

3/14/49.

58-161 x W859 mbar EMS

100 bar + tested. one bar ✓.

W859 does not carry Zet.

H189

~~471~~
477

	Lac ^{EMB}	Xyl.
1	+	-
2	-	-
3	+	-
4	+	-
5	+	-
6	+	-
7	+	-
8	+	-
9	+	-
10	+	-
11	+	-
12	+	-
13	+	-
14	+	-
15	+	-
16	+	-
17	+	-
18	+	-
19	+	-
20	+	-
21	H189	
22	H189	

This 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.

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	"	mq.	n.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	"	+	-

		Lac	Xgl
51	190c	+	-
52	"	+	-
53	"	V-	V+
54	"	+	+
55	"	+	+
56	"	V-	V

Thus, out of 56 trials here, only 6, or $\frac{1}{9}$, are still heterozygous after lac revisions.

This suggests that reverse-mutation may be more frequent in diploids than in haploids.

Label 474:1-6.

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

B1/p₁₀₅/49.

{
 1
 2
 3
 4
 5
 6
 7
 }

1490m

lac
Lat+

Xyl

		-
1	+	-
2	+	-
3	+	-
4	+	-
5	+	-
6	+	-
7	+	-
8	+	-
9	+	-
10	v	v
11	+	-
12	+	v
13	v	-
14	+	-
15	+	-
16	+	-
17	+	-
18	+	-
19	v	v
20	+	-
21	+	-
22	+	-
23	+	-
24	+	-
25	+	-
26	+	-
27	v	v
28	+	-
29	+	-
30	v	v
31	+	-
32	+	-
33	v	v
34	+	-

477-7

477-8

477-9

-10
-11

-12
-13

Since all
Xyl segregants
are Xyl-, they
need not be treated
other than Xyl, merely
fostered.

3/15 - 16/49.

H190 b + c.

Pick single + isolates to EM8 Lac and spot on EMB Xyl.
[Straining is designed with some sig gens are recognized
as ~~Xyl~~ Xyl -].

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

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

Pick from corresponding EM8 Lac spots as 477: 14-17 (b)
Also inoculate into Penassay to allow and 18-24 (c)
sig generation.

Reversions in lac-diploids

477a.

3/15/49.

Tests on 1st 6 Lec v.

Mutants from EMS to

~~EMS~~ Recovery

transf-

	Lac?	Xyl	Mtl	Prod. Lac
189	1	+		
190	2	✓		-
190	3	✓		-
190	4	✓		-
190	5	✓		=
190.	6	✓		=

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

477b From 190 A picks a number of - and + colo. from same papilla
to correlate heterozygosity.

A. Lact	Xyl	Lac-	Xyl.	C.	+	Xyl	-	Xyl	# 14
1	—	—	—	—	1	✓	—	✓	
2	—	—	—	—	2	✓	—	—	
3	—	—	—	—	3	✓	—	—	
4	—	—	—	—	4	✓	—	—	
B	—	—	—	D.	—	—	—	—	
1	—	—	—	—	1	—	—	—	
2	—	—	—	—	2	—	—	—	
3	—	—	—	—	3	—	—	—	
"	—	—	—	—	4	—	—	—	

The heterozygosity of lac+ revertants is probably due only to the fact that the chloramphenicol was randomly segregated.

477-1 turns out to be to be lac+ Xyl- not Xyl+.

Second series: Lec v. includes:

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

Reversion in Lac - diploids

4776.

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

start out Penassay cultures of these new heterozygotes.

	—	+	Pred!
2	109	22	—
3	63	25 (exag.)	—
4	4186	32	—
5	41	23	—
6	79	fewer.	—
7	Lac EMB	Xyl EMB	All of these are pred. Lac −!
8	M190 EMB	Recheck (from EMS lac brushes)	10− : 13+
9			
10			
11			
12			
13			
14			
15		+	
16		+	
17		+	
18	V−	+	
19	+	+	
20	V−	+	
21	++, −	−	
22		+	
23		−	
24		+	
25		+	

Reverses in lac- / lac+

47/c

3/19/49.

check & streaks from E45's lac bushes.

	lac EMB	Xyl EMB	MFE MB
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
9	+	- (v)	- v
10	+ (v?)	- v	- v
11	+,- (=)	-	-
12	v+	- v	- v
13	+	- v	- v
14	v+	- v	- v
15	v-	- (v)	- (v)
16	+	- (v)	- (v)
17	+	- (v)	- (v)
18	v-	- v	- v
19	v+	- v	- v
20	v-	- v	- v
21	++ (-)	-	-
22	v-	- v	- v
23	v-	- v	- v
24	+ (-)	-	-
25	+	-	-

	a: lac EMB	b: lac EMB	(bush) Xyl EMB
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	++	++	++
10	++	++	++
11	++	++	++
12	++	++	++
13	++	++	++
14	++	++	++
15	++	++	++
16	++	++	++
17	++	++	++
18	++	++	++
19	++	++	++
20	++	++	++
21	++	++	++
22	++	++	++
23	++	++	++
24	++	++	++
25	++	++	++

	b: lac	c: lac
1	v-	v-
2	v-	v-
3	v-	v-
4	v-	v-
5	v-	v-
6	v-	v-
7	v-	v-
8	v-	v-
9	++	++
10	++	++
11	++	++
12	++	++
13	++	++
14	++	++
15	++	++
16	++	++
17	++	++
18	++	++
19	++	++
20	++	++
21	++	++
22	++	++
23	++	++
24	++	++
25	++	++

	lac (?)	Reduction
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	-	/

many are -, +
Resemble +. 2 col each

10-
10+

Possibly the
+ + were not recovered
due to difficulty in
distinguishing lac+
from lac- or selection
by lac+.

3/15/49.

Inoculate Y10 8 secs. nutrient agar + EMB Lac as for mutagenic tests. Pick 100 cols and streak on W518 or EMB Lac.

1 colony (from W.A.) apparently λ -. Streak out to confirm streaked 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 dilute plating of W811.
Each lysogenic.

3/16/49

Dilute stock 1 to 10/ml. Add 1 ml of 10% Pneumocay + 1 ml ^W518.
Dispense 1 ml quantities to small tubes & incubate essay.
Incubate at 40° 1 hour; also take initial essay.

A. Initial assays. 5, 5, 4, 3, 7
B. plated after 1 hour. 1, 1, 3, 3, 2

Interval too short for a burst.

Absorption of λ .

480

3/16/49

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 absorption of the phage in B m.s., or else a wide discrepancy in plating efficiency for free phage and adsorbed phage.

lysogenicity and plaques on λ and phage 311

481

3/15/49.

No. Plaques

1-6	1
7-10	2
11-14	3
15-17	4
18-21	5
22-27	6
28-31	7
32-35	8
36-45	9
46-47	10
50-51	11
54-57	12
58-61	13
62-65	14
66-67	15
70-73	16
74-77	17
78-81	18
82-85	19
86-89	20
90-93	21
94-97	22
98-101	23
102-104	24
105-106	25

all λ -
(1 autolytic exception)

94-96: 95 autolytic; does not?
99-100: Autolytic
103-104: "
105-106: Not autolytic

on	in
10378	W811
±	+
±	++ plaque ++
++	++
++	++
++	±
-	-
-	-
++	++
++	++
++	++
++	++
++	++
++	-
++	-
++	++
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Plate 1 ml sewage with 518. 25 plaques found on 5 plate.

Picks and 2) done, streak on 518 to obtain mutants, 4-6-4-3 of each for λ or 518. b) recover from 2 plates and compare action on 518 and 311. #14 is the only phage showing a clear differential. Half from 518 plate and each half.

a) Picks 94, 96, 106 and streaks to check λ .

b) Rechecks: Phage "14" is active on 518, 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 brush of 481-106 (= 482A) for lysogenicity.

8 single colonies tested. None were $\lambda+$. Brush showed λ and faint amount of phage as streaked. General comportment like Sp14. Put on slant to store for later manipulation.

Growth of λ and bacteria in W811.

483

3/18/49

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

1. A (bacterial count) = 518 1:30 P.M. .5 ml

B. phage on 518.

2. 2:45 P.M. .2 ml

3. 3:30 .1 ml

4. 4:40 .1 ml

5. 5:40 .1 ml

6. 7:15 .001 ml

	Counts:		corrected
	A	B (k)	A - B (k)
1	386	11	2900 - 110
2	304	5	
3	462	7	
4	4000	34	
5	8500	635	
6	9000	114	

	Arc/ml	log A.	log B.
	A	B (k)	
	772	2.9	1.3
	1520	3.2	1.4
	4620	3.6	1.8
	40,000	4.6	2.5
	85,000	4.9	3.8
	9×10^6	7.0	5.0
	114,000		

high!

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

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

~~Interference of λ~~

453A

3/20/49.

1. Assay λ (ca 2×10^4) by a 10^{-2} dilution on W518.
2. Add ~~1 ml P19 (2×10^4) to 1 ml W518~~ to assay for resistance
~~Plate .2 ml samples.~~
3. Add 1 ml λ + 1 ml W518. Incubate ~~20~~ ~~30~~ 30 mins. Then add P19 1 ml. Plate .3 ml samples.
2. ~~Literally~~ 3, using broth for λ .

(4) Assay bacteria.

1. λ was $56 \times 200 = 10^4$ /ml.
- 2: Resistant to P19. 81, 113, 92, 47
3. λ - " 42, 101, 12, 84

The aims of this expt. may be injured by the presence of P19.

Virtually all colonies in ② and ③ were heavily mucoid.

3/15 + / 49.

Mix 2 tubes #2 & H168 from EM5 broths.

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 sum to be
 mixed!
Strain out the questionable on marmitol.

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+

785

94-176 "Lac -"

94, 96, 97

Counts on plating:

A: Lac.	316 +	356	B:
	<u>32</u> -	<u>55</u>	
	38 V	41	

Lac	200 +	383 +
	<u>23</u> -	<u>29</u> -
	9 V	11 V

Gal Too heavy for most part.

330 +
43 -

50 ha tested all U_5^R , but some are injure

3/29/49: stockout colonies from EMS bac pass 485:1, 5-7.

① 1 quadrants. + ped.

⑤ 4 quad. + $\frac{ca}{ca}$ -

⑥ 2 halves + $\frac{ca}{ca}$ -

⑦ 1 quad. + $\frac{ca}{ca}$ -

No persistence of predominant character.

H/68'

2-3" 4/

No.	From	Spec	Count
1-12	168-6-c-neg	397	{ 133
13-24	168-6-c-pos	2	{ 123
25-30	168-6-d-neg	1	{ 384
31-36	168-6-d-pos	1	{ 244
37-40	168-1-a-neg	1	{ 144
41-44	168-1-a-pos	1	{ 124
45-54	168-1-b-neg	1	{ 124
55-64	168-1-b-pos	1	{ 124
65-68	168-1-c-neg	1	{ 124
69-72	168-1-c-pos	1	{ 124
73-78	168-1-d-neg	1	{ 124
79-84	168-1-d-pos	1	{ 124
85-86	168-1-e-neg	1	{ 124
87-88	168-1-e-pos	1	{ 124
89-100	168-5-a-neg	1	{ 124
101-112	168-5-a-pos	1	{ 124
113-127	168-5-b-neg	1	{ 124
128-142	168-5-b-pos	1	{ 124
143-172	168-5-c-neg	1	{ 124
173-202	168-5-c-pos	1	{ 124
203-206	168-5-d-neg	1	Milk
207-210	168-5-d-pos	1	
211-214	168-5-e-neg	1	
215-218	168-5-e-pos	1	
219-224	168-7-a-neg	1	{ 124
225-228	168-7-a-pos	1	{ 124
229-252	168-7-b-neg	1	{ 124
253-271	168-7-b-pos	1	{ 124
272-288	168-7-c-neg	1	{ 124
289-318	168-7-c-pos	1	{ 124
319-338	168-7-d-neg	1	{ 124
339-361	168-7-d-pos	1	{ 124

Predans.

1:	4	-
5:	+	
6:	+	
7:	-	

$$\frac{231+}{18-}$$

March 25- 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 sihship, 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

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

Lac- ~~MX~~ selections

Lac# selections:

X#	M#	M-		X#	M#	M-
X#	10	0	10	X#	38	0
X-	1	99	100	X-	6	66
			110			72
						110

Among Mtl selections:

L#	L-		X#	X-
M#	26	30	46	10
M-	32	35	0	67
M#:::	X# 19	27	X# 0	X- 0
X-	7	3	X- 32	X- 35

Chicks of Lac+ & Mtl+
selected birds

	Lac- selection				Lac+ Selections			
	M+X+	M-X-	M+X-	M-X+	M+X+	M-X-	M+X-	M-X+
168-6	0	12	0	0	2	7	3	0
-1	5	37	0	0	3	9	0	0
-5	0	56	0	0	22	32	3	0
-7	5	24	1	0	11	18	0	0
	10	99	1	0	38	66	6	0

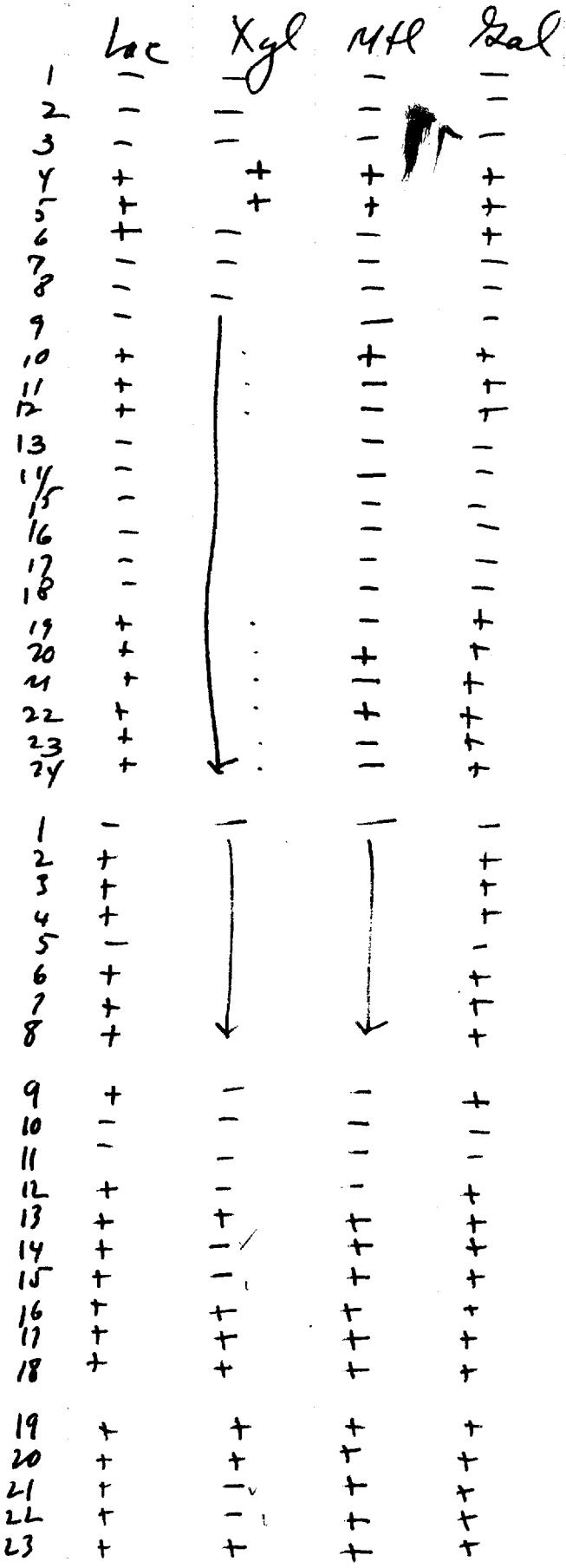
	Mtl- selection				Mtl+ selection			
	L+X-	L+X+	L-X-	L-X+	L+X-	L+X+	L-X-	L-X+
-6	12	0	5	0	7	10	0	0
-1	10	0	4	0	0	0	1	13
-5	4	0	4	0	1	5	0	0
-7	6	0	22	0	0	4	2	14
	32	0	35	0	7	19	3	27

Segregation ratios:

	lac		%	Mtl		%
168-6	+	-	+	+	-	+
6	231	18	93	61	95	39
1	21	159	13	73	98	43
5	1300	147	90%	20	690	3
7	50	390	11%	74	212	26

Note variability in all ratios.

H168



H168'

Tar Xyl mtl Gal

24 +

25 -

26 +

27 +

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 -

56 -

57 -

58 -

59 -

60 -

61 -

62 -

63 -

64 -

65 +

66 +

67 +

68 +

69 +

H168'

	Lac	Xyl	Mtl	Gal
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	- - - - -	-	-	-
101	- - - - -	-	-	-
102	- - - - -	-	-	-
103	- - - - -	-	-	-
104	- - - - -	-	-	-
105	+	-	-	-
106	+	-	-	-
107	+	-	-	-
108	+	-	-	-
109	+	-	-	-
110	+	-	-	-
111	+	-	-	-
112	+	-	-	-

H168'

	Tac	Xyl	Mal	Gal
113	-	-	-	-
114	-	-	-	-
115	-	-	-	-
116	-	-	-	-
117	-	-	-	-
118	-	-	-	-
119	-	-	-	-
120	-	-	-	-
121	-	-	-	-
122	-	-	-	-
123	-	-	-	-
124	-	-	-	-
125	-	-	-	-
126	-	-	-	-
127	-	-	-	-
128	+	-	-	-
129	-	-	-	-
130	-	-	-	-
131	-	-	-	-
132	-	-	-	-
133	-	-	-	-
134	-	-	-	-
135	-	-	-	-
136	-	-	-	-
137	+	-	-	-
138	-	-	-	-
139	-	-	-	-
140	-	-	-	-
141	-	-	-	-
142	-	-	-	-
143	-	-	-	-
144	-	-	-	-
145	-	-	-	-
146	-	-	-	-
147	-	-	-	-
148	V	-	-	-V
149	-	-	-	-
150	-	-	-	-
151	-	-	-	-
152	-	-	-	-
153	-	-	-	-
154	-	-	-	-
155	-	-	-	-
156	-	-	-	-
157	-	-	=	-

H168

Lar Xyl Mtl Gal

158
159
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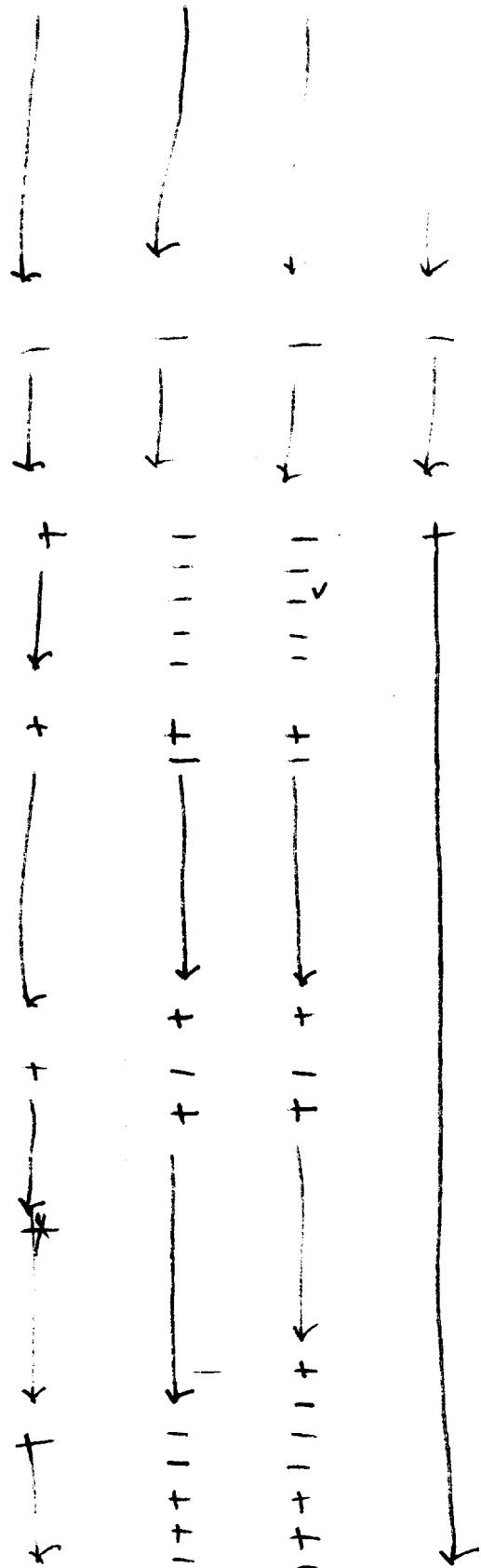
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201
202



H168'

Lec Xyl Mtl Gal

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

H 168'

Lac Zyl McI Gal

248 -
249 +
250
251
252
253
254
255
256
257
258
259 -

260 -
261 -
262 -
263 -
264 -
265 -
266. -
267 -
268 -
269 -
270 -
271 -
272 -

273 +
274 +
275 +
276 +
277 +
278 +

279 -
280 -
281 -
282 -
283 -
284 -
285 -
286 -
287 -

288 -
289 -
290 -
291 +
292 -
293 +

(?)v

H168-

	Lac	Xyl	Mal	Gal
294	+	+	+	+
295	+	+	+	+
296	+	+	+	-
297	-	+	+	-
298	-	+	+	-
299	-	-	-	-
300	-	-	-	-
301	-	-	-	-
302	-	-	-	-
303	-	-	-	-
304	-	-	-	-
305	-	-	-	-
306	-	-	-	-
307	-	-	-	-
308	-	-	-	-
309	-	-	-	-
310	-	-	-	-
311	-	-	-	-
312	-	-	-	-
313	-	-	-	-
314	-	-	-	-
315	-	-	-	-
316	-	-	-	-
317	-	-	-	-
318	-	-	-	-

Cross streaks in very heavy phage suspension.

P19:	K-12	++!	(mutant?)
	W435	++	
	W518	++	
	W877	-	

B/1	2 plaques
B/2	-
B/3,4,7	- (1 plaque?)

	T1	T2	T4	T5	T6	T7	P14	λ	P19
W518	$\pm(\lambda?)$	++	++	-	++	++	++	+	++
W877	-	++	++	-	++	++	-	-	++

\therefore P14 interferes 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 A418 and 671 into 10 ml Y2 Dext.

Plate out 10^{-3} and 10^{-5} ml, E. coli lawns (as described in prep)
Actual value $\times 10^{-2}$.

Initial, at 10^{-3} :

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

Final 2820:

19	13		
16	9		
18	12		
6	18		
12	18		
<hr/>		64	70
			134

$$\chi^2 = 2.9$$

$$p = .09$$

$\frac{63}{56} \quad 91 \frac{84}{77}$

$\frac{57}{64} \quad 70 \frac{77}{77}$

120 161

147

134

281

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

$$.016$$

$$.018$$

$$.012$$

$$.013$$

$$.059$$

$$\times 49$$

$$\underline{2.9}$$

Analysis of 4x4 data.

+		Σ
32	55	86
31	36	61
25		
56	91	147

$$\begin{aligned}
 \chi^2 &= 4 \left(\frac{1}{33} + \frac{1}{23} + \frac{1}{33} + \frac{1}{38} \right) = 4 \left(.03 + \cancel{.04} .02 + .03 \right) \\
 &= 4(.12) = .5 \quad p = \cancel{.02} 0.3
 \end{aligned}$$

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	34

a. plate totals. $\chi^2_4 = \frac{1}{29} (\frac{25+4+16+1+9}{9+16+36+25+121}) = \cancel{.02} 6.3$

$$= \cancel{.02} 2.3 \quad p = \cancel{.02} 0.6$$

agreement in aggregation: $\chi^2_4 = \frac{16}{17} + \frac{16}{15} + \frac{16}{12} + \frac{16}{13} + \frac{36}{12} + \frac{36}{12} + \frac{4}{14} + \frac{4}{16}$

$$= .94$$

$$1.07$$

$$1.33$$

$$1.23$$

$$3.00$$

$$3.00$$

$$.25$$

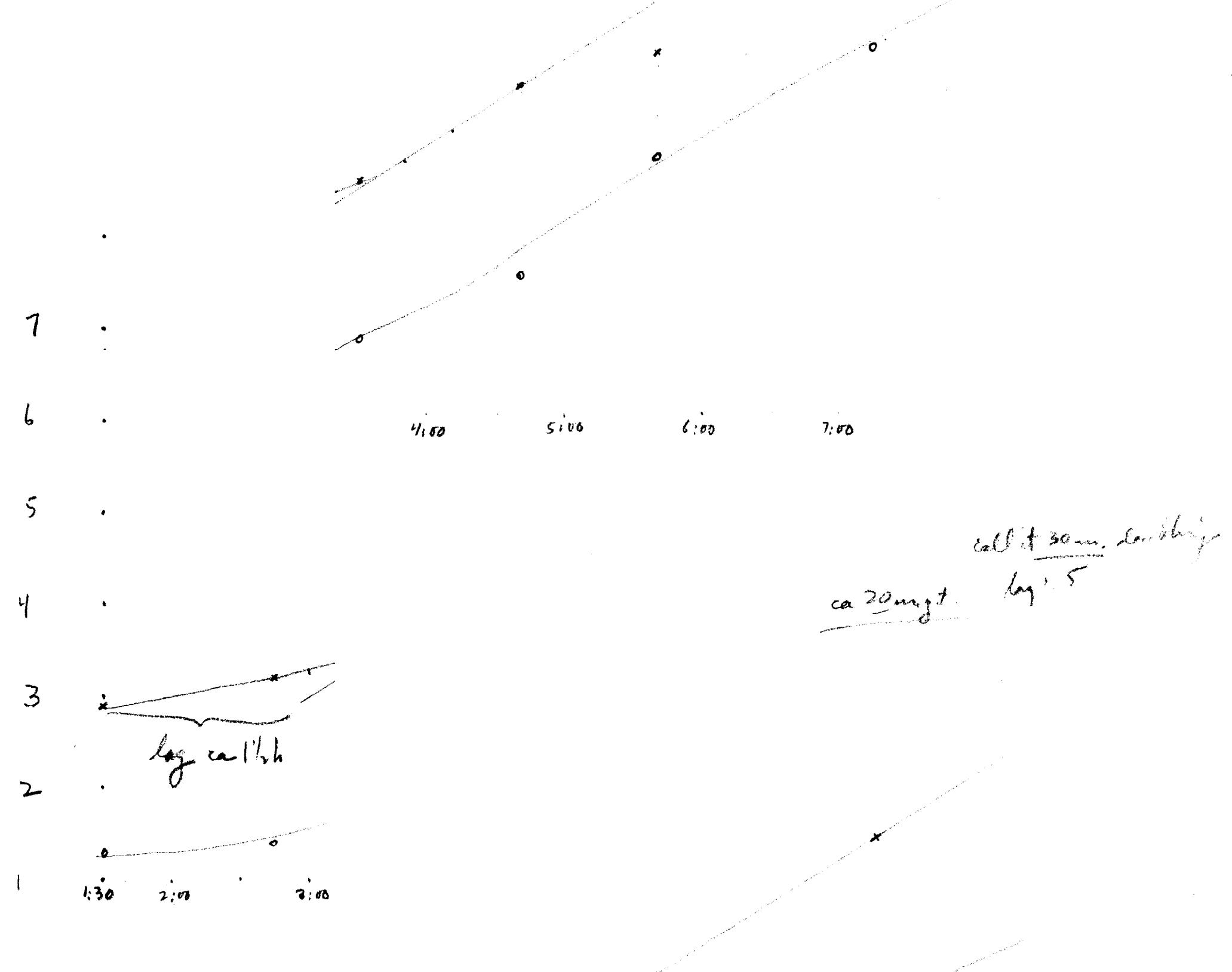
$$.29$$

$$\underline{11.11}$$

$$= 11.11$$

$p = .025$ for homogeneity.

Probably due to clumps of lac or + which are dispersed by ~~the~~ spreader



3/22/49.

Mix = 1 ml of 10^{-5} dilution of a 518 suspension (resuspended in Y_2).
= 1 ml of $[10^9]$ ~~10³~~ diluted as indicated. incubate 30 m.
refrigurate 30 m.

A. No p plate = 3 ml soft agar.

B. 10^9 C. 10^7 D. 10^5 E [↑] assay 42×10^7 (lower count than expected!)F. W518 assay. 3×10^{10} initially; should have been ca. 3×10^{11} with which is in reasonable agreement?A ca $4 \times B$. 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 at a higher dilution of cells.

↑ plaques in citrate seem to be smaller but clearer. Could this be a condition for lysogenicity.

3/19/59

Add 0.1 ml p19 (2×10^4) to col. mix W518. After 10 min. 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 mutant.

Plate out p19 at various dilutions on W811 to determine prevalence of the mutant. Do. on K-12.

3/21.

P19: 10^{-1} ca 10^2 10^{-3} : 7 plaques with 'holes' 

B/1 1 plaque picked P₁₉0, very heavy plaquring but can't pick. 'holes' noted.
Results, and pick a labeled plaque for P19hb, and P19hb₂.

P19/518 for titr. $10^{-7} \times 10^2 = 10^2$ or.

P19/811. 10^{-7} shows 8 plaques. 10^{-3} confluent at edges 10^{-1} shows plaque formation, probably in secondary growth.

[Does p19 multiply in p19's lysis?] Pick plaque at 10^{-7} and grow on W811. /K-12. Same appearance at 10^{-5} .

518/9: 7 mucoid colonies, no purified. 5 are 10^3 , with many mucoid resists. 2 are very thin non-mucoid. Strain these out for culture.

∴ p19 although it is somewhat purified with by & 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 O/1 bef on 3/22/49; grown on O/1 in NSB overnight and filtered A23. At 10^7 , no plaques noted after 5 h., 10^{-5} gave 9 plaques on 811; 37 m 518.

$\times 10^8$ plated in W811 or in W811 + W518 gave 1 plaque on these plates. This may be a contaminant, but grow out for tests.

Repeat at 10^{-5} : B/1 18
518 28
811 0

Note contradiction in 811!

P19B, then, has opt. activity on 518 or B/1 but not in 811.

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

P19. At 5 hours, 10^{-7} gave 16 hours: 136; unf. around edges 10 m 518, none on 811

10^{-5} gave 0; 10^{-3} gave about 100 vague plaques, irregularly visible on plate (probably low plating efficiency), two clear plaque picked for isolation of possible mutants. At 18 hours, repeat at 10^{-5} , 10^{-7} m 518, 811.

8 plaques noted in 811 at 10^{-5}

P19 10^{-1} /811 gave irregular complete lysis = uncoated resistant.

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

10^{-7} 217 m 518 0 m 811. $\therefore P19\lambda = 3/217.00 = 1/7090$
Plaques on 518 are large with spreading hole; on 811 are small and circumscribed

$\lambda, 3 \times .3 \text{ ml } 10^{-7} \text{ m B/1} \Rightarrow \text{no plaques}$

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.

p14

w877 is a mass culture of w518 [~~at~~] 7. and are single colony
isolates which are not lytic and are resistant to p14.
However, at regions of cross-streaks, they show a very faint increase
in opacity, but no growth inhibition. After 2 s.c.i., use for
studies on "growth in them."

--w811 Technique.

diluted w811, plated with w518 at different cell densities, gave
no plaques, either at room temperature or at 37.

3/21/49.

Add p^{14} to 10 ml so that 10^{-3} ml will yield 10 plaques. i.e.,
 10^5 particles. (1 ml 10^{-4} dilution of stock)

- A). Assay stock p^{14} to verify addition: Confluent lysis over part of plate
B). Incubate tube E W8776 to determine any growth of p^{14} .

196 plaques counted at 10^7 . Plaques generally very closely. 1 clear spot noted. Picture as possible p^{14}

$\lambda + W518$

492

3/23/49.

A. Mix 1 ml $W518$ culture \approx 1 ml $\lambda \times 10^9$ (+) Incubate 4:35 - 5:05.

Dilute 10^{-6} and plate. (i.e., 10^{-5} ; .1 ml)
 ≈ 2300 .

= 30 mins.

B. Mix 1 ml ~~$W518$~~ λ (exabove) \approx .1 ml $10^{-5} W518$. Incubate -
and plate .1 ml. 221, 260 $\bar{m} = 240$

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

D. " $10^{-7} \lambda$. $\frac{8}{14}$ (+ some scattered, unreadable); $\bar{m} = 11 \times 10^7$

C shows initial count of 2×10^9 bacteria. These were, w/A, exposed
~~to~~ (2×10^8) to ~~2×10^8~~ λ . Apparently 2×10^9 of them sur-
vived!! [probably an error in diluting A, unless λ is contaminated].

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

Nude repetition.

Picks colonies from A to determine lysogenicity.

3/20/49.

1. Dilute a fresh 518 culture to 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; ~~Test .1ml /10 (10^{-3}) + /10 (10^{-5})~~ and ~~+ /10 (10^{-6})~~. Plate .1ml sample to be comparable ~~to above~~. Wash the 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 . Dose?~~

1. (No λ). ~~55~~, 75, 67, 78. $\Sigma = 74$ m = 72 cu.

2. 30, 48 m = 39.

3: 45, 54, 80 m = 56

Thus at least 50% of 4518 cells survive attack of λ .

Colonies 1 are perhaps perceptibly larger than 2 and 3?

Fish carefully from colonies 2 and 3 and test for $\lambda +$ in W518:

- (2) (λ diluted). 29 tests. 28 $\lambda+$ 1 $\lambda-$. 9 were apparently autolytic.
- (3) λ undiluted. 26 tests 12 autolytic 24 $\lambda+$.

3/26/49.

See 517/or
reunite

89 Lac+ colonies derived from H189 papillaeon EMBS.

On Xyl EMB, these were +/ - : Chrysom Lac EMBS:

	Lac	Steale	Beech	Chrysom Lac EMBS:
	LacEMB	XylEMB		
1	16	+	-	V = +, -
2	20	+	-	V = +, -
3	26	+	-	V - +, -
4	28	+	-	V = +, -
5	41	+	-	V = +, -
6	47	-	+	H +, -
7	67	+	-	V? + +, -
8	68	+	-	V = +, -
9	79 76	(76)	+	V = +, -
10	79 78		+	V = +, -
11	79 (pop.)	+	-	+ + - - not V

Xyl -	11	19	+	+	-
	17	85	+	++	-
	18	36	+	++	-
	17	59	+	+, -	-

} evidently mutants

11 Additional

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

Resist from all of these.

Use 1-10, 12, 13 for study as Lac^v.

Isolate 10 Lac+ and 10 Lac- from 494-1. Test on Mtl EMBS + TS.

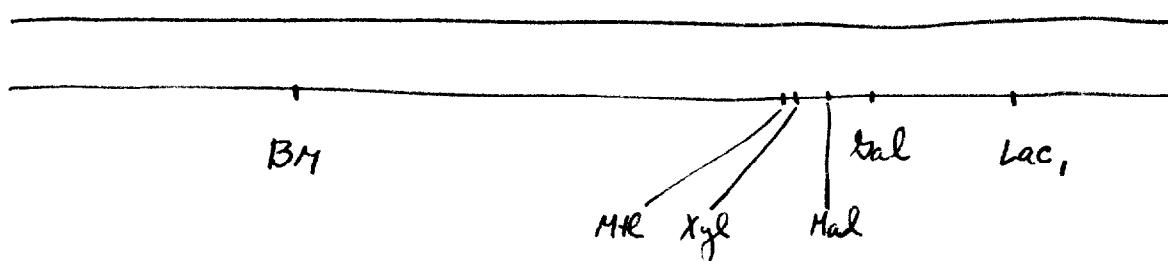
Lac+ : 10 Mtl - TS^RLac- : 7 Mtl - TS^S 1 Mtl - TS^R 2 Mtl + TS^S.The Lac+ mutation here is coupled \cong TS^R. m - 1.Ditto in 494-2. Lac+ : 10 Mtl - TS^R
Lac- : 9 Mtl - TS^S; 1 Mtl + TS^R. same as - 1.494-4. 10 Lac+ all TS^S! } All S !!
10 Lac- all TS^R! } All R !!

Analysis of 495 segregant 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_i^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

skipped

Lact	<u>Gal</u>	<u>Mal</u>	<u>Mfr</u>	<u>Xyl</u>
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.

495a.

4/1/49.

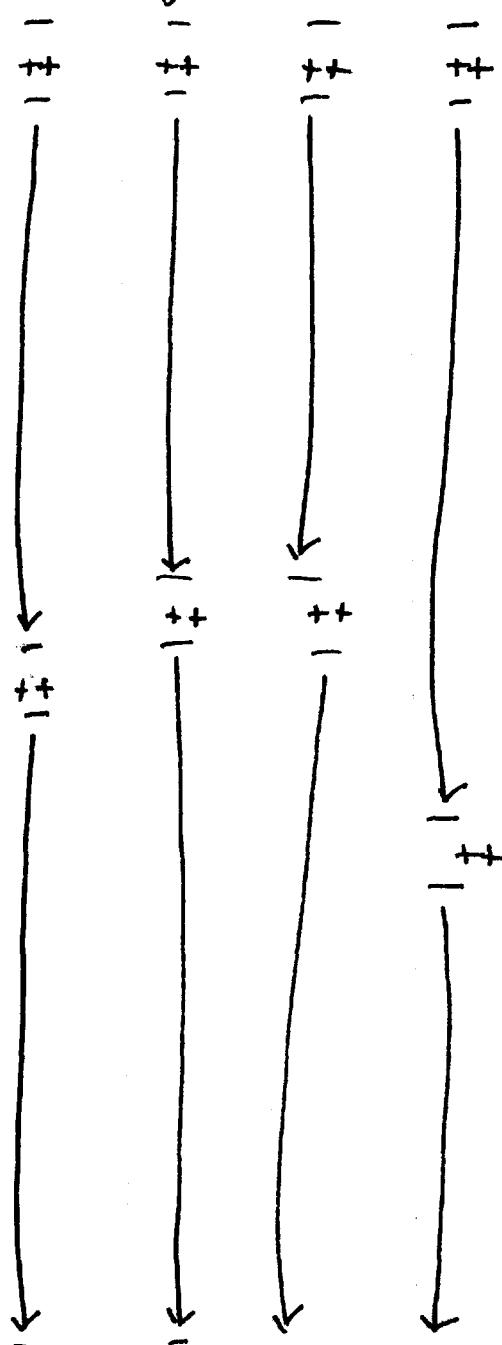
100 Lac+ prototrophs tested. No Lac₋. Purify + and -
W416 x W677. and test linkage

39 Lac- prototrophs tested or

N2]

Gal Xyl Mtl Mal.

1
2
3
4
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38
39



N2]

100 loc + photographs.

31; 33

~~32, 34~~; 41, 44, 48?, 50

①-50.

All Gal+ except: 1, 3, 5, 7; 14, 16;

~~26, 28~~~~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).

Xgl+

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.

Xgl+

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 ~~58~~52, 53, 54, 55, 57, 58; 61, 62, 67, 68, 74, 76, 79, 91Check 58
Mtl+

Absorption of P¹⁹

496x

3/28/49.

Add .1 ml 10^{-2} P¹⁹ (initially 10^4 /ml) to 1 ml (Suppl 1, 2, or 3), incubate 20 mins. Add .8 ml peptone. Assay A on WS18. Centrifuge. Supernatant: Assay B on 518. Assay by diluting (.1 ml / 10) [#] and using .1 ml sample.

1. Add NSB
2. Add WS18
3. Add W811.

1A: 6, 4 B: 21, 7. Background very granular.

2A: 27, 19 B: 1, 17. " " Counts clearly b.s.

3A: 0. Many diffuse o B: 12 p 19. Ca \leq D λ ? ca 20.
plaques, probably λ .

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

3/28/45

- A. Add $10^9 \lambda$.5 ml to .5 ml B1 suspension 3PM.
of B1, control, adding peptone .5 ml. 3:00 PM.

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

Controls: 0; cluster of mispunct lysis.
1 colony on each of two plates. Picks these for
further test.

- B. Add \cancel{to} $10^9 \lambda$ to 10ml NSB. Incubate B1. Acetate.
P30. Plate .3ml 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. Keep ① as W-883

Does P19 displace λ in reconstituted? 497.

3/28/49.

Plate W811c 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.

3/29/49.

- | | |
|---------------------------|----------------|
| A. W826 x W477 | A. W826 x W477 |
| B. W836 x W466 | B. W836 x W466 |
| C. W | C. W826 x W466 |
| | D. W836 x W477 |

Test lac + prototrophes for lac^v.

A. 48 ^{tests}	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. Other pure ++!
Retest as 498-1.

mlac EMIB, +, - and v colonies seen. Cultivate on ETYS Lac
as H- ~~192~~. 192

Total



50

60

100

30

97

337 plaques tested.

1 differential n ~~p₁₈, p₂₀~~
5/18, 8/11 (p20)

New phage?

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 hairy plaques, almost completely filled in.
Streak out residual growth to test for lysis.
4 single cols: #2, 4 autolytic (Helsing). #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 different plaque appearance on 811. Recheck. (499-5.)

None lysogenic. Throw out. Test "resistants" for lysogenicity.
None differentiated.

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

When streaked out, appears autolytic. Isolate apparently pure colonies.
None of 4 were lysogenic as W811.

4/9 97 additional & tested. Nonedifferential on W518; W811.

Dilutions of $\lambda + \epsilon$ P19

500

3/30/42

Plate 10⁷ W518 in varying dilutions of P19 10⁹.

P19		
0	ca 300	
10 ⁻¹		
10 ⁻³		
10 ⁻⁴	ca 100 small	← least aggr.
10 ⁻⁵	like control; many nibbled.	
10 ⁻⁶	as above.	

loop diluted W811. ϵ .1 ml P19 colonies

0

ca 10³

"

∴ p19 destroys individual cells of W811, although plaque formation is irregular. Thus p19 is unsuitable for studies on blockade.