G5									analog
	13 H1									
	14 H4									



— ♀

♂ Lac − Ara+/Ara−

○ Lac+ / Lac−

− pure as ind
all Mal−Xyl−MtL−SR

Still to be characterized:

- ① G11 purity on Ara (Lac−Ara+). Try V1 also
- ② Look thoroughly for recombinants of Lac/Ara in the G12 progeny. ~~(Use V1)~~

✓ ③ G24 any complementary Lac+ Ara− ?
(No − of 45 Ara−, all Lac−; do. Lac+)
other three types definite = A B C
−− ++ −+

φ = Lac$^-$ Gal$^+$ Ara$^-$ (Mal$^-$ S^R) (Xyl$^-$ MH$^-$)

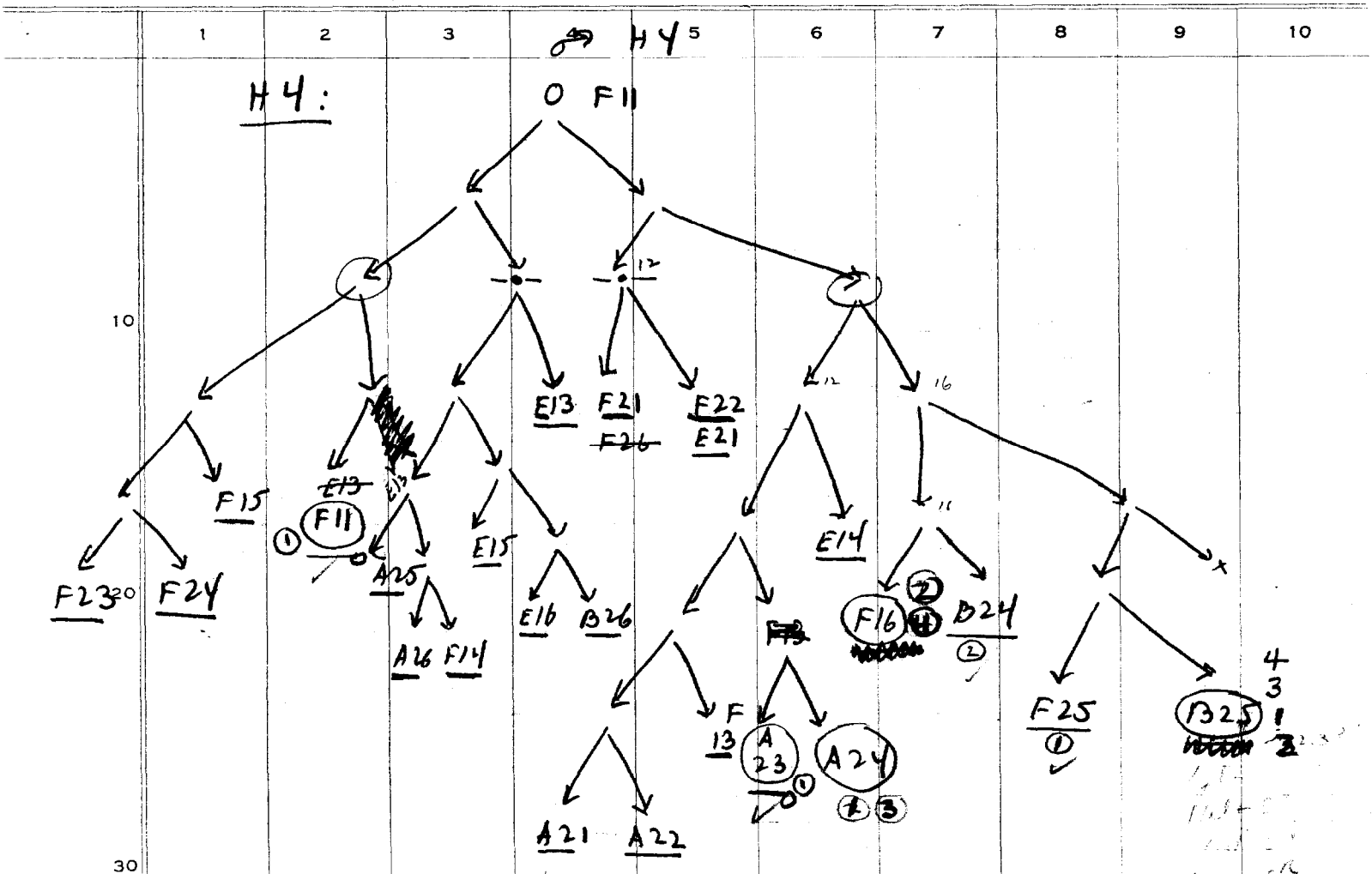
of ... + − + − S x +

Arca Ara$^-$ Gal$^+$ MH$^-$

Redraw pedigrees

1186

DATE: REF:



— = Lac⁻ ... S^R
o verified in mixture
— pure
⬭ segregating

① Mal⁺ S^S Lac⁻ Xyl⁻ ② Mal⁻ S^R Xyl⁺ Lac⁻ ③ Mal⁺ S^S Lac⁺ Xyl⁻ ④ Mal⁻ S^R Lac⁻ Xyl⁺

F25: Mal⁺ S^S Lac⁻ pure

B24: Lac⁺ Xyl⁺ pure S^R Mal⁻

F16: "Lac⁻,⁺ S^R; Xyl⁺ "Mal⁻

all are Mtl⁻ Gal⁺ H⁻ H⁻ pure
Ara⁻

F11 Mal⁺,⁻ ; Lac⁻ S^R/S^S.

A23 " "

A24 Mal⁺ S^S < Lac⁺ / Lac⁻ > Xyl⁻

B25 any Xyl⁻ S^R ~~[illegible]~~ NO
Lac⁺ S^R: all Xyl⁺

Several details

Conclusions

a) heterocaryon vs. heterozygote

b) double mutants?

B25 ctd. 10/14: 2 plates replicated to Lac ± Sm
Xyl
Mal

① all Lac$^+$ S^R are Xyl$^+$

② all S^R = Mal$^-$

③ all Mal$^+$ = Xyl$^-$
Mal$^-$ = Xyl$^+$

④ 1, 2, 3, 4 types seen.

also, mutants Xyl$^-$ S^R, 4 is absent.

Further tests on content of clones

1186

DATE: Oct. 11, 1917...

REF:

C:

~~Lac~~ Lac+ and − , to check Mal, S, Xyl.

F6. 10 Lac+ 10 Lac−

Xyl

Xyl	S	Mal
all −	R	all −
all −	R	5+ 5− .

∴ F6 includes

Lac+ Mal− Xyl− S^R

Lac− Mal− Xyl− S^R

Lac− Mal+ Xyl− S^R

Not excluded:

Lac+ Mal+ (pur. Xyl−S^R)

. . S^S.

B25 12 Lac+ 16 Lac−

Xyl	S	Mal+	repeat (might have confused F25)
−	S	Mal+	
−	S	all+	

should be some S^R!

medium?

B12 4 + 4 −

F16 2 + 2 −

−	R	−	
−	R	−	
−	R	−	
−	R	−	

? →

A11 1a − 1 +

−	S	+	to isolate Xyl− Lac+ Mal+ S^S
−	S	+	Lac− Mal+ S^S

medium. v.g.

do. in test of duplicates

C22 {Lac 3+ 3− Ara 3+ 3−}

24 " "

25 " "

C12 4++, 4−−

C12 " "

∴ C22-24-25 have Lac+Ara+ / Lac−Ara−

G24 has ++, −− and −+ ?

G24 5 Lac− 2 Ara+ 3−

all S^R 1 Lac+ Ara+

C21 (−+?) 4+−

interesting Lac+ ones not pure

all Mal+ are S^R
Mal$^-$ S^S

B25

				Type
Mal−	5 Lac−	Xyl+	S^R	B25A ④
Mal+	7 Lac+	Xyl−	S^S	B ♀ ③
	3 Lac−	Xyl−	S^S	C ① any ♀ ?
				D ②

no Xyl− S^R on streak

A23 4+ Mal, 4− " } all Lac− Xyl−

① A23B = C
♀ A23A = ♀

F11 ditto { Lac− Xyl−

A24 1 Lac+ 7 / 1 Lac− } Mal+ S^S Xyl−

③ A24A
① A24B

F16: ~~A 2~~ ⑧ − 2 (Lac+)
~~B 8~~ ⑧ − 4 (Lac−)
both Xyl+ ✓

Types seem to include

♀ =	Lac$^-$	Xyl$^-$	Mal$^-$ S^R
①	−	−	+
②	+	+	−
③	+	−	+
④	−	+	−

Note complementarity

two Lac classes included here!
~~Lac+ possibly weaker~~

mix Lac, Lac sm
Mal, Xyl,
only ① ② ③ ④ found, no ♀, no Mal/Xyl
no S^R [illegible]

Enrichments of two plates

Selected and unselected Hfr: fertility; 1187
Hfr x Hfr.

DATE: Oct. 8, 1954. REF: 181

overnight cultures:

A 11851 C1 ♂
B C2 ♀ (fertile)
C D1 ♂
D D2 ♀ (infertile)
E W2582.
M ♂ W2344M1
F ♀ W2401

Conclusion - no gross difference in fertility of a fertile vs. unfertile pair! Also no prolonged adolescence required for mating.

1:40 PM. Mix ~~with~~ 7 ml broth 0.1 ml each of ♂'s and ♀'s. 4:10 plate out on EMS lac ± sm.
(E+M at 1:1).
Counts are lac+/total in lac sm

AB	5/293	(1.7%)		
	10/493	(2.0)		
CD	16/648	(2.5)	39/1090	(3.7)

A9.

EM. Ca 3/1000 lac+ sm. Try for pair isolation

A9. Mix same (2 day) cultures ~~10~~ 1E 10E:1♂ 945 ♂M

About 10 "pairs" isolated (181).

But all proved allogenate. However, crossing only late (1:15), i.e., at least 3½ hours.

Hfr x Hfr

1188

DATE: October 12, 1954. REF: 182

W2582 x W2344/M1. 9:30 - 11 AM exc.
("♂").

Most pairs illegitimate. 3 Legit, 1 shows recombination (W2582 as ♀); F1 indeterminate because mixed.

Note

			Lac	Gal	Mal	Xyl	Swarm.	Ara	S	
1	A1	♂	±	−	+	+		+	S	
X	B1	•	++ mix	+	+	+	+	+	S	Not ally.
	e1	♂	±	−	+	+		+	S	ally ✓
	C1	♂	±	−	+	+		+	S	T
	D1	♂	±	−	+	+		+	S	
	D2	♀	−	+	−	−		−	R	Legit
	E1	♀	−	+	−	−		−	R	×
E3	E2		+ mix	+	+	+		+	+	
	F1		+ mix	+	+	+	+(-)	+	+	Recomb?
X	F2	♀	−	+	−	−		−	R	
X	F3	♀	−	+	−	−		−	R	
	G2A	♀	−	+	−	−		−	R	
	G2 (X)	♀	−	+	−	−		−	R	
	G3	⊗	+	+	+−	−		+ —	+ R	(Lac+)
	G4	♂	±	−	+	+	+	± ✓	S	
	H1	♀	−	+	−	−			− R	some ♂+?
X	H2		+?	+	+	+	+	+	+ R	+, − mixed how?

mixed ±, ++

parents are W2344 = Gal− Lac+ + ... " ♂ "
W2582 = Gal+ Lac− .... " ♀ "

Interest: G3 = Lac+ Gal+ Ara+ Mal− Xyl−
(18 ♀ (G3 = W2401))

may need a reexistation for motility of W2344

Restrich Lac and Gal # B1, E2, F1, G3, H2

B1 − "both motile"

E2 − "x"

F1 0 < 1, (E3?)
00 F2 { may be recomb in opposite sense?

G3 { 10 → 0
G4 { 01 → ♂ rich purity in Lac

F1 may represent
~~ffx~~ W2344 × W2582 ♂ } Probably not. Dominance of Gal+Lac− and Gal−Lac+ rarely expressed

G3 is evidently W2582 × W2344 ♂

	Lac	Gal	
B1	mostly ±	mostly −, few +	
E2	± and − ? are all Lac− Gal+ ?	− and +	F1: Gal+Lac− pool all ran motile
F1	mostly all ±	mostly −	some F1 is mostly Lac−Gal+, it is presumably ♀ + few ♂
M2	"	" "	
G3	pure +	pure + ; legs on Ara	
G4	all ±	~~all +~~ not tested	

plus. —

• terminate
• Carbine

♂ × ♀ to prolong.

1189

OCT 14 1954

DATE:

REF: 183

	A	B	C	D	E	F	G	H	9	10
1	10 10	10	10		10		10			
2	0									
3				10						
4	10	10	10	10	(10) ?	10	0	0		
5										
10 6										

183

grew:?

	A	B	C	D	E	F	G	H
11	0, d	0, d	0, d	+ nm	+ m	0 :	+	0
12	0, d	+, d	0	+ m	0	+ mg	0	+
13	0	+ nm	0	0	0	0	0	0
14	not	0	+ m	0	0	0	0	+ sparse
15	0		0	0	0	+ nm	0	+ nm
16			0			0	0	

183 pic.

21

22

30

23

24

25

26

40

abandon

why poor or no growth

① low temperature

② capillary tip too sharp? or too acutely bent

but same as 1190, 1191.

Abandoned owing to growth failure (temperature?)

50

1190 Sww.

	♂	♀	Zyg.	types.
B4	✓	✓	no	
C4	✓	✓ part	—	
	✓	✓	0	

pedigrees

DATE: Oct 19, 1954 REF: [185-186-187]

1	2	3	4	5	6	7	8	9	10

W-2344M1 x W2401 10:30- 12N 10;1 ♀ Collect numerous pairs and follow for 1-3 exconjugant generations. P19: refrigerate[185] after transferring three pedigrees to [186-187]. B4, C4, E4

	1	2	3	4	5
10					
		B5	A15	A15	A15 ✓ 1
					A24 ✓
				B15	B15 ✓
					B13 ✓
			A16	A16 ✓
				B16	A22 ✓
					B16 ✓
					B14 ✓
	B5				
20		A2	A13	A13	A13 ✓
					A25 ✓
			A14	A14 ✓
					A23 ✓
			A11	A11 ✓
					A26 ✓
			A12	A12 ✓
					A21 ✓
				C11	C11 ✓
			C11		D12 ✓ 18
				D11	D11 ✓
30			C11		D23 ✓ 19
				C12	C12 ✓ 20
					C26 ✓ 21
				C13 ✓	V slow 22
	B6				
				✓	V.S. 23
			C14	D14	D14 ✓ V.S. 24
					D22 ✓ 25
			C15	C15 ✓ 26
40			C15		C25 ✓ 27
				D15	D15 ✓ 28
			D15		D13 ✓ 29
50					

B4 → B4 ✓

These 3 pedigrees: all ♀ on Lactal Ag Mal 1%+1 sm.

C14? B26? ? replaced with C25, 226? ? recirculation?

29 cells

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
		1	2	3	4	5				
10							M			
			E15 ✓							
20	C4 C5 C6	25		E13	✓	E13 ✓ 1 E22 ✓ 2 B26 ✓ 3				
					E26	E26 ✓ 4				
				E14	✓	E14 ✓ F21 ✓ 5				
					F15	F15 ✓ 6 F23 ✓ 7				
30				E11	✓	E11 ✓ 8 E23 ✓ 9				
					E25	{too many} 10				
				E12	✓	✓ 11 E21 ✓ 12 13 14				
		C6			E24	E24 B23 B24 B25				
					✓	n/ ✓ 15 16				
40				C16	D16	D16 ✓ 17 D24 ✓				
				E16	✓	E16 ✓ 18 F24 19				
					F16 ✓					
50										

Exhaust

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
10									★ K	
						G11	G11	G11 ✕ G24 ✓ 1		
							H11	H11 ✓ 2 H25 ✓ 3		
						G12	G12 ✕			
				E5			H12 ✕			
20						G13	G13 ✓	4		
							H13 ✓	5		
						G14	G14 ✓	6		
		E4 ↗ E4 → E5, 6					H14 ✓	7		
						F11	✓	F11 ✓ 8 F25 ✓ 9		
30							G16	G16 ✓ 10 G25 ✓ 11		
				E6		F12 ⇔	? NF	G15 ✓ 12		
							G15 ✓	G15 G26 G26 13		
						F13	✓	F13 ✓ 14 F26 ✓ 15		
							H16	H16 ✓ 16 H26 ✓ 17		
40						F14	✓ ✕			
							H15 ✕			
50										

A. aerogenes / D-Ara. 1191

DATE: Oct 22, 1954 REF:

By UV/EMB, 8 st obtained 3 lac⁻ mutants from W2654. A is nearly full- ; B + C are slow but entirely scorable

P21 from D(0) of W2654, ~~[illegible]~~ W2663. Keep A as W2663

---

215 P22 Prepare mixture of W2654 + W2663 1:1

from D(4) with .01 and .001 ml / 10 of each & mixture. 37°.

		Tube		
A	W2654	.01	1-3	A1 became + 11/7 AM. A2 ± 11/8. almost pure lac+ ~~and streak~~.
B	"	.001	1-3	
C	W2663	.01	1-3	
D		.001	1-3	
E	Mix	.01	1-5	11/8: 1: ± 2: ± 3: 0 almost pure lac+ mutants
F		.001	1-5	

also tube #0 = D(0).

P23 (outer bench all day). Streak on EMB lac - 0 - citrate and DA faber. Turbidity 0 except in D(0).

A - D(6) as pure E cal: 1 F > 20:1 Lac⁻ : Lac⁺

~~F~~ (DA no arabinose) cal: 1

E, F1 (Ara.) cal: 1

P24 0 turbidity.

~~P~~ P9 streaks of E1, E2 E1 now ++ E2 ± E3 0 E4 ±

~~E~~ now + B1 + B2 ± B3 ±

P 2 P A2, 3 ±

structure of P9:

E1
2
3
4
} almost pure +

F1
2
3
4
} almost pure +

A1 ++

C1 −−

E0 almost pure +

F0 almost pure +

Clear that "baz − " does not persist in subsequent S^{R}!

1192

DATE: 10/23/54 see 1190 REF: 188 - 189

188

189

Picks 188: A11 B11 D11 E11 F11 G14 H13 B26
13 13 14 12 14 D26
14 16 16 16 H23
15 16 25
16 26

A24. — = growth
× n.g.

also wrote: A4, B4, C1, C4, E4, F1 G1 H1 H4
& A6 B6 D2 D5 D6

[185]

A26 { all ♀ except G15 are Lac−
all ♂ Lac±.

G15 pure ? Lact

No good pedigrees after 3 day refr. storage

over.

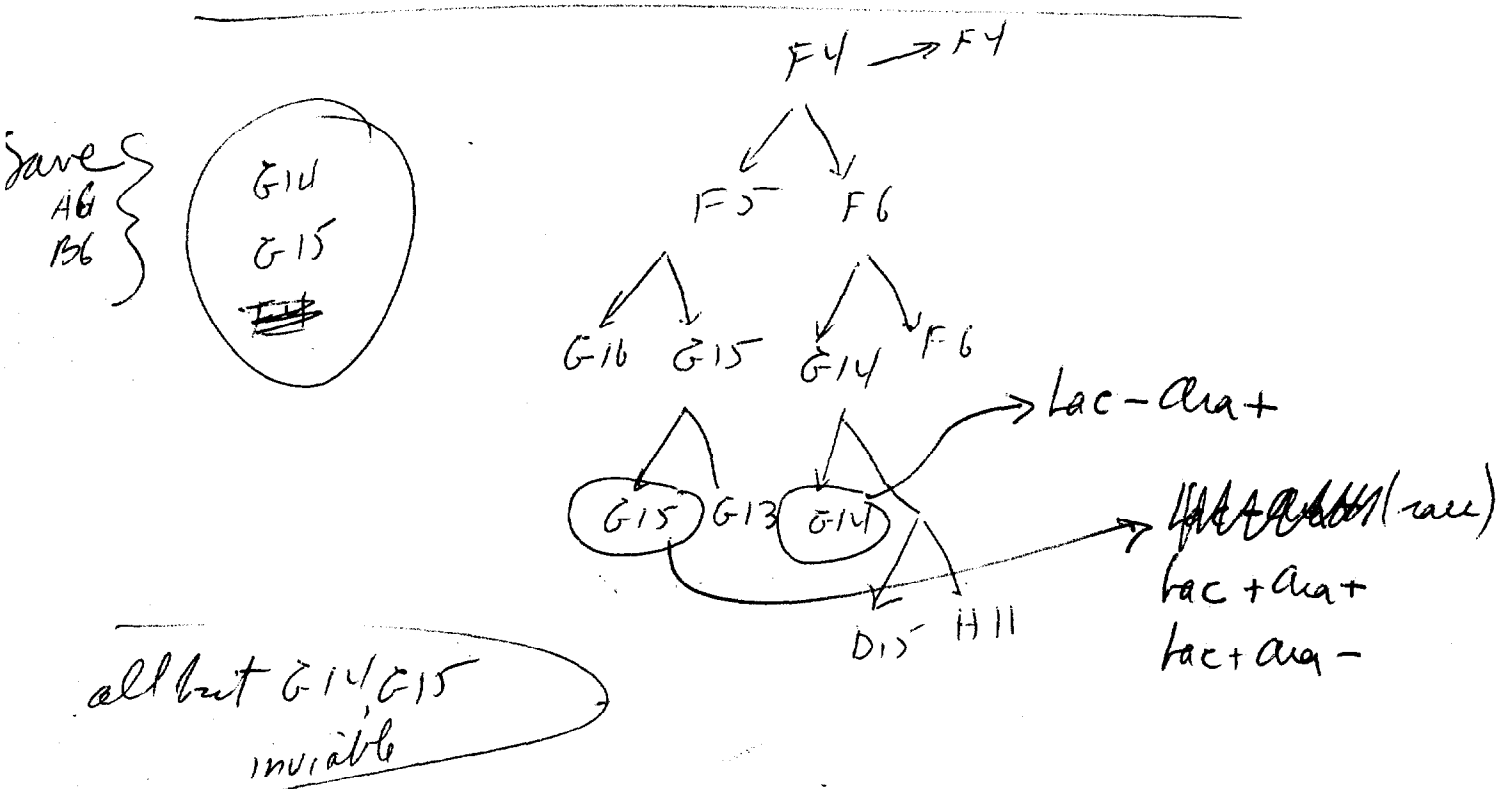
A27 all viable clones from 185, 188, 189.

are parental on Lac, Mal, Xyl, MTL, Ara, Gal sm.

except #19 = Lac+ S^R Ara+ } Gal+ MXM−

#18 = Ara+ Lac−

185-1-2 should be enriched on EMS Gal for purity.



G14: pure Ara+ Gal+ Lac−

G15 Ara+/− "

For future tests, use "MXM" (Mal+ Xyl+ MTL) EMS in preliminary screening.

also

ca 7/14 – 16

Look at Caulobacter.

Distant from Hutner
is contaminated with
interesting motile rod
(s) ② grows better
~~than~~ in pyruvate
than NSB, and OK
at 37°

26-2a FEB 26 1955 Photo
26-2b
27B4 (small plate, range #)
27B1
27B2
27B3

29B2
29B1 FEB 26 1955

28D

Available 28D1.

~~494~~

1192 ctd.

DATE: REF:

G14: pure lac- Gal+ Ara+

G15: pure Gal+. Reported rare lac- among many Lac+.

of 12 Ara+ all Lac+
12 Ara- all Lac+.

at least 1 lac- Ara+ identified. Reexamine for lac- Ara-. V_1 should also be scored: both V_1^R on Lac.

On retrials, G15 was pure Lac+. (Previous lac- colony probably came from G14.) Absence of lac- Ara- is notable but might be in other parts of pedigree, undetected. If segregant is lac- V_1- Ara, implies crossover of V_1-Ara. Ara- should be checked also. Some lac+ did come from late ~~ward~~ restrain! — These are all lac+ V_1^r Ara- are lac+ V_1^s. ∴ Types recovered are

lac+ Ara+ V_1^r lac+ Ara- V_1^s and lac- Ara+ V_1^r as if 3/4 strands from $\frac{+ \; +r}{- \; -s}$ hybrid with c.o. between Lac/Ara. Unfortunate that others were not recovered.

Note A5 and A6 showed mottled appearance on EMB Gal, but DCO failed to find any evidence of segregation.

Logical, if one c.o. is recovered, should other be also?

Luca - J. Indent selection 1193

DATE: P25 10/5/54. REF:

7-36 assays at 125/ml. (wrong: per 0.1 ml)

8- Thinfoe samples of .5 ml will have 62 R.
dilute .2 + ~~6.1 ml per assay~~ + 12.2 ml Per
Distribute .5 ml samples ~~to~~ 10 ml H2O, sal

Found most cultures had mutants — note error in planning above! (average inoculum was 1 mutant per tube.

DCG assayed all tubes (1-10 = ~~H2O~~ sal) (11-20) = water.

8-1 = 169,160 x 10^6 (1.6×10^8)

8-11 = 142,144 x 10^6 (1.43×10^8)

DCG notes 1/4 of colonies sal-!

Streptomycin sensitive

on SM, per .1 ml:

8(1-11)		11-20	
	7		27
	32		31
	15		12
	34		9
	17		27
	3		1
	12		16
	9		0
	11		12
	23		17

presumably variability in lag.

Note adverse selection! Parent cultures had ca 125/.1 ml.

8(2) series: dilute 7-36 by 1:6250, incor. .5 ml into each of 20 tubes of saline. Found 1 tube (8(2)-3) to have cols. by loop assay. But 0.1 ml assays shows only 34, 20 colonies per 0.1 ml.

Remember: 8(1)-3 = 8-C 8(2)-3 = 8-D (Rom)

Is periodic selection coming in?

Recontinued by Luca

Stage 14-5

11/27 Picks 109 singles from EMB-0
also 8 ≠ clusters of 5., to EMBlacI ons.

None of singles, 2 of 5's showed S^R

Restreak these on EMBlac for final conf.

= total of 2/149. A, B.

10 colonies on EMBlac. Pick at random
3/10, 2/10 S^R

(02716)

Parallel platings at last stage showed S^R = Cal−
(if S^R suppresses Su^+_{Cal4}). This isolate has weak if any response to s/4.

Note ≠ Cal + and ± ? together noted as
Cal − − not on second head, probably matter of folding

C1 = S^R Cal−
C2 = S^S Cal?
C3 = repeat isol S^S.

Gal+ reversions of W2716 were noticed to be Gal$\underline{U}$!

---

passed through 20 passages (ca 10^{-3} ml/10 - 10^{-4} max.), i.e. for
1/10/55 about $\log_2 10^{80}$ = about 250 generations, then plated.
DCG examined ca 1000 colonies (5 plates); all were SR on replica. One isolated as W2716-20 for quantitative comparisons.
About 0.1% of colonies at this stage were weak Gal+. Proved stable SR and, as above, unstable +. Save ①.

T2 and crosses

1195 #44

DATE: OCT 27 1954 REF:

Some preliminaries (1,9c) not recorded.

Grow ♂, ♀ overnight.

8:55 add 1ml broth culture to .1ml .05% T2

10:05 – treatment 2: spin down + resuspend

" 3: use stained cells per se

ca 10^{25}

(1)	♀ + ♂ 3	1ml : .5	7ml Penassay	
(2)	♀ + ♂ 2	" "	"	
(3)	♀ 2 + ♂	1ml : .5	"	37°.
(4)	♀ 3 + ♂	" "	"	

Also note .2ml T2 went slightly faster.

addition of 1ml fresh broth at 8:55 delayed (.2ml T2) coloration about 1 hour

♂ reduced T2 > ♀.

Exp. n.g. – label (T2) insufficient for low power determination.

Conclusions – so far, T2 label has not been satisfactory. In growth overnight in T2, much of the label is extracellular. In buffer pools so far, there has generally been just too little label to be workable. Needed: some pulmin. expts on incorporating the label, especially in line 28. This should not be allowed to interfere with pedigree work and cytology

DATE: Nov. 2 1954 REF: 192

11/ Prepare ♂, ♀ TT for pect. study

General conclusion. ① TT label can be introduced in ca 2 hours (in old broth .005% TT)

② It interfers considerably with motility but some pairs may still be obtainable.

P1 prepare labelled cells. Let stand in frig.

#2 Most of TT had settled. Supernatant may contain the most active labelled ♂♂. Take off about ½ and mix with ♀ unlabelled + ca 1 ml broth 10:30 AM.

(also prepare freshly labelled ♀♀ and fists ♂♂

In general, only a few labelled ♀ proved satisfactory.

1196-97

DATE: REF: 192 - 193

Comment: 192

E1♂, E6♀ are ♀ & ♂

C2, C5 are ♂ ♂
F2, F3

193 C5 was given to me as a male unless contaminated. Not examined in detail; ~~and~~ has chromosomes ~~illegible~~ pair. (Examine now)
C5 ♂ OK. But examine for motility of present culture.
F2 - F3 no record!

192 was all right to follow pedigrees & T2 only the following are of interest. Most ♂ died not grow

C1♂ ✓
C1 → ♂ → D3 ♂ ×
→ D4 ♂ ×

D2♂
D2 → F5 ♂ ×
→ E5 ♀
→ E6 → E6 F6 ♀
E6 ♀

C2 → C2♂ ✓
→ D5 inviable ×

no zygote

C3 → C3♂
♂ survived at least 3 generations but no zygotes.

♂ C3 C4 ♂
C3 B4 ♂ ♂
♂ ♀ B5 C4
♀ ♀ × ♂♂♀
♂ A5 ♀

1196-97

DATE: REF:

	1	2	Lac	Gal	Ara	M&M	Scy	8	9	10
1		C1	+	−	+	+				
		D2	+	−	+	+				
		E1a	I	−	+	+				
→	92	F1b	+	+	−	—	R Lac −			
→		E1b	−	+	−	−	R Lac −			
		193A1	+	(+)	+					
		2		error?	+					
		3			+					
		4			+					
10		5			+					
		A6								
		C1								
		C2								
		C4								
		C6								
		E2								
		3								
		4								
		5								
20		6								
		G1								
		2								
		3								
	193	H2		+−			R −+			
25	192	E2	+	[redacted]	+		R −+		[redacted]	
31	192	A2	−	+	−	−	R −			
2		3	−	+	−	−	R −			
3		5	−	+	−	−	R −			
4		B4	−	+	−	−	R −			
35	192	C2	+	−	+	+	S			
36		3	−	+	−	−	R −			
37		4	−	+	−	−	R −			
→ 38	192	C5	+	−	+	+	S			
39		E5	−	+	−	−	R −			
40		E6	−	+	−	−	R −			
41	192	F2	+	−	+	+	S			
2	192	F3	+	−	+	+	S			
3		F4	−	+	−	−	R −			
4		F6	−	+	−	−				
45	193	B1	−	+	−	−				
6		2	−	+	−	−				
7		5	−	+	−	—				
8		6	−	+	−	−				
9		C3	−	+	−	−				
50		C5	−	+	−	−				
51		D3	+	+	−	−	R +−			
2		4	−		−	−	R −			
3		5	−		−	−				
4		6	−		−	−				
55		F2	−		−	−				
6		3	−		−	−				
7		F4	−		+	—	R −			
8		5	−		−	−				
9		6	−		−	−				
60	17	1	−		−	−				
61-62		H4-H5	+− / −		—	—	R+− / R−			

♂♂

all ♂♂ ok

00 → 39 (1)

N.R.

1196-97

DATE: REF:

Conclude: It has no particular value and tends to impair viability as well as motility.

192 E2 needs review! Plated as mixture! Only part may have been picked up!

193. Five random pairs were picked & pedigree analyses.

H2 mixed as recorded! ~~What are H4-H?~~ ~~plot A6~~

Pairs completed are

Total score then is:

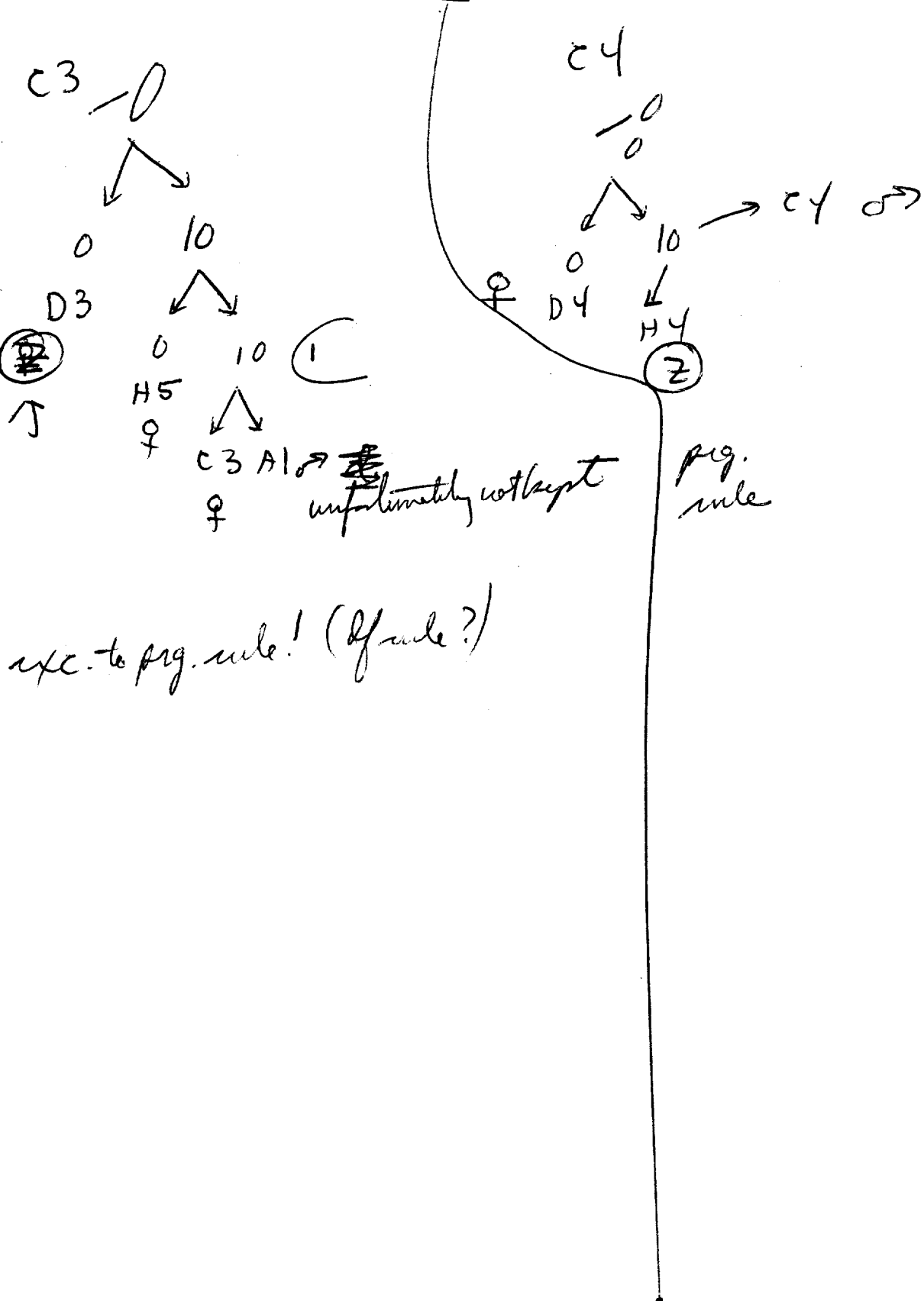
3 (R) from 14 assessable pairs! Why so low? Pedigree analyses have indicated a higher incidence! Maybe based on selection for clonal integrity in the pedigrees!

	♂	♀	♀2		
	A2 ✓	B1 ✓	B2 ✓	1	
A1♂?	A3 ✓	B3 X			
	A4 ✓	B4 X			
	A5 ✓	B5 ✓		2	
	A6 ✓	B6 ✓	H6? ✓	3	
	C1 ✓	D1 X		4	
	C2 ✓	D2 X			
A1	C3 ♀ ✓	D3 (R)	H5	may have F♂	5 4
	C4 ✓	D4 ✓	H4		6
	~~C5~~	C5 ✓			7
	C6 ✓	D5 ✓	D6 ✓		8
	E2 ✓	F2 ✓			9
	E3 ✓	F3 ✓			10
	E4 ✓	F4 (R)			11
E5 ✓	~~E5~~	[illegible]			
	E6 ✓	F5 ✓	F6 ✓		12
	G1 ✓	H1 ✓			13
mixed	G2 ✓	H2 mixed.			
	G3	(R)			14

14 viable pairs
4 inviable ♂ ♂
no pedigree

owing to temporary mistaking of one petrol plate, not all pedigrees were clearly started and some subs thrown out. See over.

A1-C3-D3-H5
C4-D4-H4
E4 - F4



DATE: 11/7/54. REF:

Productive pairs were

C3 - D3 — {large → C3 (pres. Fate of male ?)
                 10 OD3

E4 - F4 10, small 50 / E4 OF4.

G3 - H4 10 : (11) G-3 OH4

There is no ambiguity in the history of these, though it is unclear why H4 should have been chosen for G3, unless prompted. Note inversal of pairing relationships in C3.

(Zygotes were ~~prepared~~ carried rather late)

Recombinants in ♂♂ recognizants would be difficult to detect. Should routinely restreak ♂♂ on EMB Lac.

Save ♀♀ above and ♂♂ for recheck

C3 ♂ ♀ D3 Lac+/- MXM - Arg - SR. Score V, R / S { V, S / V, S

E4 ♂ F4 Arg+/- Lac - MXM - SR ♀ V, S ♂ V, R

G3 ♂ H4 Lac+/- MXM - Arg - SR. ♀ V, S ♂ V, R

Rec. not of very great interest.

192 E2 - mixed! But no record of separation of clones.

More pairs, simple.

1197

DATE: Nov. 4, 1954 REF: [193]

1	2	3	4	5	6	7	8	9	10

J: 10 ♂: 9 cg 12[45] - 12[15] setup. Cg. 24 pairs isolated and allowed to separate. Minimum of prodigine analysis except in re intra-current fission during conjugation. Pick misses with [192] for tests.
:7 ml

pairs isolated as.

	17	→ sur.	♀ sur.	Zyg.	types
11/5[4]	10	✓	✓	no	
	0	×	✓	no	
	3	✓	✓	✓	
	4	✓	×	—	

Filter indicator comparisons 1198

DATE: Nov 8, 1954 REF: Jacob Lettvin, MD & CRA Catalog

of 1079

24 hour cultures as mounted 1 ml q : .2 ml ⇒ : 7 ml Penassay

Inc. 37°8²⁰ — 10¹⁰ PM.

Plate ca 10^3 / EMB Blacom.

A W1603 (W1177 Lp^s)

B W1177

C W1895 M1 ($O_2^?$)

D W2344 M1 ($O^?$)

E W2401 (φ)

F W2578 (W1607 Lp_2).

Est SR+ / SR−

AC	54/1000
AD	37/1000
BC	22/300
BD	28/300
EC	10/300
ED	8/300
FC	too variable to score +
FD	

Note no marked diffusion inefficiency of recombinants cf. Lp^+, Lp^s Hfrs!

Try Hayes' Hfr:

A10 Repeat with W2~~30~~ ~~al~~ (= G) old cultures

1:1:7 ml 11¹⁵ AM — 12 ~~6~~ PM.

Refrigerate to 3 PM.

		SR+ / R−	Plaques/5T8
1603 A	C 1895	AC 17/300	ca 50
1177 B	D $O^?$	AD 14/300	
W01 40 E	G 2344	AG 3/300	ca 20
		BC 15/200	+++ (10^2-10^3)
		BD 14/150	
		BG 7/200	ca 100.
		EC 10/300	
		ED 6/300	
		EG 0/300+ ←	

Results: measurement of W2344 M1 and λ^s × W2401

Note $Lp^+ \times Lp^+$ gave more λ than $Lp^+ \times Lp^s$. In all combinations W1895 was more potent than W

also plate AC and AG, BC, BG on λ^s indicator. (2401)

(over)

Repeat P15 use old cultures as
mostly 1:1; 7 ml
2½ hours.
streak out E143 bacteria. Score SR+

W2324/Motility	× W1177	0
"	× ~~W2344~~ W2401	0
W2324	× W1177	++ (>1%)
"	× W2401	++ also note plaquing!
W2344	× W2401	++

---

again P17 1:1:7
(A) old cultures 7:30 - 9PM
(B) fresh cultures (from above) 9PM - 10PM

ca 1% in all B. In A, ~~W~~ W2324 ♂ × ♀ was ca. 1/10 % SR+
but × W1177 and ♂ × ♀ gave ca 1%. ∴ ? W2324 is more affected by aging than is W2344.

(C) A18. overnight cultures 1:1:7 9:30 - 11:30

♂ × ♀	ca 1%
♂ × 1177	>1%
W2324 × ♀	>1%
W2324 × 1177	+, <1% Some plaquing again. What is this phage? Which auto on W2401?

Then why no zygotes from pairs?

Conclusion: W2324 may be slightly less sterile than W2344. No clear evidence of erotic induction. Should use Jacob's conditions, count in/c.

Ecolec induction: preliminaries 1198

DATE: 11/19/54. REF:

	1	2	3	4	5	6	7	8	9	10
	Motilize W2324 for crossing purposes — 1 passage.									
	~~too~~ Cross culture was almost sterile									
	S.C. gave no SR+ × W1177. Candida probable F — (W2696)									
	P20	W2693	× ♀		SR+: 1 papillomby in third x-cross!			Reserve for fertility!		
10		W2324	♀		ca 0.2%.					
1 hour		"	W1177		2%					
1:1:7		2344M1	♀		2%					
20										
30										
40										
50										

W2324 (Hayes Hfr) × W2401
single cells

1199

DATE: Nov 11 1924

REF: [194]

cross ① 9:40 - 10:30 then same time at K T
② 11:15 - 11:35.

A1. Unseparated pair → ♀♀ only.

~~A1~~ - 6 ✓
~~A2~~ - 2 ✓
B3 - A3 - B4
C1 - C2 ~~C4~~ C5
C3 - C4
D1 - D2 ✓
D3 - D4 ✓
~~E1~~ - E2
~~E3~~ E4
~~F1~~ - F2
F3 - F4 ✓
G1 - G2 ✓
G3 - ~~G4~~
H1 - H2 - ~~H5~~
H3 - G4 - H4 - H6

Complete pairs: 9
Pro (♀ OK) :

no zygotes

No lysis seen!
Numerous pairs despite indefinite motility of W2324. Cells of latter are shorter than W2324, & harder to distinguish from W2401.

---

all parental are lac± sm, 14 x M. (Gal+).
~~exp~~ except B3 which is lac+/- (lac-SK) pairs mixed.
lac- : A1, A6, B2, B6, C1, C2 C4, D2, D4, E2, E4, F2, F4 G2, H4, H6.

lac+ : D3, F1, A3, B4, B5 C3, C5, D1, E3, G1, G3, G4, H1, H2, H3.
Non-motiles in F1? (mic. segg)

presumed pair ↓ mixed (non c chain)
B3 - A3 - B4
Save F1, C1-C2-C5, D1-D2 as exconjugates.

slight cross ♂ × ♀
W1895M1
♂

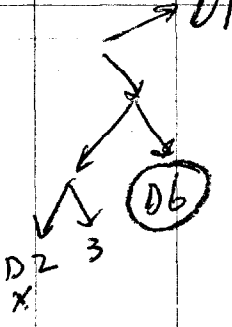
1200

DATE: Nov 13, 1954

REF: [191]

gave

D1 -0→ (1) D1, 0 0 0 / 2 3 6 Presume.



all fluid Cal+ Lac+/- V, S

(1) 0E2 0E6 -0→ E1. 0 → 0E3 0 (A3)

E1 -0→

A3
E3
3
E6

S^R Lac+/-

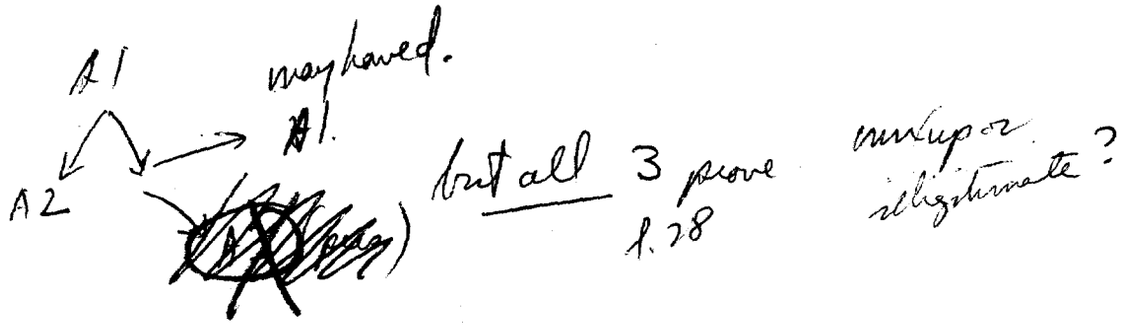
parental ♂T, ♀ resp except A3 Lac+/- and D6 Lac+/- B4 4.9.

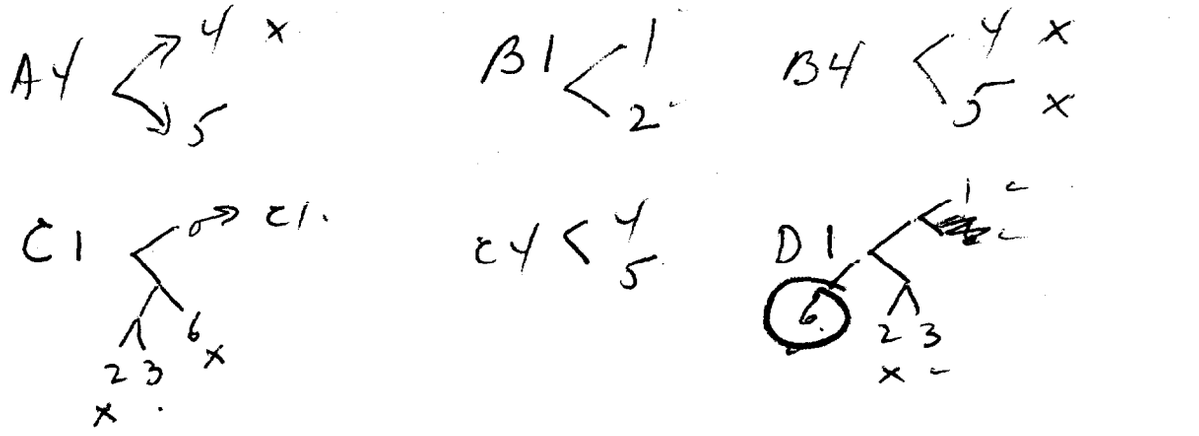
all parental on M×M Arat~~ck~~

Presumed to have been W2344M1 × W2401. However, all ♂♂ were Cal+ and at least D1, E1 were T1^S. . . must have been W1895M1 visited, ~~&~~ which had been set up concurrently! Confirm on ~~the~~ D10).

Yielded 1/9 c. } 2/11 zygotes
1/2 Inc }

195





♀♀ complete: A5, B2, C5, (E 2,3,6) E5, (F5,6), G1, H4, (A3) (G5,6,E6) : (9)

♀ partial: (C3), (D2(3)6) : (2)

∴ 2/11 zygotes. save E1-2-3-6-A3 and D1-3-6

F x ♀ 1201

DATE: 11/18/54. REF: 196

W2206141 Re-isolated and checked by EML. Mass culture is F+. Use overnight inoculum 1:2 both 9:50 – 11:25 Then 2 ml W2401 : 0.1 ml W2206M1 : 7 ml Remeasure 37° 11:25 AM.

A. Streak out at time of setup (11:50 AM) } Single colonies from EMB streak
B. " " " " Conclusion (12:52 AM) } and pools of 10
C. Single cell clones (v.i.) 1-24 ♂♀ 31-41 ♂♀.

♂.♀

	C		C
A1-2-3	(1)-2	E1-2-3	37-14-(15)
A4-5-6 sip	3	E4-5-6-D6	38--16-(13)
B 1-2	(4)-(4)	F1-2-Φ	17-(18)
C1-2-3	7-(8)	F4-5-6	20-21-39
C4-5-6	34--9	G1-2-3	
D1-2-3	35-(10)-(11)	G4-5-6	40-(22)-23
D4-5-F3	36-12-19	H1	
B 4-5-6	5-6-33	H4-5	41-(24)

Then to EML. cultures (1-), 2401

0 = F+ by EML test
– = –

Note Babroe on EMB streak
No SR+

ca Jan 10, responded coli isolations to cope
with Salmonella hangovers in re
B.A.O.S. draft mss.

Summary - Exps. i TC N diplords. 1202 5

DATE: Jan. 4, 1955. REF:

1	2	3	4	5	6	7	8	9	10

(1) MLM tested sergeants d gave hims from IV-2 and VII-94 by preparing lysates. Also gave hims 3-144, but these sera as not being full Cal - (modifie!)

JAN 16 1955

20

30

40

50

Genera TCN diploids

1202
1

DATE: 1/20/54. REF:

(1)

3-22 (W2406 × W583) gives as Gal^v Mal− S^S Xyl^v (Ara^v) lac^v

To check Ara^v and if so, confirm Gal quality (see over)

res. ara- immediately + from pigtail

For Gal + 4367

7-294 (2406 × 945) Gal$_2$ + Mal+ Ara^v Xyl−/+ Mtl+ lac^v

3-148 Gal$_7$+ Mal+ Xyl^v Lac^v Mtl+$^{v?}$ send to Clark!

4-2 Gal$_7$ = Xyl− Lac^v Gal− (to confirm Gal character).

Started

4-76 Gal$_7$ = Xyl^v Lac^v mal− Gal−

7B-94 Gal$_7$ ^{v} lac^v Mal− Xyl−

H346 Lac^v Mal S^v (Gal etc +) maybe Hfr^v ? G+S^S autonit or segregants.

(W1895 × W1590)

H351-5 Lac^v Gal$_1$ + (Hfr?) Mal, S$^{+/S}$ Ofr − 1470 M-Lac, SR^v, Hfr

	lac	Mal	S	
1	✓	✓	S	Gal+ ✓ and streaks also → Preliminary trial × W2401, no SR+ but culture was largely segregated.
2	✓	−	S	Gal+ ✓
3	✓	+	R	Gal+ ✓
4 (mostly)	✓	−	S	Gal+
5	✓	+	R ?	Gal+ ✓

Hence: interesting diploids in D(Lac) [initiate roller for 2 generations!]

∴ H351-5 check out OK. Next to be tested as possible Hfr. Most auxotrophic segregants should be OK. Retrieve single colonies. Note: SR's maybe more encouraging as parent type!

Hfr 1, ✓

note again correlation of Gal$_7^{+}_{+}$ / Mal+ !

3-22 Repeat LacV are pure Arg− !

Y/papillae seen on original streak — mucoid, non-segr. probably contam. Check on Mal, : Arg+ prove Mal+ mucoid

Arg− Mal−

3-72 is Arg−

H351. May be either Hfr/F− or Hfr/− or F−/−.

① test segregants − mud auxotrophs.

② test diploid itself. It is LacV Mal− S^R prototroph.

a) If S^R/S are formed, these are phenotypically S^S. N.G.

b) Try for Mal+ prototroph × Mal+ auxotroph!

DATE: REF:

H351-5 repeat

11/24 (1) Mal⁻s are revertible, occ. papillae in thick streak.

H351X W2401 papillae on thick streak (EMBlac on) and ca. 1% SR+ /
H352, X } was largely segregated at time of plating
10 H354X } no SR+ also 4 plaques. Streak on Lac A.

(2) H346. EMBlac mostly ⊙, some ● and O
on EMBlac on, mostly O, a few ⊙ (maybe autonits, prototrophic). Spot some from each on EMB to look for S^s or X⁻
20 deplords.

(3) Replate both cultures of H351, H353, H355 for segregants.

30

11/26. Notes: 3-11 seems Lac+, Rev possible Lac⁻.
replate 3-14 seems Lac+/slow " " .

(microscopically)
H352 (EML)(1×23) - lac⁻ segregates mostly motile lac⁺ apparently
40 non-motile with occasional exceptional cells (ca 10^{-6}). On motility agar 4 Lac⁺ and 1 lac⁻ each gave clusters or tracks, then isolated swarms. Reisolates are pure as inoc. except 1 stab Lac⁺ now pure⁻. (Probably uplift polygenic control of motility). Line-1 parent may have been poorly motile to start also.

50

DATE: REF: 11/26/54

H351 - segregants: almost all prototrophs by replicator. Pool auxotrophs for purif + crossing tests.

also check possible SR or auxotrophic diploid & haploid from H352, H355. same stuff in works

H355 2 Lac- 6 Lac+ ξ all Mal- SR. Pool +, - for crossing tests.
1202B1 1202B2

H346/EMB lac sm: Some Lac ⊙ sm purified + tested.

Disregard prototrophs; leaves 5 Mal- auxotrophic. Restreak to recover Lac+, if possible. (mid rather to test Mal+ S^s segregants ~~for Lac~~ Hfr!)

11/29. (1) Now available Lac+/- auxotrophs from ~~H352~~ H352+5

(2) H346 SR auxotrophic Mal- dipl (2 isolates = 1202C1-2)

(3) 3-M4 has some modified Gal- ; 3-~~M4~~ 11 is pure Gal+ !

(4)

Replate H351 and check for crossability

H346 plating: Pick Mal+ and - to Lac.

11/30- 8 Mal-: 7 Lac- 1 Lac+/- (segregating ? sybil) } prototrophy.
16 Mal+: 6 Lac+
10 Lac-

1202
Obd. 4

DATE: 4/28/54. REF:

1 2 3 4 5 6 7 8 9 10

Segregant crosses

H355 : Lac-, + = B1 - B2 resp. } x W2401

H352: B3 - B4

and H351 x

(all crosses run on Sm indicator plates + W2401 or W1177) in 2 ml runs. 2 TU per ml.

12/7/54. Recap. (Not all protocols recorded).

H351 x W1177 } → ca < 1% SK+. ∴ This is or carries Hfr by mixed donor.
x W2401 }
control N.G.

(should also check for F+)

(H351 = Lac+ Gal- Sm^R ...)

(Reisolate W1177 colonies 1202D from SM plate, restreak on EMB/Gal for purity + ✓).

and continue to look for segregants.

♂ x ♀

1203

DATE: 11/20/14

REF: 197

Cross 1:1:7 $1^{10} - 2^{20}$ Then selfs.

all pairs to except #3 (Loc⁻ S^R ...); all MXM - dies - or ♂↗

∴ Save

likely cross somewhat hetty manip. interactions

Keep probes for other pairs.

S.S. A4 10 ↗ 1 :: A4 (42)

0 A5 (3)

0 A6 (4)

3: Loc⁻ S^R Ala - MXM - Gal - (partly not checked)

4: Loc - ...

42: Loc + Gal - ...

Viability very high!

♀ complete : 12 only 1 zygote

♀ incomplete : 3

15

Yields:

A1 sap

B1

C1

D1

E1

F1 sap

G1

H1

A4

B4

C4

D4

E4

F4 sap

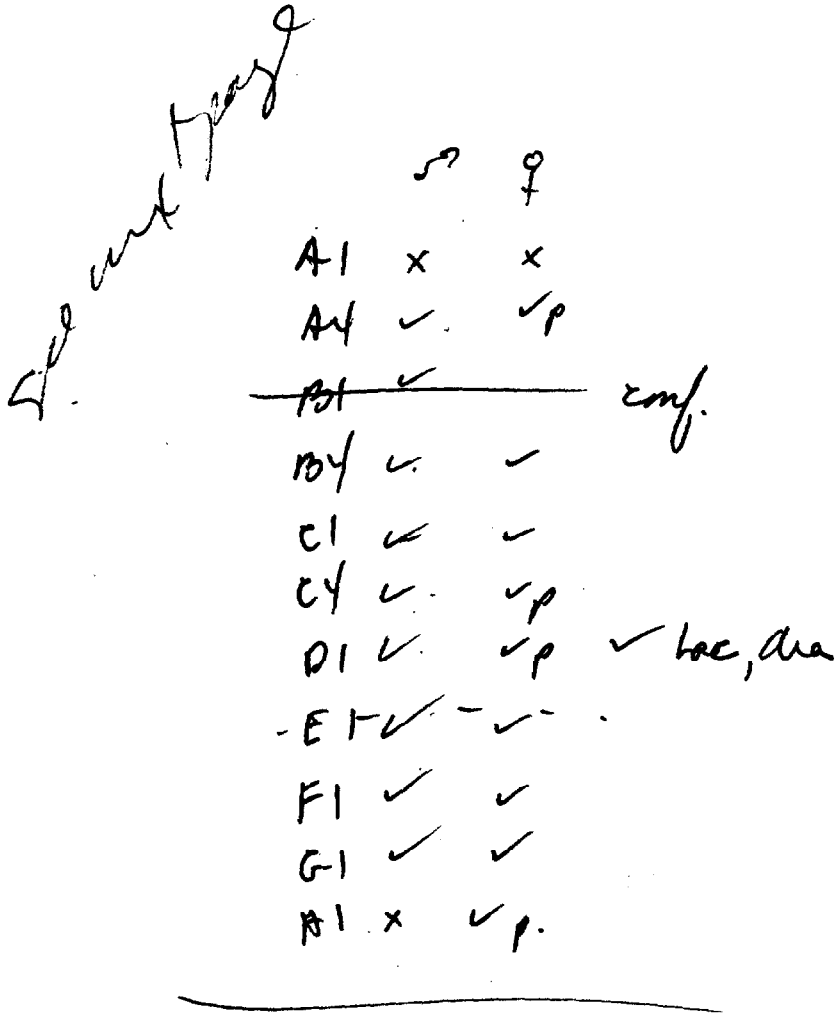
G4 sap

H4 leg?

manip. strongly

D

(See comment 1806



✓✓ 5
✓✓p 4
9
x ✓ 1
x x 1
11

11630 × C3 (♀ × ♂)

Casual.

1204

DATE: Dec. 2, 1954 REF: 202

all parental or backcross, Cal, MXM except D3(18) = Cra + MXM — backcross (B2 org-grein as. is ♂? combos. Includes mutility; not — no record of separation). all Cal+ checked for purity.

D3: — ♀ → (11) 0 0
D1 D2 D3
♂ X (2) ♀

Pedigree unfortunately incomplete.

(also demonstrates for Dolly handling & procedure generally, rather hasty & desultory Care. manipul. to separate in time.

check all ♀♀ for presence of Cal —.

Yield:

A1 sib

A4 sib

B1 ? (♀)

B4

C1

C4.

D1

E1

F1

G1

H1

♀ complete: ~~B5~~ B4 C1 E1 F1 G1 } 9 / ~~♂~~ 9

♀ incomplete: A4 C4 D1 H1

Invisible ♀♀: A1

Lower yield! again. May likely be due to haste in manipulation in sequences of last several crosses. Compare pedigrees!

Thesis 120 ✗ for protocols

1~~2~~ - summary Flue may be meant. As I

read protocols (focussing on persistent pairs)

	♀ →			
A1	x	x	complete < ♀	3
			♀ ↛	3
				6
C1	✓	✓		
C4	x?	✓	no ♀	~~4~~ 2
A4	✓p	✓		
B1	x?	✓	no ♂ or ♀	1
				~~10~~ 9
B4	✓	✓		
D1	✓p	✓		
E1	missed rest seq.			
F1	✓	✓		
G1	✓p	✓		
H1	✓	x		

∴ ~~2~~ =

compare pairs.

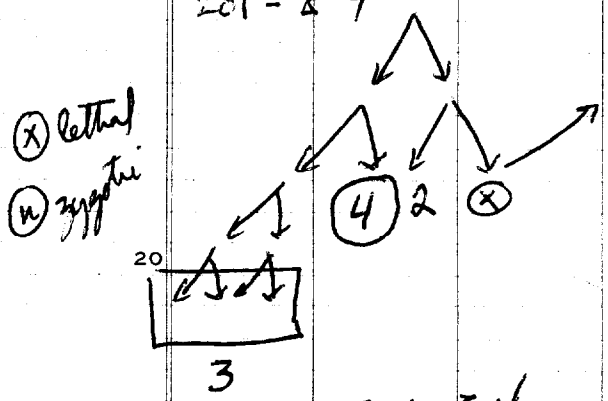
DATE: Dec 6, 1954 REF: /201-203/

This pair used in recent experiments as 1) pretested fertile sibs and 2) for possible selection of potentiality of Gal paratypes.

Protocols do ~~not~~ give timing of cultures, probably mixed early, about 10:15 AM by DCG during class. Isolations were begun at 11:10 AM, therefore quite fresh. Cultures also presumably overnight. Isolations completed 12N.

Yields low (0/3 complete; 2/6 incomplete) (3 illegitimate!) 2/9 total. See protocols for details. Successful (productive pairs) saved as:

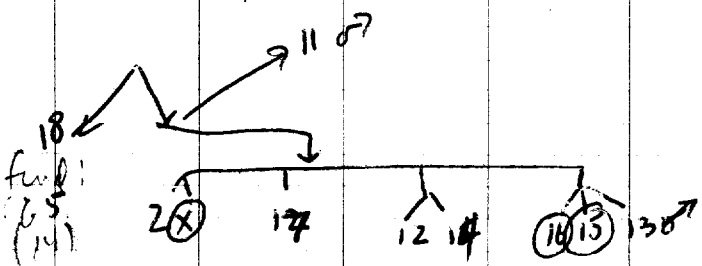
201-E4



all cultures parental
Lac +sm, Gal, M, X, M, Ara

(4) = Lac+/- Ara+/- V, R

201-C4



(15) = Lac+/- Ara- S

(16) appears mixture of ♂'s; not technically accounted for but this is a poor pedigree (R+/S-) unless otherwise confirmed

201-16 is a puzzle. Not fully analyzed but contains motile and non motile elements; Lac+/-; Gal+/- and seems surely a mixture. It is impossible to be certain whether this is an artifact; until a pedigree can be obtained from such an individual, it must be left in doubt, even though there have been no reasons for it.

Neg, V, : streaked out cultures but not analyzed in detail for the present.

1205

~~16 put. initials~~ 3 ill → 13

~~♀ sec wi ♀~~

	♂→♀	
A1	✓	✓p
A4	x	✓
B1	✓	✓
~~B4~~	ill.	
C1	✓	✓p
C4	✓	✓
E4	✓	✓ ✓ big+
D1	✓	✓
~~D4~~	ng.	
E1	✓	✓p
E4	✓	✓
~~F1~~	~~ill~~	
~~G1~~	~~λ not pair~~	
G4	✗	✓ (R)
H1	✓	x
H4	✓	x

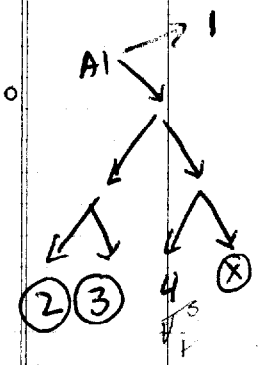
12 total pairs coll.

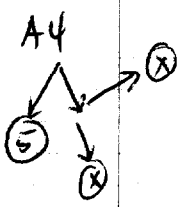
♀+♂→:	8	1(R)
♀ no ♂→	2	1(R)
♂ no ♀→	2	
	12	

DATE: Dec. 7, 1954 REF: [illegible]-204+205

Note, use old suspensions to start cross (cf. 1205, day before)
No record of timing, presume around 90 minutes. Note very favorable yields here.
Yields (3/5 complete; 4/7 inc) 7/12 total! See protocols for full details.
Coverglasses of 1206 and 1205 were held over for isolations and scoring together.

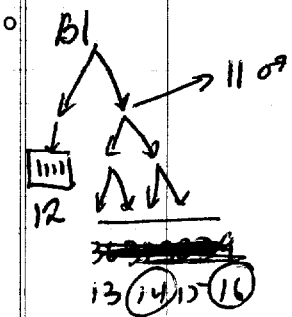
Productive pedigrees, as saved:

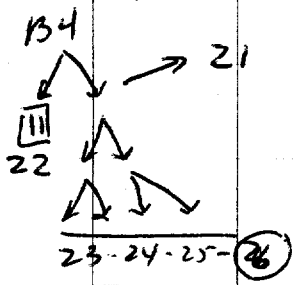




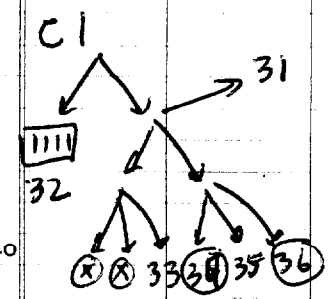
all parental lac+ses Mal, Xyl, MH Gal ara except:

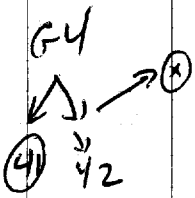
(2) = Lac+/- Ara+/- R
(3) = Lac+/- Ara- S
(5) = Lac+/- Ara- S





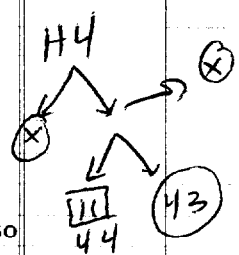
(14) = Lac+/- Ara+ R
(16) = Lac+/- Ara+ R
(26) = Lac+/- Ara- S





(34) = Lac+/- Ara+ R
(36) = Lac+/- Ara+ R
(41) = Lac+/- Ara- S
(43) = Lac+/- Ara- R/S

V1 - Ara [illegible] affirmed. But full analysis held off.



interrupted since NRC-100 meeting and reports, theses, etc. Resumed work ca 12/23.

♂ × ♀ 56 wells. 1207

Dec. 21, 1954 - 206
Dec 22, 1954 - 207

(206), (207)

2/. 1206. Use suspensions incubated P19.

	Loc	MXM	stl	Ara	Gal	SM		Loc	MXM	stl	Ara	Gal	SM		
1	—	—	—	—	+	R-		+	—	+	—	+	R+		A1
2	+					R+	A2	+		?	—		R+-		D1
3	—							+			—		R+ -		F1
4	—							—			—		R-		
5	—							—			—		R		
6	—							—			+		R		A2
7	—							—			—		R		
8	—							—			—				
9	+			+		R+	C3	—			—				
10	—							—			—				
11	—							—			—				
12	—							—			—				
13	—							—			—				
14	—							—			—				
15	—							+			—		R+ -		C4
16	—							—			—		R-		
17	+					R+	D5	±			—		R+		A5
18	—							+			—		R+		B5
19	—							+			—		R+		C5
20	—							+			—		R+		A6
21	+					R+	A6	+			—		R+		B6
22	—														
23	—														
24	—	+				R-+	D6								
25	—														
26	—														

31-42 ♂♂♀	±	+	+	+	—	S	31-43	±	+	±	+	—	S	

3-4 mg kanamycin

Reichle - 1 Lac, 1 Gal⁻ 24. V. Ara. (4%) Gave as MA.

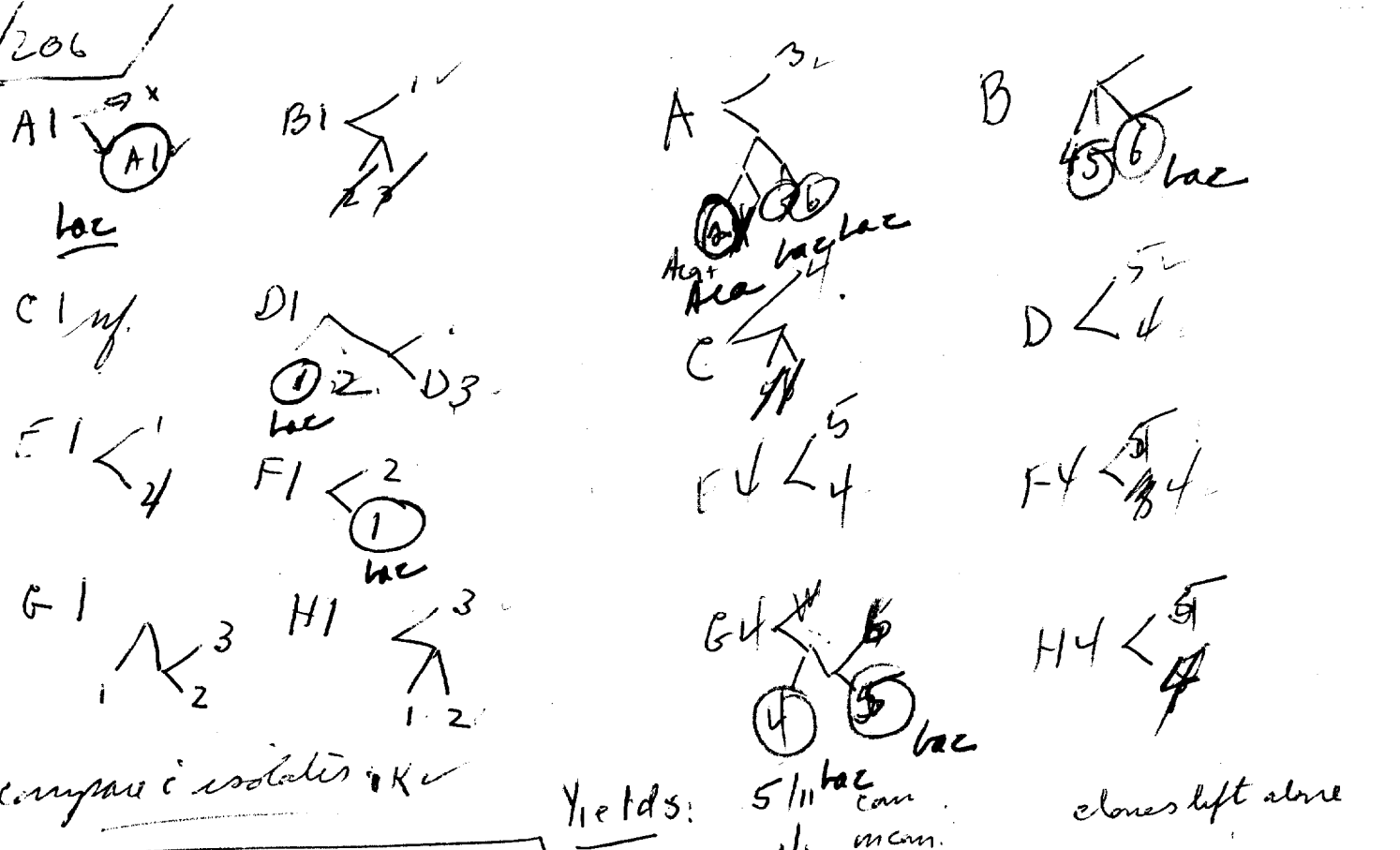
34 mg streptomycin (8).

Yields 5/11 ; 5/11.

reichle subtol otherwise - +

su 1209

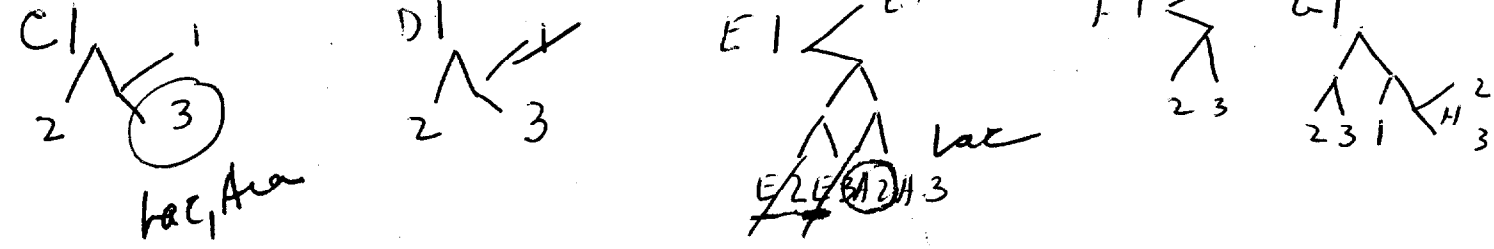
206



compare isolation Ke

207

D1



E1, G4 1/4 each.

DATE: Interesting projects REF:

206

A4 0 → 0 05 Lact 06 Lact 24 hrs ~3/4!

B4 0 → 12 cells. 1 loose B4 ♀

0 ⇒ B6 (n=3) ♂

100. B5 (n=3,4) ♂

partly emulated ~2/8.

D1 5 → ~160. D1 twins D2 D3 0 ♀ sic

unemulated

G4 0 → 160. 150 4 → 5 → 60 FRACTION UNKNOWN ~2/16

partly emulated

207

E1 10 → E2 3 A2 A3

1/4.

G4. sib. < 0 D6 C6 0 C5

1/4

note C1 ♀ ②

In practice, cannot rely on prolonged emulation of joined pairs ∴ fatally subclone

Transmission of fragments.

Hfr (Val+ × –) × F – (Val–)
(W2730)

Dec. 21 ff. 1914

Objective: test for transmission of fragments

EML Val+( ) × W2344M1 (for Val⁺ fragm/Val₂⁻.)
p.800:1-7 Tsolates.

Plan: a) SR+ crossing tests b) progeny analysis of
(Val+ × ♂) × W2405 (Val4⁻).
+/Val₂⁻

A. On 1st pul um, 800-1 × W2405 (24-48 h. cultures) (10:1 ratio ca 3 hours incubation necessary)

12/21. cross
EMB Val, Lac + >> –
lac sm. No + in 18 hours. Probable slow + thereafter.
after 36 h. some probable Lac+

Point: 800-1 is mostly Val+ or Val⁰, ca 15% –.
.. presumably still Hfr. (an Aur). No lac++ or Val+ SR
implies no transmission of fragment. → of probably Lac+ "SR, {15 Aur+ / 13 Aur –}

b 12/23 as above, all 7 isolates, streak × on Calsm. tc screen.
× 4⁰⁵–5⁴⁵ PM
pul – all Val SR.
12/25 – dilute. #1 on lac sm again shows SR work +.
Conclude: at least #1 is certainly Hfr but is not transmitting the Val fragment with appreciable frequency. Should be repeated under conditions selecting Val+ prototrophs. Label 800-1 as W-2730

c). 4 papillae in thick streak of #1 above. (sm)

D. Should check Val character of recombinants above, but probably not best suited material if Hfr Lp_2^{R+}

c)

4 papillae streaked out, each gave Gal+ dn repurification, 3 apparently segregating occasional (1-3)

Gal−, ≠ 4 pure Gal+. None were Lac+ (nor were crude papillae) and ∴ unlikely to be related to recombinators. Might be direct transductants from sp. lysis of W2730 parent. Review of possible complications, abandon expt. (Save for test on Mal− Mal, Arg. (stab?). Might still be one c prototroph selection) and Lp^s ~~resist~~ F$^-$

Transmission of allotype fragment
Hfr × F⁻ prototrophs.

1208

DATE: 12/29/54 REF:

Design

Isolate Gal+ prototrophs and, if segregating, determine whether they are 2⁻ or 4⁻.

This will reveal conclation of segment when fragment is selected. Since the cross is so strongly orthotypic, recovery of 2⁻ segregants will indicate close association.

E, F W2405⁻ × W2730, W1895. (latter as control for parotype incidence). 1:1:7 ca 10AM – 5PM. Then plate 10^{-3} ml, 10^{-4}, 10^{-5} resp. on EMS Gal

A2: Fertility was very low – ca 1-2 prototr at 10^{-3}! probably low recombination of $M_1^- \times M_2^- H^-$.

A3 Use old susp. for mixing, recross as above 9:30 – 5:10 PM
Wash, plate 0.1 ml samples on S Gal.

A5-6. Count E: 8 plates 12+/722 F: 3 plates:
(about ½ + are sectored) 3+/204 (41, 75, 88)

↪ (81, 73, 112, 95, 99, 89, 108, 75⁻)

E1 both Gal V
E2 Lac+

F1, F2 } pure Gal+

test segregants

	Gal	Lac
3	+ − V?	+ − ✓
6	+ − +(−)	+ −
7	+ −	−
8	+ −	−
9	+ −	−
10	+ −	−
11	+ −	−
12	+ −	−
4	N.G.	

Redrich 4 cols: pure ++ 3 + , +

Lac−

.. all pure + when purified.
5 maybe some Gal +

Tests on segregants.

1208

DATE: REF:

		Hfr: +	1⁻	2⁻	4⁻	
E1	1	+	+	+	−	diagnosis
	2	+	+	+	−	all Gal 4⁻ (ottie)
	3	+	+	+	−	
E2	1	+	+	−	+	shouldn't test for Hfr!
	2	+	+	−	+	
	3	+	+	−	+	all Gal 2⁻ (para)
	4	+	+	−	+	
	10	+	+	−	+	
	6	+	+	−	+	
leaky	3	+		−	+	2⁻
	5	+		−	+	2⁻
	6	+		−	+	2⁻
	2p	+		−	+	2⁻
	8	+		−	+	2⁻
	9	+		−	+	2⁻
	10	+		+	−	4⁻
	11	+		+	−	4⁻
	12	+		+	−	4⁻

(EML finds in a comparable situation)

2⁻	4⁻	?
10	III	I

; when fragment appears it is usually associated with parental locus (not always), i.e., fragment is "linked" to locus.

2⁻ 4 4⁻

should cross + Lp's to examine Lp situation of the fragment.

~~know~~ selected

It remains possible that some 4⁻ are transductants.

Stl - of W2401.

1209

DATE: ~~Nov.~~ Dec. 28, 1954. REF: see 1207

also prob. temp-sens.

In tests for Stl reaction (see p. 1207) exconjugant clones varied. On one plate, only 2 clones were negative, remaining 4 being variable.

P27 ♂ strain ♂, ♀, ~~Hfr~~ 206-11 and 206-12 on EMB Stl.

P28 ↙: ♂ is weak + ♀ is − (5) & occasional + or V (6) colonies.

206-12 appears to be − (1)

206-11 is mostly − (2), has ± (3) and + (4) colonies!

B. P28 Strain as indicated ♂ or ♀, ~~Hfr~~ F'lac.

P29 all Lac+. Though 1 and 5 are weaker.

On Stl 1 and 2 are pure −

3 is −, + (still segregating?)

4 is +, − (" " ?)

5 is pure − (some spotting in thick portion)

6 is +, − may still be segregating.

Held over weekend for further study: incl. small broths also.

~~A2~~ F

On replating from colonies, read by REW

#3 scaled as − #4 as +, − #6 as + and −.

P3 Conclusion is that W2401 is a mixture of unstable + and more stable −. Since stock culture is predominantly Stl − while most clones from pairs in that experiment were V three may be some selective difference with reanalyzing. For further analysis, replate B5 and B6 broths; discard the others.

DATE: Jan 4, 1955 = C1 = W2401 pur. stl⁻ REF:

P4: B5 now seems pure. save stock and replate (1? colony on plate)

C)

B6. 90+ % stl+ " , remainder —.

Replate these = C2 = C3 = stl⁻ "segregant"

= stlv ✓

D1

Selectivity? Streak out W2401 parent of cross this date, and also the cross on EMB lactose, stl. for later comparison of cross with D2 parent. D1 is ca 0.1% + D2 is mixed.

On testing SR+, 0/14 were stl+, ∴ no evidence of selectivity. How account for [206]? Note [206] spread all-, while [207] was mostly +. Without further data, must assume that ♀ parent of [206] had simply accumulated high incidence of stlv type. But why would this not reappear in [207] which was same susp. the next day?

A11 On replating 9C1 shows ca .1% +. Repeat, to test stability of C4 ✓ shows stlv on two restreakings.

Conclusion: W2401 carries an stl⁻ —ca 1/1000→ stlv —ca 20%→ stl-

♂ × ♀

12 10

DATE: Jan 4 1955 REF 208 - 218

36 hour mor. 10:1; ~~#8~~ 7 10^{44} — ca 12-12^{30} total.

OCC pilus/clonear

Summary of results: (Tested on Lac±sm, Gal, MXM, ara, std).

208: 21-31 are ♂, but 22, 31 probably mixed with ♀ (S^R Gal+...)

1-13 all ♀ except: #7 Lac+ (S^R) #2 MXM+,... Lac−

218; 1-20, 3♀ all ♀ exc: 3, 10, 20 Lac+... +/−

: 21 ♂

Std reactions punctal (i.e., ♀♀ are ~papillate) but 208-1, 12 and 218-19 are more strongly +! cf. with W2401.

Interesting pedigrees thereof:

D4 — O → O O (1 1) D4 (28) ♂

♀ 1-4 — 5, 10, X, 18

♂ 1-4 — 6, 11, 15, 19

) = 208−

— = 218

C1 — O → O O → C1 (22) ♂ + ♀ ?

C2 (2) C1 (3) C2 C3 X X

Lac− MXM+ ♀ ... Lac+

save for later identity checks.

pure Lac+....

Note only 1/8 but g^3 might have made 2.

No large segregating.

Yields:

D1 × O → (111) O X

(1111) D2 (2,7) — d6 ♂ 21 — d5 20 — O3 (7) — d2 8 — d3 14 — d4 17 — d1 3 — Lac+, −

Lac+, − ← Lac+...

pedigree reconstructed. pure+ not ± 3/8 and not yet purified.

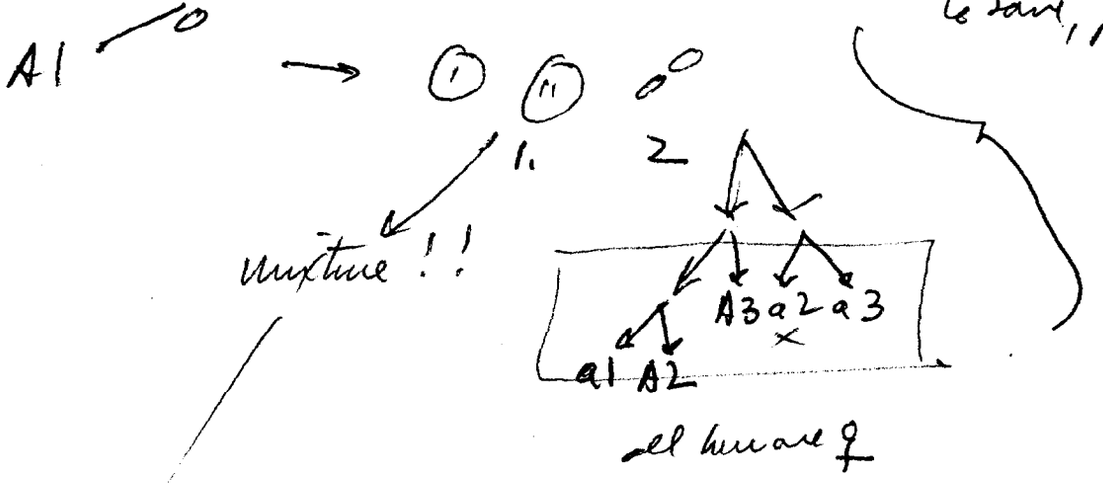
(over)

Mixed clones. By design,
218-31 is listed as N+M but ♀♀ only formed morulates.

208-22 (C1) listed as M, few cells. ♂ and ♀ appr. form.

208-31 A1 is listed as N+M and behaves as mixture. This is not accountable in pedigree unless a cell was overlooked which is neither likely nor is it countable.

to save, pool as A2.



1210 → B5♀.

88	A	~~208C1~~	208C1
	B	~~208C2~~	208C2
	C	~~218~~	218 C1

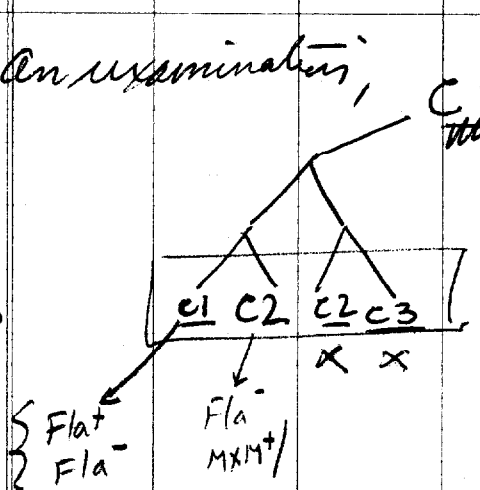
presumably no ♂ these cells was a motile ♀! (abortive phenotype?)

208-C1 analysis (motile recombinant) (W2732)

1210

DATE: ~~Feb~~ 1/9/55. REF:

An examination;



C1 has ♀♀ mainly but some motile cells. Gal+, - mixed. ~~From one motile passage may mean merely that 2 cells of diff. kinds were present.~~

C2 has (MXM)+. An examination of culture and on motility agar, no Fla+ were found. Pure Gal+ -

C2 and C3 were viable. C3 was recorded as ♂? when ? may have been motile org?. No record of separation.

218C1 is equally motile (in agar) ± from an agar. ∴ probably all still S^R. ~~also~~ also pure Gal+ ∴ Fla+ ... is presumably the only recombined character. Note: there was no suggestion that this cell was motile at time of isolation. Isolate W2732 from agar passage:

218C1 streaked on Gal EMB. >10 s.c.; and ~~grass~~ streak: no motility seen when directly transferred. Inhibition by temperature?, medium?

~~pass~~ passage through motility, Strain W2732 is selected for high motility, again to 218C1 for original

8 s.c. all motile (flares) in motility agar

208C1 is listed as ♂+♀. Look for ♀ Fla+ component via S^R selection, then motility agar. (Not seen in first dilut.)

nm

218C1 Total of 28 colonies tested: (= W2732)
27 motile
1 nonmotile [218-C1A]

: still segregating

208C1 — from spot on lacon, select through motility agar to recover a motile, Cat+ isolate.

Most colonies (e.g. from tMRS Cat5m) are Fla⁻. Basic possibility ① real but mutated with ♂+ ♀ heterozygote for Fla⁺/Fla⁻ or ② mix-recombinations from ♂×♀ (Tues should be tested further) or ③ ♂×♀ (motile chrom?)

---

motile isolated (see 210B) saved as [208C1A].

1210B

DATE: JAN 13 1955 REF:

1 2 3 4 5 6 7 8 9 10

Previous attempts (7/54) no motility observed. In view of this last experiment, renew search.

P13 – Inoculate mobility tubes from 1108E plated on EMB Lactose. Heavy growth. P15 Motility noticed in 2/10 tubes.

10

20

30

40

50

Hfx xF only

Isolations to 9/23

~~Dsol Intact~~

Dsol.	Brd-	Zygotes
229	144	37

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1185	8	7	1
1186	16	9	7
1190	3	3	0
1192			not clear
1200	11	11	2
1201			
1203		14	1
1204	10	9	1
1205		9	2
1206		12	7
1207		12	6
1210			3

Incomplete 324; 230; 67

67/230 rough datum.

SALMONELLA INDEX

Organoid summary.

JAN 2 4 1955
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Pedigrees :

—× Sw578 — 1151

FA12—× Sw666. — 1131-2-3-4-6-~~7~~8-41 ; 1212

FA60—× Sw666 — 1142-3-4-7-8-9-50

—× Sw967 — 1137

Planting's — 1140-3

Chemotaxis 1139

TRAILS. CROSSOVERS? 1033 1031 990 1149

Segregation 1033

INCIDENCE 1073 1075 990 (flares) 987 1212 981

(in H, ×) 1044

Serum effect 975 1003

O-CROSSES 1149? 988

Motility methods 1048 980

X_p' age 1047 972 942

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$H_1^{1,2}$ and H_1H_1 dupl. strains and macrophage pairs. 1002 1074 1053 991-2 1036-38-46 1051. 1049 976

Misc ϕ —X 1071 978 1063-8 1024 1016. 983

Som. antigens 1869 1034 1062 980

$H_{1,2}$ X— 989 1044 (tails)

TM2 Macrophage 1026e 1067 1053

SW666 X— 1067 1030 (autoant.) 985 984 1001 988 992-3 971 974 (993) 999

Galv 993 977 1032

SW967 X— 1027

Index notes on Salmonella
9/52 – 1/55.

Pedigrees: 1212 (① → swarms ⑤ → 20, 11, 9, 9, 8 12 → #1). 18 isolated; TM2 × 666 4-S

Details on T/S 1212-14 (FA37 × SW666 (Jan 55).

Apr 54.

1151 Pedigree × SW578 (paralytic). ; ca = T/S.

1150 FA60 × SW666 (40) cool. ① → swarms ⑪ 12, 17, 4, 36, 50, 20, 12, 24, 26, 27, 12 (28) #1.

1149 60B × SW666 (37) [+ pool (11).] → swarms. ② → swarms ⑥ → "large amicrobes" Remainder #1.
DCC also looked for crossovers.

1148 " " (10) 2 inv., 4 #1, (20), (30).

1147. " " (2) ①#1; (5).
(35): 3 inv; 4 swarms; 27 #1; (48).

1149. Dot also noted that SW967 × SW666 gave swarms (Gal+, gen) ambiguity of 60c! Presumably these were all saved.
But all isolates seemed idiotypic. Question of crossing over, esp. in swarm sites, should be reviewed.

1146 9 × SW967. "This gives largest tracks". Only (7). ; 6 0/10³ 1 2/10³...

1144. 60B × SW666. (10): 1x; (2); (1); (16); (46); SW, (14).

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1143 60-xsw666 – Same isolations of motiles to plates
16 isolates, no trails.

other isolates polyvid: see protocol

1142 60-x sw666 Same isolations to plates. No clear trails.
also (9): 0, 0, 1, 1, 2, 3, 10, >50 (++). → but not swarm!

1141 FA12-x 666 (1): 1 (14); others ↓1.

Note FA92 expt., no Pla^r seen microscopically.

1140 (1) platings: 130 (1) plated → 2 swarms, 7 cols. ↓ motility

1139 ϕOH ~~taxis~~ taxis

1138 12-x 666 mostly d. do not count, though some polymixis useful.

1137 9-x 967. (12): all ↓ (1)

1136 Plant 12-x666: 1 swarm; 4 colonies / 5.

1134 12-x666 detailed pedigree
1133 " " mic. 1 swarm (not crossover)
1131-2 " "

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1073, 1075 S. gallinarum + pullorum for H, identity 10/53.
1043 =gm

→ SW967 "few long tracks, 1 swarm"; Ø1→ "single tracks".

→ 957 "numerous tracks, few swarms".

N97: H_1, H_1, H_2 tests (monophasic) 1074. ~~reads 1072~~ 1000
?

1071 "Ø K" → . Note FA84 → SW166 : 5-6 swarms
Mustke → 0901 ca 100 tracks.

1069 Vi+ S. typhi → and → Misc. phages 978

8/53. S. bongendorf; and misc nonsp. & Jean Thomy 1068, 1063
para B phages : BAOR
TM2 monophasic SW1067. ~~SW 1067~~ 1067 1063 of 1026e (SW986)
Note: SW1067 → SW666 gave only H_1^b's.
TM2 → SW1061 gave no non-1,2.

UV, heat / phase variation: monophasic 1065

S. abortus-equi (anelbourn) 1042
1062, 1052

Somatic selection (monophasic) 1034 1062

Resume's at 1053:

SW1047 (N97...) H_1 H_1 1046 1038 1036992-991

Misc. on S. wien ek. mcard. (cf Aleuk; PRE)

→ SW1061 → still/mannopheric enx: —

but SW1057 → SW1061 (same?) did give c:1,2

S

1049: misc para B strains.

1045 S. para A.

1051 : H_1-H_1- Fla linkage

Used duplication status → . No clear result.

1048 Motility agar changes — mcard

1047 X phage

1044 H_1, H_2 transductions (TM: miami albany) + ; note of trailsome!

1041 - Phase variation: resume 1035 979

1037 S napoli

1033 Fla regulation in tracks tested. Somatic on T:S ratio. Serum effects (975)

SW967 Fla linkage. Ratios of gm: i ck.

1032 Calv? Looks for Hft (misguided). 977 973B1

1031 Resume

JAN 2 4 1955

1031 – Resume

Fla,-H,... linkage tests 1008. ; crossovers?
Monophasics (976)
abortus equi, javiana, pullorum
Z33, Z6

1030 "autoantibody" – inhibition of Fla$^+$ H,^{b} by Fla$^-$ H,^{b}?

1027 9–×967 "extensive teach" (This is probably the one saved)

"numerous T, no S" "numerous T, 1S".
heat-list of Fla$^-$ types. typing of SW970, 972...

Statement that 9–×967: numerous T, rare swarms } linkage?
10–×967: " and " . }

[Has there ever been adequate test for Fla,$^-$ in 9–×967?]

1024 S. cholerae suis, javalis ~~bebb~~ labile antigen; 1020
Misc & tests + stability tests

1019 1/53. Cetrimedium for inactivating phage n.g.

1018 "Evaporation of phage"; lysogenization 1009 994/ protection - transduction 1017, 1012 980 988

1016 Felix O phage. n.g.

1014 PLT22V. 1006

1013 host adaptation

1011 adsorption

1010 999 uv'd PLT22; TM2 Val-mutants;

1008 S. para A

1007 XRay PLT22

1003 Serum

1002 buffers 999

1001 b/i ratios: Fla⁻ linkage? Bais? 993, 992 986

0998 uv'd phage → Fla⁻. Val⁺/Fla⁺/unfeathered

990 Flaes + Trailes.

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7

989. Sendai x—
Eastbourne x—

988 "O form crosses" Never completed. Cf. OCG 53/3.

987 SW905-908 FA21-x908 "Tumorally long & prolific".
—FA9 : good yield of T+S. (better parabytic?)

985 SW666 (rough?) - b agglutinable?
984 " lysogenic? FA26-x666 28T/0s!
64T/2s!

983 Boyd phages. & h.r. tests

982 Rectify SW534 series

981 T/S/Gal+

980 Methods for O forms

974/ SW666x— back crosses ; 971

972 Xphage

992 5/6 → × typhi H901 → 3/3 SW930 1,2: —

→ × abony 7/7 1,2: elix

1000 Eduardo N25 (SW942?)

and wi FA15 – × SW942b → 1,2 – gam!

1023M Misc on Manoptenco; ; 526 – × miami → 1,2; 1,5
SW973

1025 z_{33}

1[illegible]1 Expts on N97, N25 same xxx b: 1,2)

SW942 /b → 7/7 z_{33}: — only

do 959-960-961

1036 N97 = SW1007, /b → 1036B1 → 1,2 !
Mass stock 1,2 slowly ∴ reversible ! reputable mutation

1036E i → × SW1007 → i : b and cycled

1038 Immunology ~~tests~~ N97 → × miami → b: 1,5 $H_{\downarrow}^{b}$
→ × TM2 → b: 1,2 incl $H_2^b H_{\cdot}^{1,2}$

E TM2 → × SW1007 → 3/3 i : b

→ × 942 → c : —

10385 SW10B6x — sendai → a:b:a SW1031
:b

Check 1026i → x mating → 1/2 i:1,5

mei. shigushi
no b's reformed

see summary of 1044

1046 Fresh N97 = SW1043 → b → 1,2 1046A1

and check single colonies 6/6 → b → 1,2

1046B1=b → SW1043(1,2)
(SW1043b)

1,2's poorly viable.

---

c) TM2 → SW 1043 2/2 i:1,2:(i)

Schrödinger notes 1/61

Is thruway turnover?

H_2 protein as repressor of H_1 synthesis.

Levin found a strain which was permanently in the phase-1 state.

$$Ph_2^+ H_2^{1.2} Ah_2^- \quad \text{or} \quad Ph_2^- H_2^{1.2} Ah_2^- \quad \text{or} \quad Ph_2^- H_2$$

$$\updownarrow \qquad\qquad \neq Vh_2^- \qquad\qquad \text{or stable allele.}$$

$$Ph_2^- H_2^{1.2} Ah_2^-$$

? Can $Ph_2^+ Ah_2^-$ be obtained by recombination.

$$Ah_2^- Ph_2^- H_2^{1.2} \xleftarrow{\times} Ph_2^+ H_2^{enx} Ah_2^+$$

$$\downarrow$$

$$H_2^{enx} Ph_2^+ Ah_2^- \quad \text{i.e. stable phase } \underline{\underline{2}}.$$

how select ~~in presence of~~ $H_2^{enx} Ph_2^+ Ah_2^+$ unstable phase $\underline{\underline{2}}$.

? Is phase variation going on?

$$Ah_2^- Ph_2^+ H_2^{1.2}$$

$$\updownarrow \qquad \xleftarrow{\times} Ah_2^+ \underline{\underline{Ph}}^{del} H_2^{del} \longrightarrow \text{both phases.}$$

$$Ah_2^- Ph_2^- H_2^{1.2}$$

about 5
APR ~~[illegible]~~ 1955

A loves duplications in Salmonella

Genetics of phases. (Z[33])

① Phases of Salmonella ② CDC - ... as (H,).
992 → (for SW534)
6

989 SW546

91 Tal2 × 546 → (11) i:—
16
27 i:—

b:— × → 3 b:—

b:enx → b:—
↘ enx:1,2 × i:1,2
↳ i:enx

Tal2 × 537 12 i:—

All 180 CDC 137 behaves as $H_1^{1,2} H_2^{0}$.

{ contra other pseudo's + monophasic 1,2's:

+ caused by N25 → CDC137 by mutation of $H_1^{b} \rightarrow H_1^{1,2}$