3/14/49.

58-161 x W859 on Lac EMS

100 Lac + tested. One Lac ♀.

W859 does not carry Het.

H189

~~477~~
477

		EMB Lac	Xyl.
1	H189 1	+	−
2	"	−	−
3		+	−
4		+	−
5		+	−
6		+	−
7		+	−
8		+	−
9	H189 2	+	−
10	"	+	−
11		+	−
12		+	−
13	H189 3	+	−
14	"	+	−
15		+	−
16		+	−
17		+	−
18		+	−
19		+	−
20		+	−
21	H189 4	+	−
~~22~~	~~H189 5~~		
~~23~~			

The appears to have been uniform segregation!

Pick + papillae from H189 - 190 on EMS Lac.
Streak out on EMS Lac to purify. Test single + colony derived from 1 papilla for Lac, Xyl v.

~~474~~.
477

Set

		Lac	Xgl
1	189a	+	—
2	"		
3	"		
4	"		
5	189b		
6	"		
7	"		
8	"		
9	189c	V+	V
10	"	+	—
11	"		
12	"		
13	189d	+	—
14	"		
15	"		
16	"		
17	189e	+	—
18	"		
19	"		
20	"		
21	190a	+	—
22	"		
23	"	+, −	—
24	"	+	—
25	"	V−	V
26	"	+	—
27	"	+	—
28	"	+	—
29	"	+	—
30	"	+	—
31	"	m.g.	m.g.
32	"	+	—
33	190b	+	—
34	"	+	—
35	"	+	—
36	"	V−	V
37	"	+	—
38	"	+	—
39	"	+	—
40	"	+	—
41	"	+	—
42	"	+	—
43	"	+	—
44	"	+	
45	190c	+	—
46	"	+	—
47	"	+	—
48	"	V−	V
49	"	+	—
50	"	+	—
51	190c	+	—
52	"	+	—
53	"	V−	V
54	"	+	+
55	"	+	+
56	"	V−	V

Thus, out of 56 trials here, only 6, or 1/9, are still heterozygous after lac reversion. This suggests that reverse-mutation may be more frequent in diploids than in haploids. Label 477:1-6.

1	9
2	25
3	36
4	48
5	53
6	56

B/15/49.

	Lac	Xgl	
1	1190	+	—
2		+	—
3		+	—
4		+	—
5		+	—
6		+	—
7		+	—
8		→	—
9	+		—
10	V	V	477-7
11	+		—
12	+		—
13	V	V	477-8
14	+		—
15	+		—
16	+		—
17	+		—
18	+		—
19	+		—
20	V+	V	477-9
21			
22			
23	V	V	-10
24	V	V	-11
25	+		—
26	+		—
27	+		—
28	+		—
29	+		—
30	+		—
31	+		—
32	V+	V	-12
33	+		—
34	+		—
35	V+	V	-13
36	+	→	

Since cultures
Xgl segregants
are Xgl-, they
need not be streaked
out on Xgl, merely
tested.

3/15-16/49.

H1906 + c.

Pick single + colonies to EMSLac and spot on EMB Xyl. [Streaking is deepened with since segregants are recognized as ~~Lac~~ Xyl − J.

b. 37 tests 4 Xyl v [6, 13, 14, 37 J.

c. 35 tests. 7 Xyl v. [1, 7, 5, 21, 27, 31, 33 J.

Pick from corresponding EMSLac spots as 477: 14-17 (b) and 18-24 (c)
Also inoculate into Penassay to allow segregation.

Reversion in lac-diploids 477a.

3/15/49.

Tests on 1 of 6 Lac V. Inoculate from EMS to Runaway

from H-		Lac	Xyl	Mtl	Pud. Lac
189	1	+ ?			
190	2	V			—
190	3	V			—
190	4	V			—
190	5	V			=
190.	6	V			=

On lac EMS, 1 shows a sheen; others do not. Has one become Lac+/Lac+?

477b From 190 A pick a number of - and + cols. from same papilla to evaluate heterozygosity.

		Xyl	Xyl.
A. Lac+		—	
	1	—	—
	2	—	—
	3	—	—
	4	—	—
B	1	—	—
	2	—	—
	3	—	—
	4	—	—

		Xyl +	Xyl -
C.	1	V	V
	2	V	—
	3	V	—
	4	V	—
D.	1		V
	2		V
	3		V
	4		V

#14

The homozygosity of lac+ mutants is probably due only to the fact that the bacteria were already segregated.

477-1 turns out to be to be Lac+ Xyl- not Xyl V.

Secondaries: Lac V includes:

	#
7	10
8	13
9	20
10	23
11	24
12	32
13	35
14 C+	#1

Reversion in Lac- diploids

3/15/49.

All valid lac +/- from lac -/- came from M-190.

2-6 first series. Recover from brushes on EMS lac.

7-14 second series

streak out Penassay cultures of these new heterozygotes.

	−	+	Pred!
2	109	22	−
~~3~~	63	25 (exag.)	−
4	~~486~~	32	−
5	41	23	−
~~3~~	79	fewer.	−

All of these are pred. Lac -!

	Lac EMB	Xyl EMB	Mal EMB	Recheck (from EMS lac brushes)
7				+
8				+
9				++
10				++
11				++
12				++
13				++
14				−
15				+
16				+
17				−
18	V−			+
19	+			−
20	V−			+
21	++, −			−
22				+
23				−
24				+
25				

10 − : 13 +

3/19/49.

Check 5 strains from EMS lac bunches.

	lac EMB	Xyl EMB	MTL EMB	EMS Lac	Pred (?)	Recheck
2	V-	— V	V	many are −, +	−	✓
3	V-	V	V	Results + 2 colonies each	−	✓
4	V-	V	V		−	✓
5	V-	V	V		−	✓
6	V-	V	V		−	✓
7	+	−(v)	−v		++	−
8	+	−v	−v		++	−
9	+	−(v)	−(v)		++	
10	+(V?)	−v	−v		+	
11	+,−(=)	−	−		?	
12	V+	−v	−v		+	
13	+	−	−			
14	V+	V	V		+	
15	V-	V	V		−	+ ?
16	+	−(V)	− V		+	
17	+	−(V)	−(some +)		+	
18	V-	V	V		−	−
19	V+	V	V		+	
20	V-	V	V		−	−
21	++,(−)	−	−			
22	V-	V	V		−	−
23	V-	V	V		−	
24	+,(-)	V	V		+	−
25	+	−	−			

10 −
10 +

	a: lac EMB	lac EMS	Xyl EMB. (bunch)	b lac	lac S	Xyl
2	V-		+V	V-		+V
3	V-		+V	V-		+V
4	V-		+V	V-		+V
5	V-		+V	V-		+V
6	V-		+V	V-		+V
7	V-		+V	++		
8	V-		−	++		+V
9	++		—	++		—
10	++		—	++		—
11	++		—	++		—
12	++		—	++		—
13	++		—	++		—
14	++		−	++		—
15	V+ ?		+V	V+?		+V
16	++		—	++		—
17	++			++		—
18	V-		+V	—		—
19	++			++		
20	V—		+V	V—		+V
21	++		—	++		—
22	V-		+V	V-		+V
23	++		—	++		—
24	V—		+V	V —		+V
25	++		—	++		—

Possibly the V+ was not recovered due to difficulty in distinguishing Lac+ from Lac−, on selection for lac+.

Disinfection of λ - Y10 & 811

3/15/49.

Irradiate Y10 8 secs. on nutrient agar + EMB Lac as for mutation runs. Pick 100 cols and streak on W518 on EMB Lac.

1 colony (from N.A.) apparently λ -. Streak out to confirm. Included as λ+ (weak) and λ^R

2d. sample of 100 tested. No disinfecta seen: (i.e., all λ+).

3/28/49. 35 single colonies from a dilute plating of W811. Each lysogenic.

Single toxin: λ. 4/9

3/16/49

Dilute stock λ to 10/ml. Add 1 ml to 10 ml Pneumococcus + 1 ml W 518.
Disperse in ml quantities to small tubes ~ 1 ml per assay.
Incubate at 40° 1 hour; also take initial assay.

A. Initial assays. 5, 5, 4, 3, 7

B. Plated after 1 hour. 1, 1, 3, 3, 2

Interval too short for a test.

3/16/99

A. Assay	No plaques!
B. 518 4.5 ml	561
B supernatant	60
C. 518 0.5 ml.	176
C supernatant	106

.5 ml λ + 4.5 ml W518 (or 0.5 W518 + 4.0 water).
Absorb 10 m.
Centrifuge 5 m. .1 ml aliquots + .1 ml W518 plated (except for B which contains W518 already.)

This is a poor experiment since no assay was obtained, and there is a large discrepancy between the total recovery in B and C. The results do suggest, however, either marked adsorption of the phage in pellets, or else a wide discrepancy in plating efficiency for free phage and adsorbed phage!

3/15/49.

nos	plaque		on W518	on W811
1-6	1	all λ−	±	±
7-10	2		++	++
11-14	3		± ~~+±~~ 1 plaque	−
15-17	4		++	++
18-21	5		++	++
22-27	6		++	±
28-31	7		−	−
32-35	8		−	−
36-45	9		++	++
46-49	10	all λ− (1 autolytic exception)	++	++
50-53	11		++	++
54-57	12		++	++
58-61	13		++	++
62-65	14		++	−
66-69	15		++	++
70-73	16		++	++
74-77	17		++	++
78-81	18		++	++
82-85	19		++	++
86-89	20		++	++
90-93	21		++	++
94-97	22	94-96: 95 autolytic; others not?	++	++
98-101	23	99-100: Autolytic	++	++
102-104	24	103-104 "	++	++
105-106	25	106 Not autolytic	++	++

plate [illegible] sewage with 518. 25 plaques found on 5 plates. Pick and a) [illegible], streak on 518 to obtain [illegible], test 4-3 of each for λ on 518. b) recover from plates and compare action on 518 and 811. #14 is the only phage showing a clear differential. Pick from 518 plate and recheck.

a) Pick 94, 96, 106 and restreak to check λ.

b) Recheck: Phage "14" is active on W518, forming very large plaques, but not on W811. Culture as Sp-19

a) 94 gave many colonies; only 2 plaques. 96 was autolytic
106 may be truly lysogenic. Test single colonies for λ.

3/18/49.

Test single colonies and bunch of 481–106 (= 482A) for lysogenicity.

8 single colonies tested. None were λ+. Bunch showed λ and fair amt. of phage as streaked. General comportment like Sp 14. Put on slant to store for later manipulation.

Growth of λ and bacteria in W811.

3/18/49

Dilute a broth culture of W811 10^{-6}. Add 1 ml to 10 ml Permease. For initial assay take .5 ml from tube, then aerate at 37.

1. A (bacterial count) 5 518 — 1:30 PM — .5 ml
   B. phage on 518.
2. — 2:45 PM — .2 ml
3. — 3:30 — .1 ml
4. — 4:40 — .1 ml
5. — 5:40 — .1 ml
6. — 7:15 — .001 ml

	Counts. A	B (4)	~~control A B (4)~~ ~~290 110~~	Calc/ml A	B 4	log A.	log B.
1	386	11		772	22	2.9	1.3
2	304	5		1520	25	3.2	1.4
3	462	7		4620	70	3.6	1.8
4 high!	4000	34		40,000	340	4.6	2.5
5	8500	635		85,000	6350	4.9	3.8
6	9000	114		9×10^6	114,000	7.0	5.0

Very clearly, the plating method used does not recover all the phage present, especially that found to bacteria. Need expts. to work out necessary refinements of technique.

As a check, 30 cols. from A1 tested. Each carried λ.

Interference of λ

[redacted]
453

3/20/49.

1. Assay λ (ca 2×10^4) by a 10^{-2} dilution on W518.

~~2. Add 1/2 ml P19 (2×10^4) to 1/2 ml W518 to assay for resistance~~ Plate .2 ml samples.

3. Add 1 ml λ + 1 ml W518. Incubate ~~30~~ 30 mins. Then add P19 1 ml. Plate .2 ml samples.

2. Like ~~the~~ 3, using broth for λ.

~~(4) Assay bacteria.~~

1. λ was $56 \times 200 = 10^4$/ml.

2: Resistants to P19. 81, 113, 92, 47

3. λ- " 42, 101, 12, 84

The aims of this expt. may be impeded by the presence of p19h.

Virtually all colonies in ② and ③ were heavily mucoid.

Segregates of H168

3/15+/49.

Inoc. 2 tubes Vz ± H168 from EMS bunch.

Plate out when grown.

1-17 Mtl+. 18-93 Gal+ 94-176 Lac+

A. 1-17: Mtl+ 1, 2, 4, 5, 9, 15 are mixed Lac+, −. others are Lac−. Do. Gal.
1, 4 Xyl−; others are Xyl+. Do not seem to be mixed!
Streak out the questionable on mannitol.

The colonies picked from these expts. are too contaminated to be useful.

B. 18-93. Gal+

18-39. 20, 21, 24 are apparently mixed ± Gal+
#25 is Xyl+, others are Xyl−.
20, 21, (22) 23+, 24, 26, 34 badly mixed ± Lac+
others are Lac−
all are Mtl−.

40-81. ~~43~~, 46, 47, 48, 50 53, 54, 57, 60, 61, 62, 63, 67 68, 69,
badly mixed ± Gal+
40, 58, 76, 78 may be Mtl+, others are Mtl−
40, 51, 58, 67, 76, 78 are pure Xyl+; others are Xyl−

42, 45, 46, 47, 49, 53, 54, 56, 61, 62, 63 badly Lac+

94-176 "Lac-"

94, 96, 97

Counts on plating:

A: Lac. 316+ 356
32 − 55
38 V 41

B: Lac 200+ 383+
23 − 29 −
9 V 11 V

Gal Too heavy for most part.
330 +
43 −

50 lac+ tested all V_5^R, but some are mixed

3/29/49. Streak out colonies from EMS bac from 485: 1, 5–7.

---

(1) 4/quadrants. + pred.

(5) 4 quads. + ca =

(6) 2 halves + ca =

(7) 1/quad. + ca =

No persistence of predominant character.

H/68

485

Nos.	From
1-12	168-6-e neg
13-24	168-6-e pos
25-30	168-6-d neg
31-36	168-6-d pos
37-40	168-1-a neg
41-44	168-1-a pos
45-54	168-1-b neg
55-64	168-1-b pos
65-68	168-1-c neg
69-72	168-1-c pos
73-78	168-1-d neg
79-84	168-1-d pos
85-86	168-1-e neg
87-88	168-1-e pos
89-100	168-5-a neg
101-112	168-5-a pos
113-127	168-5-b neg
128-142	168-5-b pos
143-172	168-5-c neg
173-202	168-5-c pos
203-206	168-5-d neg
207-210	168-5-d pos
211-214	168-5-e neg
215-218	168-5-e pos

Predom.	
1:	~~+~~ −
5:	+
6:	+
7:	−

231+
18−

March 2 5- 28, 1949.

H-168 was streaked out on EMS Lac. Single colonies were picked to YZ and also streaked out on EMB Xyl to ensure heterozygosity. Broth cultures 1, 5,6,7, corresponding to variegated streaks were diluted 10^{-8} and plated on EMB Lac or EMB Mtl. Approximately equal numbers of # and - colonies were selected from these plates. The selections were made as indicated on following sheets.

Summary of colony counts:

	Lac#	Lac-	% #	Mtl#	Mtl-	% #
-1	21	159	13	73	98	43
-5	1300	147	90	20	600	3
-6	231	18	93	61	95	39
-7	50	390	11	74	212	26

These samples are clearly heterogeneous, probably because of sibship, and too small a number of independent segregations. This internal correlation is also seen in runs, e.g., of the rare Lac#Xyl-Mtl# in the Mtl# selections of No. 6.

Pooled Summaries:

Among Lac selections

	M#	M-	S	X#	X-	S
L #	44	66	110	38	72	110
L-	11	99	110	10	100	110
			220			220

Lac- selections

	M#	M-	
X#	10	0	10
X-	1	99	100
			110

Lac# selections:

	M#	M-	
X#	38	0	38
X-	6	66	72
			110

Among Mtl selections:

		L#	L-
M#		26	30
M-		32	35
		L+	L-
M#	X#	19	27
	X-	7	3

		X#	X-
M#		46	10
M-		0	67
		L+	L-
M-	X+	0	0
	X-	32	35

Checks of Lac segregants

Lac- selections

168- 6	M+X+	M-X-	M+X-	M-X+
	0	12	0	0
-1	5	97	0	0
-5	0	56	0	0
-7	5	24	1	0
	10	99	1	0

Lac+ Selections

	M+X+	M-X-	M+X-	M-X+
	2	7	3	0
	3	9	0	0
	22	32	3	0
	11	18	0	0
	38	66	6	0

Mtl- selections

	L+X-	L+X+	L-X-	L-X+
-6	12	0	5	0
-1	10	0	4	0
-5	4	0	4	0
-7	6	0	22	0
	32	0	35	0

Mtl+ selections

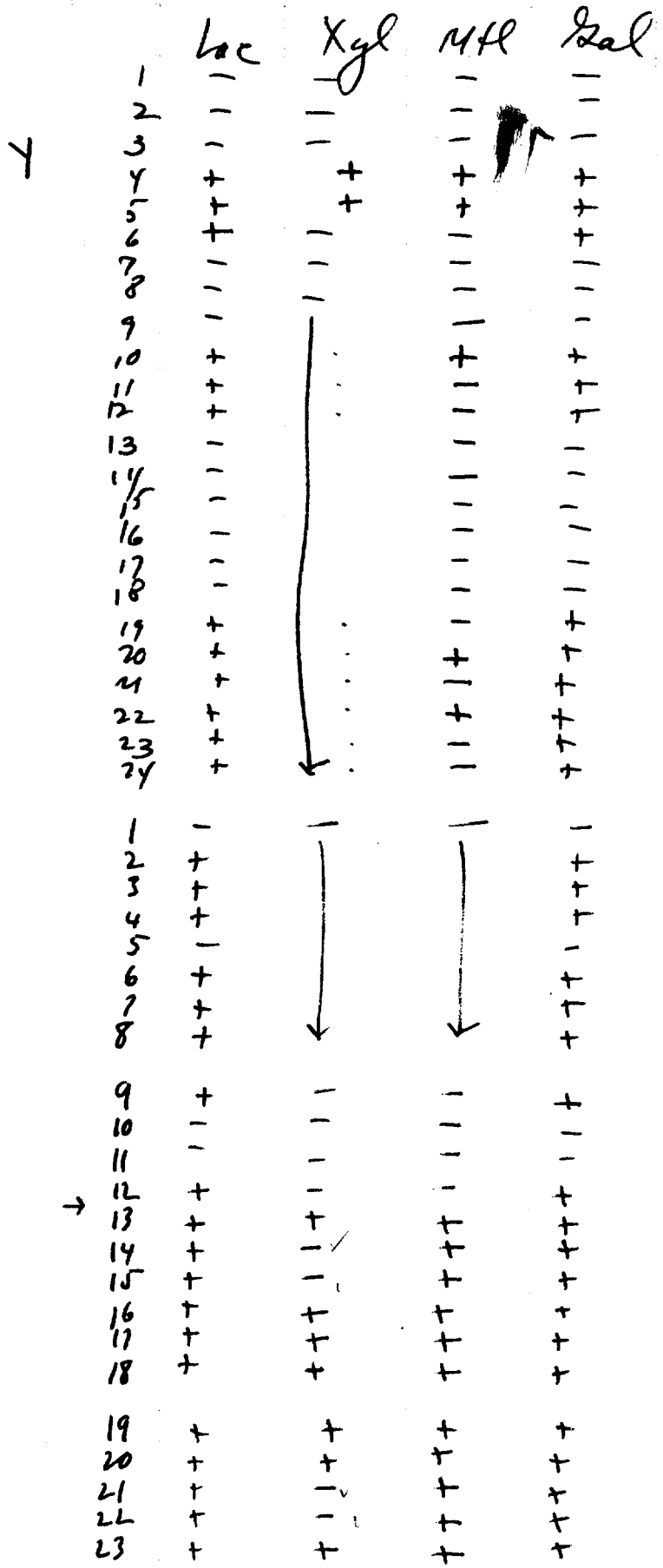
	L+X-	L+X+	L-X-	L-X+
	7	10	0	0
	0	0	1	13
	[illegible]	5	0	0
	0	4	2	14
	7	19	3	27

Segregation ratios:

168-	Lac +	Lac -	Lac % +	Mtl +	Mtl -	Mtl % +
6	231	18	93	61	95	39
1	21	159	13	73	98	43
5	1300	147	90%	20	698	3
7	50	390	11%	74	212	26

Note variability in all ratios.

H168


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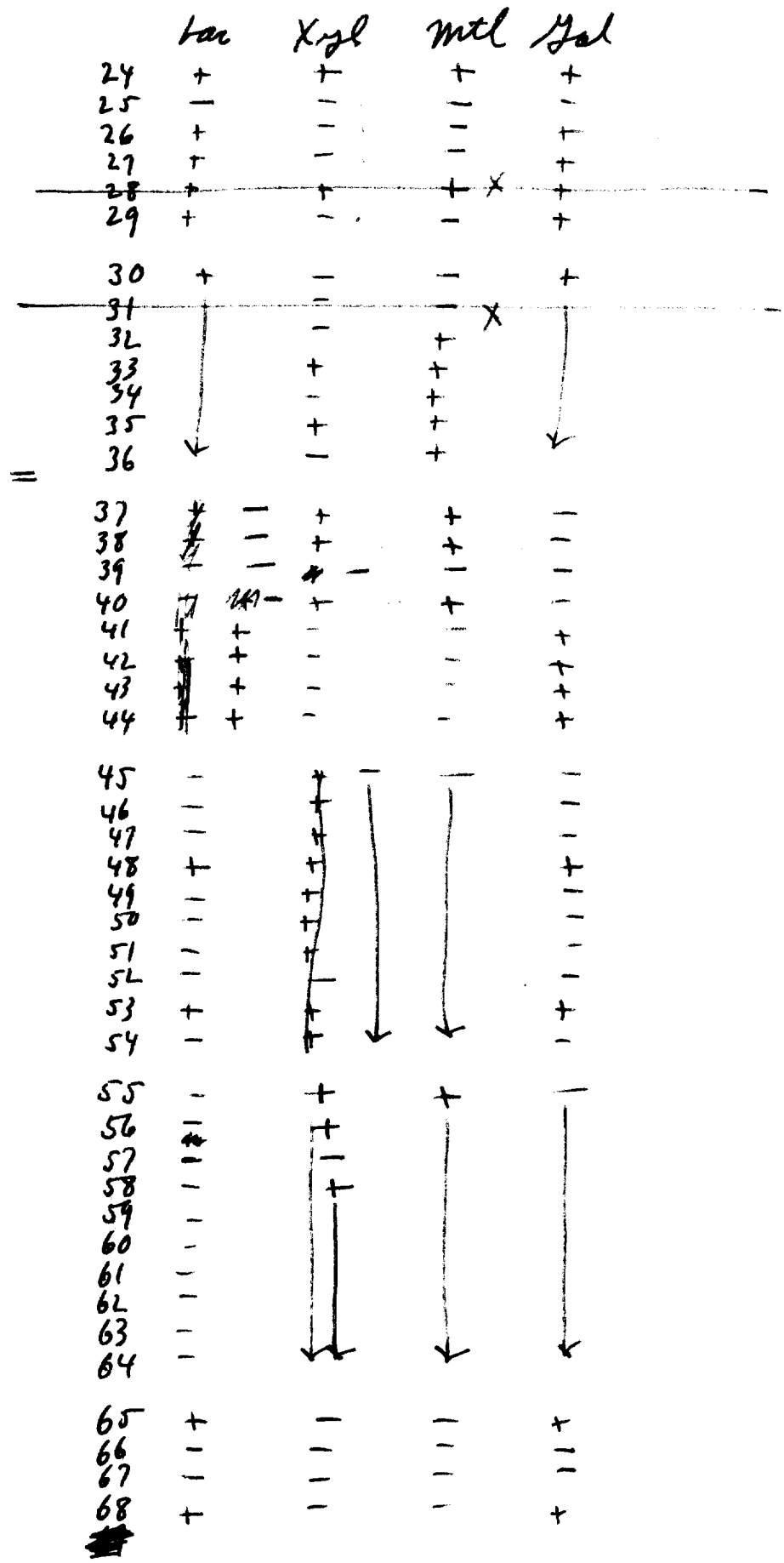
23 + + + +

H168'


Lac
Xyl
Mtl
Gal
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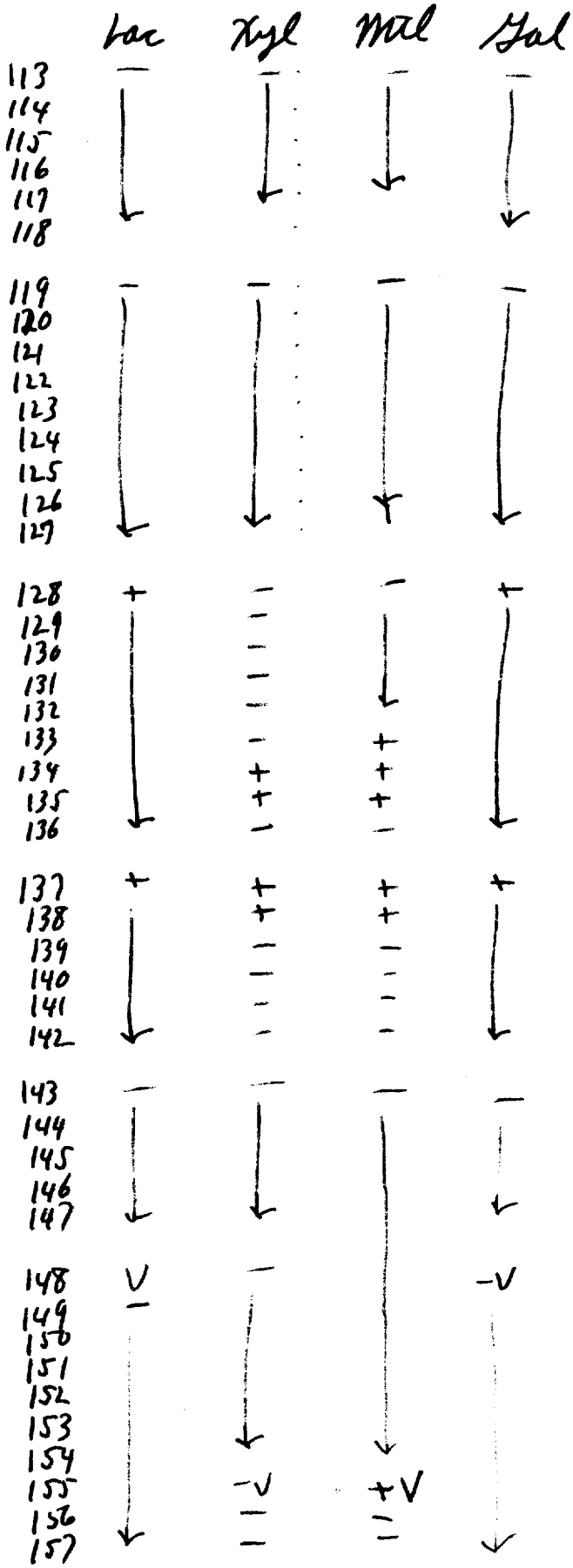
64
65
66
67
68

H168

	Lac	Xyl	Mtl	Gal
69	−	+	+	−
70	−	+	+	−
71	−	+	+	−
72	−	+	+	−
73	−	−	−	−
74	−	+	+	↓
75	−	−	−	↓
76	V	↓	↓	↓
77	−	↓	↓	↓
78	−	↓	↓	↓
79	+	↓	↓	+
80	+	↓	↓	+
81	+	↓	↓	+
82	+	−	−	+
83	+	−	−	+
84	+	−	−	+
85	−	+	+	−
86	V	−	−	− v?
87	+	↓	↓	+
88	+	↓	↓	+
89	−	↓	↓	−
90	−	↓	↓	−
91	−	↓	↓	−
92	−	↓	↓	−
93	−	↓	↓	−
94	−	−	−	−
95	−	↓	↓	−
96	−	↓	↓	−
97	−	↓	↓	−
98	−	↓	↓	−
99	−	↓	↓	−
100	−	↓	↓	−
101	+	+	+	+
102	+	+	+	+
103	+	−	+	+
104	+	−	−	+
105	+	−	−	+
106	+	+	+	↓
107	+	−	−	↓
108	+	+	+	↓
109	+	+	+	↓
110	+	−	−	↓
111	+	+	+	↓
112	↓	−	−	↓

H168


Lac
Xyl
Mal
Gal
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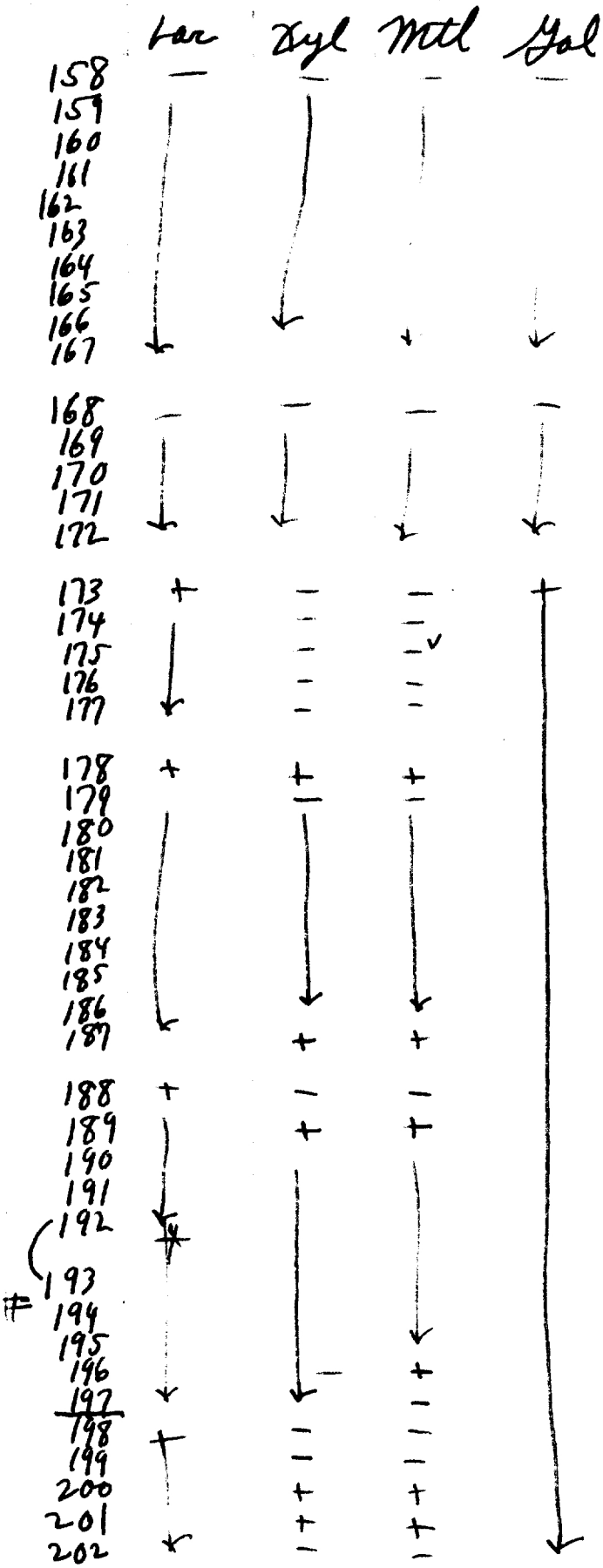
H168


Lac
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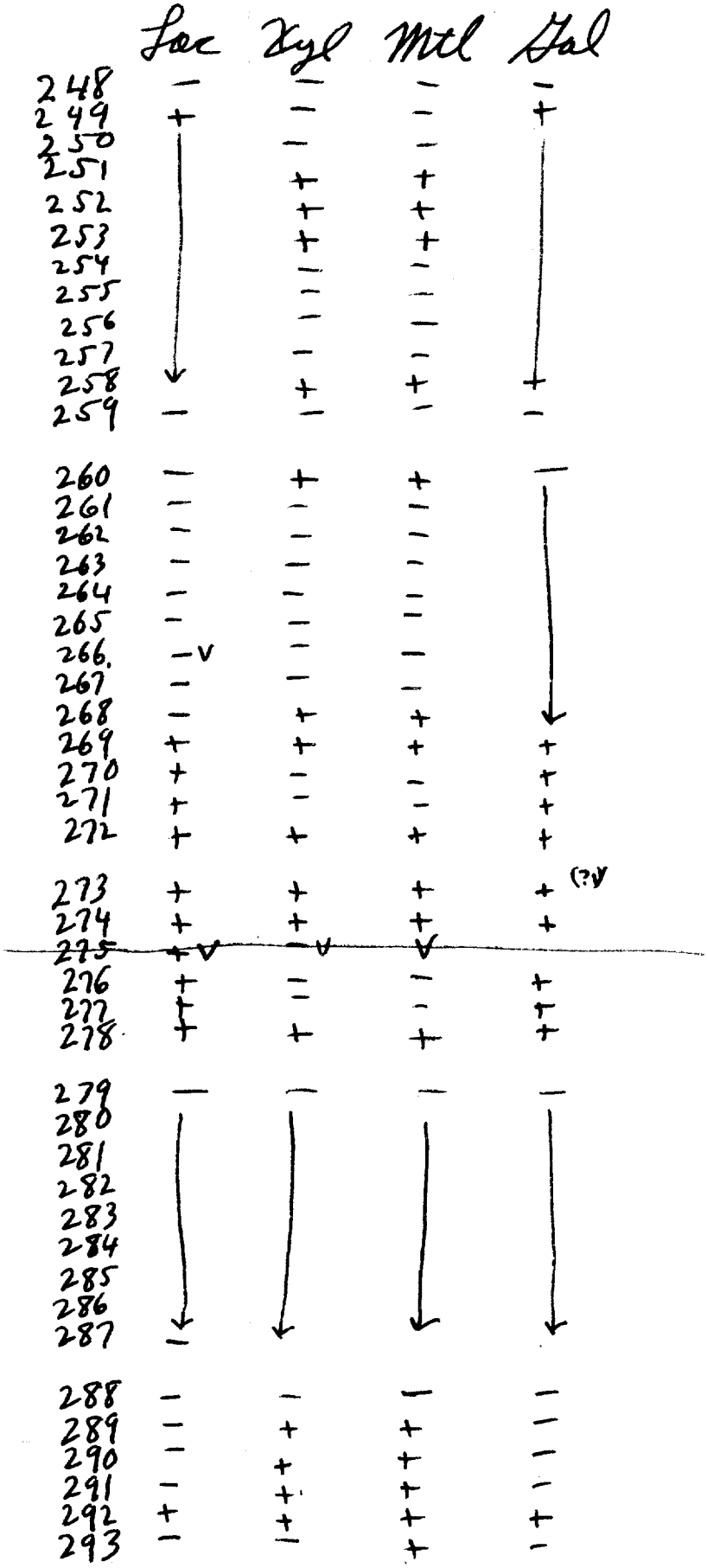
H168

	Lac	Xyl	Mtl	Gal
203	—	–	–	–
204	~~+~~ –	–	–	–
205	+	–	–	+
206	+	–	–	+
207	+	+	+	+
208	– v	–	– v	–
209	+	–	–	+
210	+	+	+	+
211	–	–	–	–
212	–	–	–	–
213	+	–	–	+
214	+	–	–	+
215	+	+	+	+
216	+	–	+	+
217	+	+	+	+
218	+	+	+	+
219	– v	–	–	–
220	–	+	+	–
221	–	–	–	–
222	–	–	–	–
223	– v	–	–	– v
224	–	–	+	–
225	– v	–	–	–
226	–	–	–	–
227	– v	+	+	–
228	–	~~+~~ –	–	–
229	+	–	–	+
230	+	–	–	+
231	+	+	+	+
232	+	–	–	+
233	+	–	–	
234	+	+	+	
235	+	–	–	
236	+	–	–	
237	+	–	–	
238	+	–	–	
239	–	–	–	–
240		–	–	
241		–	–	
242		–	–	
243		+	+	
244		–	–	
245			–	
246			–	
247			– v	

H 168


Lac
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292
293
V
(?)V

H168

	Lac	Xyl	Mtl	Gal
294	+	+	+	+ ✓
295	+	+	+	+
296	+	+	+	+
297	−	+	+	−
298	−	+	+	−
299	−	−	−	−
300	−	↓	↓	−
301	−	↓	↓	−
302	+	↓	↓	+
303	−	↓	↓	−
304	−	↓	↓	−
305	+	↓	↓	+
306	−	↓	↓	−
307	↓	↓	↓	↓
308	↓	↓	↓	↓
309	↓	+	+	↓
310	↓	+	+	↓
311	−	+	+	−
312	↓	+	↓	↓
313	↓	+	↓	↓
314	↓	+	↓	↓
315	↓	+ (s?)	↓	↓
316	↓	+	↓	↓
317	↓	+	↓	↓
318	↓	−	+	↓

Cross streaks ≡ very heavy phage suspensions.

P19:

K-12	++ ! (mutants?)
W435	++
W518	++
W 811	−
B/1	2 plaques
B/2	−
B/3,4,7	− (1 plaque?)

	T1	T2	T4	T5	T6	T7	P14	λ	P19
W518	±(λ?)	++	++	−	++	++	++	+	++
W877	−	++	++	−	++	++	−	−	++

∴ p14 interfères with λ possibly, but not with P19 or other

This interference may be genetic cross-resistance.

λ :	B/1	B/2	B/3,4,7	W518
	−	−	−	+

Plate p19 on B/1 to isolate hb mutant.

3/18/49.

P19: Mix .1 ml each of an 18 hour culture of W418 and 677 into 10 ml YZ broth.
Plate out 10^{-8} and 10^{-5} on EMB staining background of ...
Actual value $\times 10^{-2}$.

Initial, at 10^{-8}:

	+	−	Σ	% −
a.	31	55	86	64
b.	25	36	61	59
Σ	56	~~10~~ 91	147	62

Final 2P20:

+	−	
19	13	
16	9	
1~~8~~	12	
6	18	
12	18	
64	70	134

$\chi^2_1 = 2.9$

$p = .09$

56 (63)	91 (84)	147
64 (57)	70 (77)	134
120	161	281

$\frac{1}{63} + \frac{1}{57} + \frac{1}{84} + \frac{1}{77}$

.016
.018
.012
.013
———
.059
× 49
———
2.9

Analysis of [illegible]

a.

	+	−	Σ
	31	55	86
	25	36	61
	56	91	147

$\chi^2 = 4\left(\frac{1}{53} + \frac{1}{23} + \frac{1}{53} + \frac{1}{38}\right) = 4(.03 + .04 + .02 + .03)$

$= 4(.12) = .5$ $\quad p = 0.3$

b.

19 15	13 17	32	27
16 12	9 13	25	27
11 11	12 12	23	27
6 12	18 12	24	27
12 14	18 16	30	27
64	70	134	

a. plate totals. $\chi^2_4 = \frac{1}{27}\left(\frac{25+4+16+1+9}{16}\right) = 63$

$= 2.3$ $\quad p = 0.6$

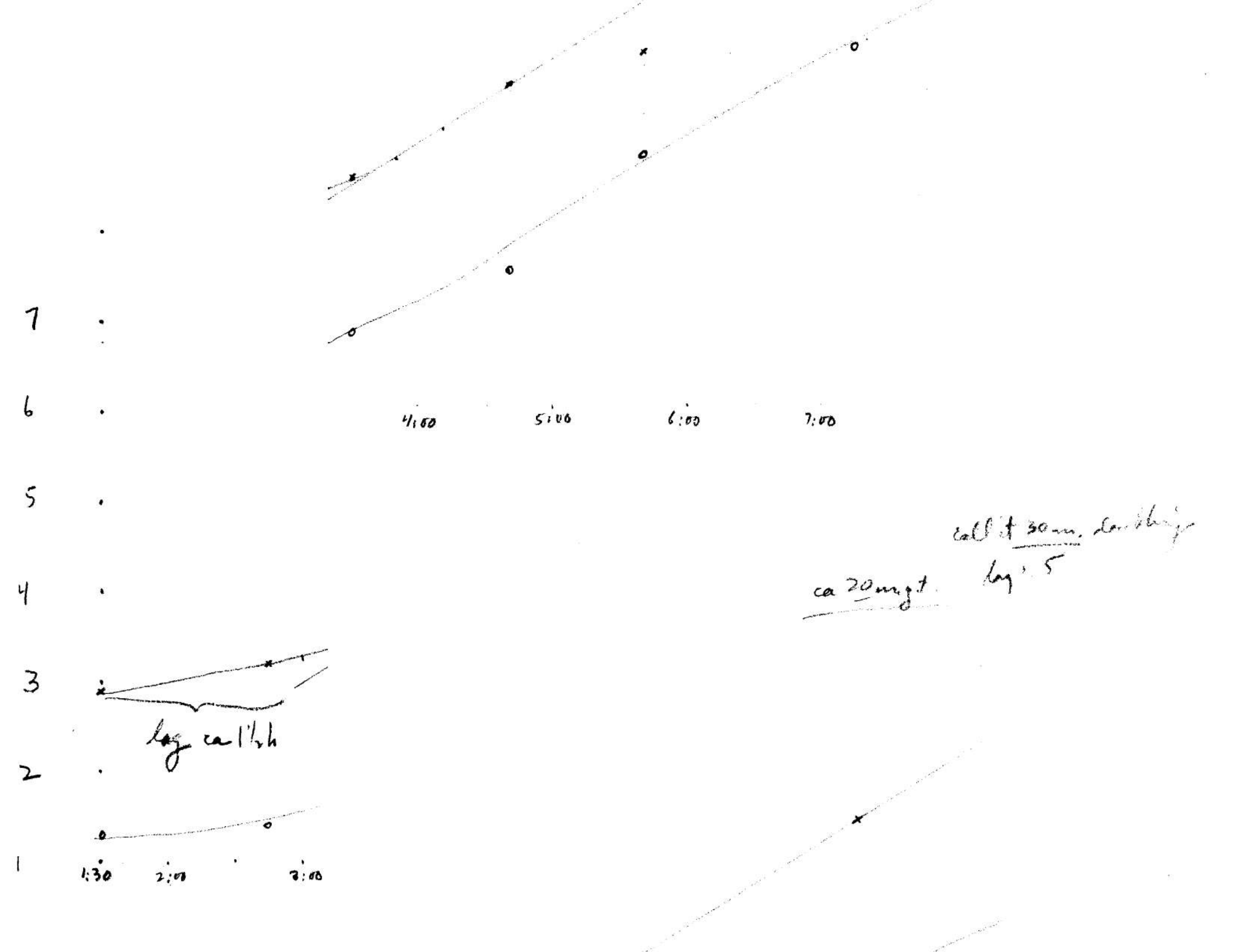
agreement in segregation: $\chi^2_4 =$ $16/17 + 16/15 + 16/12 + 16/13 +$
$36/12 + 36/12 + 4/14 + 4/16$

$= 11.11$

$p = .025$ for homogeneity.

Probably due to clumps of Lac or + which are despised by spreader

.94
1.07
1.33
1.23
3.00
3.00
.25
.29
11.11


7
6
5
4
3
2
1
4:00
5:00
6:00
7:00
1:30
2:00
3:00
ca 20 mg t
log ca 1½h

λ + λ− : Results.

988.

3/22/49.

Mix = .1 ml of 10^{-5} dilution of a 518 suspension (resuspended in Y2).
= 1 ml of [10^9 λ] diluted as indicated. Incubate 30 m.
Refrigerate 30 m.
Plate = 3 ml softagar.

A. No p

B. 10^9

C. 10^7

D. 10^5

E λ assay 42×10^7 (lower count than expected!)

F. W518 assay. 3×10^{10} initially; ~~should have been ca. 3×10^4 not 4 which is in reasonable agreement?~~

A ca 4× B. i.e., ca 5,000 and 1200 respectively.

C, D again approach value in A.

This expt. indicates that a fairly large proportion of cells of W518 escape lysis. Should be repeated also at higher dilution of cells.

λ plaques in citrate seem to be smaller but clearer. G might be a condition for lysogenicity.

P19 : [illegible] titer.

3/20/49

Add 0.1 ml p19 (2×10^9) to 0.1 ml W318. After 10 m. Add .1 ml W811. Plate .2 ml samples

Nearly complete lysis was obtained. W811 is only relatively resistant to P19 or else there may be a frequent h mutant.
Plate out p19 at various dilutions on W811 to determine prevalence of the mutant. Do. on K-12.

3/21. P19/B1: 10^{-1} ca 10^{-2} 10^{-3}: 7 plaques with haloes 

1 plaque picked P19-0, very heavy plaquing but center picked. haloes not noted. Repick, and pick a haloed plaque for P19 hb_1 and P19 hb_2.

P19/518 for titer. $10^{-7} \times 10^{-2} = 10^9$ o/r.

P19/811. 10^{-7} shows 8 plaques. 10^{-3} confluent at edges 10^{-1} shows plaque formation, probably on secondary growth.
[Does p19 multiply on p19 $\frac{S}{S}$ lysis?]. Pick plaque at 10^{-7} and grow on W811.
1K-12. Same appearance at 10^{-5}.

518/9: 7 mucoid colonies, me purified. 5 are 19^S, with many mucoid revertants. 2 are very thin non-mucoid. Streak these out for isolate.

∴ p19 although it is somewhat intrafused with by x does not show a complete specificity.

3/20/49 ff.

when p19/811 plaque was plated, no plaques were seen.

Repeat plating of p19 into W811: no plaques [The 811 used may have become contaminated.

p19B was readily plated and subjected to 3 single plaque isolations on B/1 ~~bef~~ on 3/22/49; grown on B/1 in NSB overnight and filtered A23. 37 on 518. At 10^{-7}, no plaques noted after 5h., 10^{-5} gave 9 plaques on 811;

$> 10^8$ plated ē W811 or ē W811+W518 gave 1 plaque on three plates. This may be a contaminant, but grow out for tests.

Repeat at 10^{-5}:	B/1	18	P19B, then, has opt. activity on 518 on B/1 but not on W811.
	518	28	
	811	0	

Note contradiction on 811!

It also lyses B/2; B/1,5; B/3,4,7.

P19. At 5 hours, 10^{-7} gave 10 on W518, none on W811 16 hours: 136; well around edges

10^{-5} gave 0; 10^{-3} gave about 100 vague plaques, irregularly visible on plate (probably low plating efficiency), two clear plaques picked for isolation of possible mutants. At 18 hours, 8 plaques noted on W811 at 10^{-5}

Repeat at 10^{-5}, 10^{-7} on 518, 811.

P19 10^{-1}/811 gave irregular complete lysis ē mucoid resistants.

10^{-5}. CL on 518. 3 on 811 0 on B/1

10^{-7} 217 on 518 0 on 811. ∴ P19λ = 3/217.00 = 1/7000

Plaques on 518 are large with spreading halo; on 811 are small and circumscribed

λ, 3x.3 ml 10^7 on B/1 ⇒ no plaques

p14 and p19 Resistance

490

3/21/49 ff.

W518 plated with p19 gives virtually all mucoid colonies. Usually, these are autolytic when streaked out.

A1-2 gave resistant colonies when first streaked. Second streak: A1 was sensitive; A2, resistant.

B1-3 all sensitive.

A2 gives a very thin semi-mucoid growth.

W877 is a mass culture of W518[p14]. a-d are single colony isolates which are not lysogenic and are resistant to p14. However, at region of cross-streak, they show a very faint increase in opacity, but no growth inhibition. After 2 s.c.i., use for studies on ϕ growth in them.

W811 Techniques.

diluted W811, plated with W518 at different cell densities, gave no plaques, either at ~~room temperature~~ 29° or at 37.

3/21/49.

Add p14 to 10ml so that 10^{-3} ml will yield 10 plagues. i.e., 10^5 particles. (1ml 10^{-4} dilution of stock)

A). Assay stock p14 to verify addition: Confluent lysis over part of plate

B). Inoculate tube ē W8776 to determine any growth of p14.

196 plaques counted at 10^7. Plaques generally very cloudy. 1 clear spot noted. Picked as possible p14'

3/23/49.

A. Mix. 1ml W518 culture ≈ 1ml λ 10^{9}(+) Incubate 4:35 – 5:05. =30 mins.

Dilute 10^{-6} and plate. (i.e., 10^{-5} ?; .1ml)

ca 2300.

B. Mix 1ml ~~W518~~ λ (as above) ≈ .1ml 10^{-5} W518. Incubate –

and plate .1ml. 221, 260 $\bar{m}$ = 240

C. Plate 10^{-8} W518 ; 31; 7 ; $\bar{m}$ = 19. Count: 2×10^{9}

D. " 10^{-17} λ. $\overset{8}{14}$(+ some scattered, uncountable); $\bar{m}$ = 11×10^{7}

C shows initial count of 2×10^{9} bacteria. These were, in A, exposed ~~to~~ (2×10^{8}) to ~~2×10^{8}~~ 10^{8} λ. Apparently 2×10^{9} of them survived!! I probably an error in deluting A., unless λ is contaminated I.

In B, where 2×10^{3} were exposed to excess λ, likewise all survived.

Needs repetition.

Pick colonies from A to determine lysogenicity.

3/28/49.

1. Dilute a fresh 518 culture ~~10^{-8}~~ 10^{-6} and plate .1ml for bacterial count

2. Add .1ml to 1ml λ (labelled 3/23: 3×10^9). (dil. 10^{-1}). Incubate 30 mins; ~~Take .1ml / 10 (10^{-2}) .1/10 (10^{-3}) and 1/10 (10^{-4}). Plate .1ml sample to be comparable to above.~~ Wash this tube into 10ml; 2 further 10^{-2} dilutions, then plate .1ml

3. To .1ml sample of 1, add .3ml λ and plate.

4. ~~Assay λ. 10^7~~

1. (No λ). ~~[illegible]~~, 75, 67, 78. ~~Σ = 71~~ m = 72 c.u.

2. 30, 48 m = 39.

3: 45, 54, 80 m = 56

Thus at least 50% of W518 cells survive attack of λ. Colonies 1 are perhaps perceptibly larger than 2 and 3? Pick carefully from colonies 2 and 3 and test for λ+ in W518:

(2) (λ diluted). 29 tests. 28 λ+ 1 λ−. 9 were apparently autolytic.

(3) λ undiluted. 26 tests 17 autolytic 24 λ+.

3/26/49. See 517/or resume

89 Lac+ colonies derived from M189 papilloma on EMS.

On Xyl EMB, there were +/-: Streaks on Lac EMB:

		Lac	Streaks Lac EMB	Streaks Xyl EMB
1	16	+ -	V=	+, -
2	20	+ -	V=	+, -
3	26	+ -	V-	+, -
4	28	+ -	V=	+, -
5	41	+ -	V	+, -
6	47	- +	~~Atypical~~ ✓	+, -
7	~~67~~ 67	+ -	V? +	+, -
8	~~68~~ 68	+ -	V=	+, -
9	~~77~~ 76	+ -	V=	+, -
10	~~79~~ 78	+ -	V=	~~+ -~~
11	79 (pap.)	+ -	++ -	— not V

Xyl - : 14 19 + - ++ - — ; 15 85 ✓ + - ++, - — ; 16 36 + - ++ — ; 17 59 + - +, - — ; coordinately immune.

11 Additional

12	1	Xyl V	Lac V	V+	+, -
13	8	"	"	V?+	+, -

Reisolate from all of these.

Use 1-10, 12, 13 for studies as Lac V.

Isolate 10 Lac+ and 10 Lac- from 494-1. Test on Mtl EMB & T5.

Lac+ : 10 Mtl- $T5^R$

Lac- : 7 Mtl- $T5^S$ 1 Mtl- $T5^R$ 2 Mtl+ $T5^S$.

The Lac+ mutation here is coupled & $T5^R$. mi -1.

Ditto on 494-2. Lac+ : 10 Mtl- $T5^R$

Lac- : 9 Mtl- $T5^S$; 1 Mtl+ $T5^{***S}$. same as -1.

494-4. 10 Lac+ all $T5^S$! } All S !!

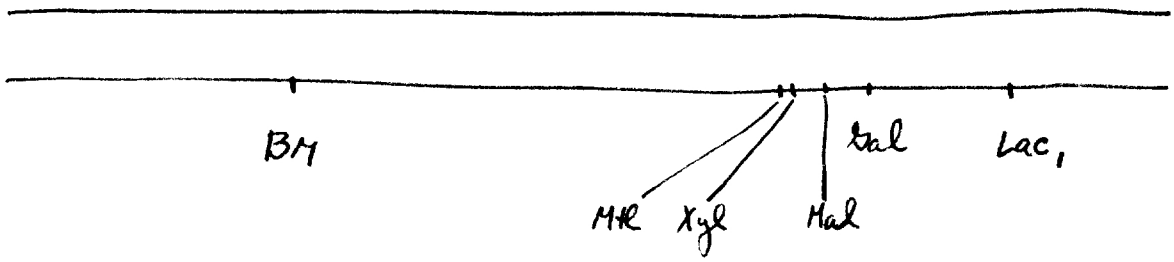
10 Lac- all $T5^S$! }

Analysis of 495 segregation data.

Among 100 Lac+ segregants, following were +

Gal	80
Mal	75
Xyl	70
Mtl	69

This suggests the map order



although Mal - Mtl Xyl - Lac is not excluded. Both hypotheses give 4% of a triple crossover (Mal - Mtl + Xyl + Gal + and Mal + Mtl - Xyl - Gal + respectively).

There would also be 4 other triples.

Determination of V_1^R would not generally be useful except in Lac, - group.

The non-vacant classes include: (Lac+):

X	Mtl	Xyl	Mal	Gal	#
5	+	+	+	+	64
4	−	+	+	+	1
3	−	−	+	+	4
2	−	−	−	+	6
1	−	−	−	−	17
5·4·1	−	−	+	−	2
5·4·2	−	+	+	−	1
5·2·3	+	+	−	+	4
5·4·3	+	−	+	+	1

not observed

Lact+	Gal	Mal	Mtl	Xgl		
1	−	−	−	−	−	
3	−	+	−	−	−	✓
5	−	−	−	−	−	
7	−	−	−	−	−	
11	+	−	−	−	−	
14	−	−	−	−	−	
15	+	+	−	+	−	
16	−	−	−	−	−	
22	+	+	−	−	−	
31	−	+	−	+	−	✓
33	−	−	−	−	−	
35	+	−	−	−	−	
38	+	−	−	−	−	
41	−	−	−	−	−	
43	+	−	−	−	−	
45	+	+	−	−	−	
48	−	−	−	−	−	
50	−	+	−	−	−	✓
52	−	−	−	−	−	
53	−	−	−	−	−	
54	+	−	+	+	×	✓
55	−	−	−	−	−	
57	−	−	−	−	−	
58	+	− ?	+	+	−	✓
60	+	+	−	−	−	
61	+	−	−	−	−	
62	−	−	−	−	−	
67	+	−	+	+	×	✓
68	−	−	−	−	−	
74	+	−	+	+	×	✓
76	−	−	−	−	−	
79	+	−	−	−	−	
91	−	−	−	−	−	
98	+	+	−	−	−	
99	+	+	+	−	−	✓
44	−	−	−	−	−	
64 Others	++	++	++	++		

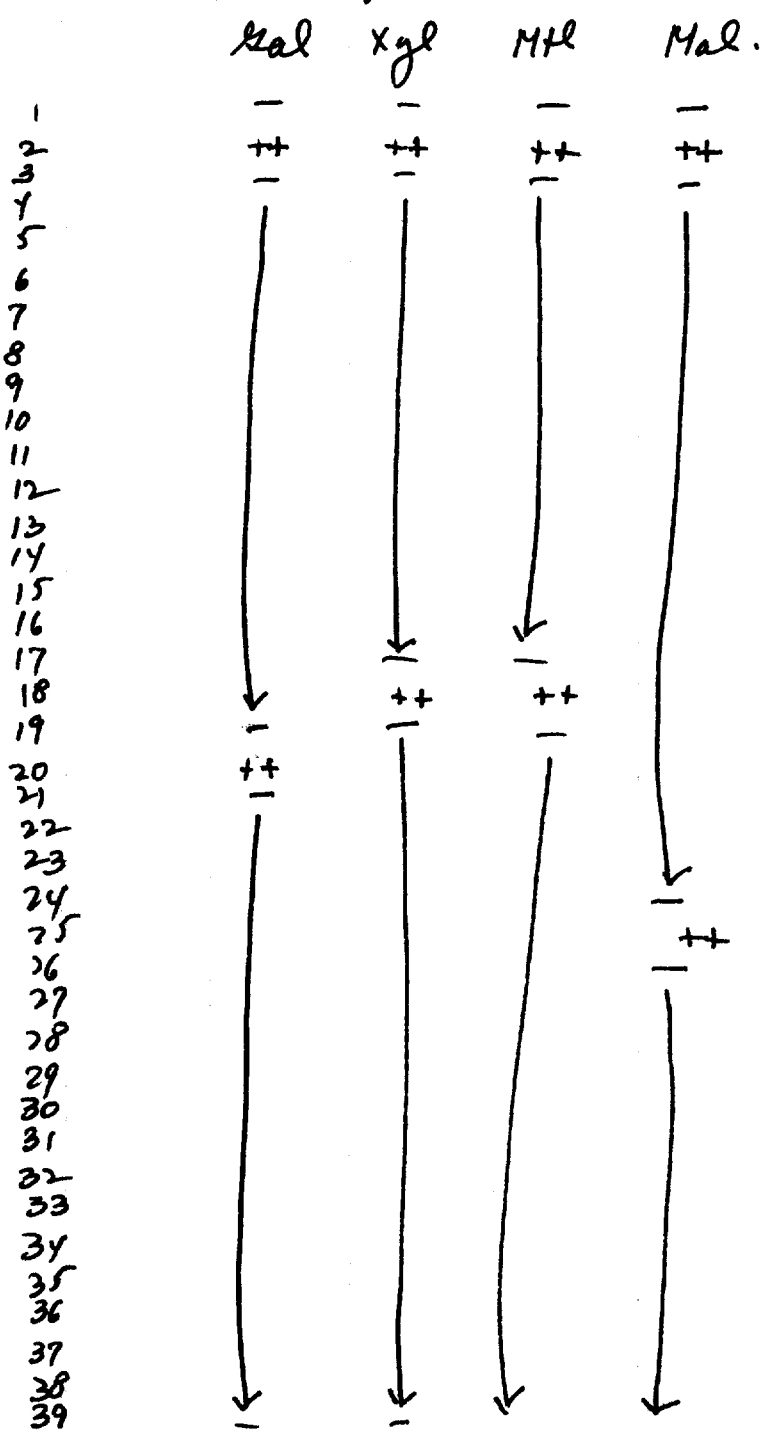
Lac⁻ Gal⁻ linkage test.

4/1/49.

W416 × W677. 100 Lac+ prototrophs tested. No Lac±. Purify + and − and test linkages

~~39~~ Lac− prototrophs tested on

NZ]



NZ]
(1)
100 Lac+ prototrophs.

1-50. All Gal+ except: 1, 3, 5, 7; 14, 16; ~~26, 28~~; 31; 33; ~~32, 34~~; 41; 44; 48?; 50; ~~31, 34, 38?, 40~~

Mal+ " : 1, 5, 7; 11; 14, 16; ~~28~~; 33; 35, 38; 41; 43, 44, 48.

Mtl+ except: 1, 3, 5, 7; 11; 14, 15, 16, 22; 31; 33; 35; 38; 41; 43, 44, 45 48; (50).

Xyl+ 1, 3, 5, 7 11; 14; 16; 22; 33; 35; ~~38~~; 41; 43, 44, 45, 48; 50

51-100. All Gal+ except. 52, 53, 55, 57; 62; 68; 76; 91.

Xyl+ 52, 53, 55, 57, 60; 61, 62, 68; ~~71, 74~~ 76; 79; 91; 98, 99.

Mtl 52, 53, 55, 57, 60, 61, 62, 68; 76, 79, 91, 98.

Mal ~~Xyl~~ 52, 53, (54), 55, 57, (58); 61, 62, 67, 68, 74, 76, 79, 91

Check 58 Mtl+

Adsorption of P19 — 496x

3/28/49.

Add .1 ml 10^{-3} P19 (initially 10^9/ml) to 1 ml (Suppl 1, 2, or 3); Incubate 20 mins. Add 8 ml peptone. Assay A on W518. Centrifuge. Supernatant: Assay B on 518. Assay by diluting (.1 ml / 10) and using .1 ml sample.

1. Add NSB
2. Add W518
3. Add W811.

1A: 0, 4 B: 21, 7. Background very granular.

2A: 27, 19 B: 1, 17. " " . Counts clearly h.g.

3A: 0. Many diffuse plaques, probably λ. B: 12 & 19. Ca 50 λ? ca 20.

This experiment unsatisfactory due to granularity of background. Agar used was probably too old and dry.

Blockade by λ of B/1. 496.

3/28/49

A. Add 10^9 λ .5 ml to .5 ml B/1 suspension 3PM.
If B., control, adding peptone .5 ml. 3:00PM.

At 3:30, Plate .5 ml ē ca 10^5 P19B to test for blockade.

Controls: 0; cluster of minute lysis.
1 colony in each of two plates. Pick these for further test.

B. Add ~~10⁷~~ 10^9 λ to 10 ml NSB. Inoc ē drop B/1. Incubate.
P30. Plate .3 ml of each with ca 10^5 P19B.
No colonies in either!

↓ are resistant to p19 but do not carry λ. Probably spontaneous V_{19}^R mutants. Key ① asw-883

Does P19 displace λ in resistants?

3/28/49.

Plate W811ē excess (10^9) P19. 3 plates.

Picks "resistant" colonies and streaks out to purify. Test for sensitivity to P19, λ and for λ+.

No confluent lysis. Patchy plaques at one corner.

Continuation of H.T. W498.

3/29/49.

A. W826 x W477
B. W836 x W466
C. W

A. W826 x W477
B. W836 x W466
C. W826 x W466
D. W836 x W477

Test Lac + prototrophs for Lac v.

	tests		
A.	48	52/117 Lac−	= 44%
B.	48	143/207 Lac−	
C.	48	19/188 Lac−	= 10% Lac−
D.	48	112/134 Lac−	=

B showed me unlikely but suspicious Lac v. & thus gave ++!
Retest as 498-1.

m Lac EMB, +, − and v colonies seem. Cultivate on EMS Lac as H− 192.

Total

50
60
100
30
97
---
337 plaques tested.

1 differential on ~~p18, p20~~ 518, 811 (p20)

New phages

499.

3/29/49.

Plate .02 ml Chicago sewage filtered with W518. Picks 50 plaques and test on W518 and W811. No differentiation was noted. 1 phage gave very hazy plaques, almost completely filled in. Study as 499-1. Flask out residual growth to test for lysis. 4 single cols: #2, 4 autolytic (flaking). #3 not lytic. #1 slightly lytic. Pick cols. from 1. None lytic. No lysogenicity.

4/1. 60 additional plaques picked and tested on W811; W518: 1 showed a few plaques on W518; none on W811. Restreak as 499-2. Confirmed. Grow out as P20. 4 resistant colonies picked and streaked from zone of CL as 518/p20

4/2. 100 plaques picked and tested as above

4 showed possible differentiation on W518. 1 may show diffuse plaque appearance on 811. Restreak. (499-5).

~~None lysogenic~~. Flow out. Test "resistants" for lysogenicity None differential.

4/6 30 additional & tested. #6 may show differential. check. Not differential.

When streaked out, appears autolytic. Isolate apparently pure colonies. None of 4 were lytic on W811. T.O.

4/9 97 additional & tested. None differential on W518; W811.

Deletions of λ + ε P19

500

3/30/49

Plate 10 W518 ε varying deletions of P19 10⁹

	P19	
1.	0	ca 300
	10^{-1}	
	10^{-3}	
	10^{-4}	0
	10^{-5}	ca 100 small like control; many nibbled. ← least required.
	10^{-6}	as above.

Loop deletion P19 W811. ε .1 ml P19 colonies

0

ca 10^3 "

∴ P19 destroys individual cells of W811, although plaque formation is irregular. Thus P19 is unsuitable for studies on blockade.