

June 14, 1948.

Test, on T1 + T1h (recd from Kovitch):

	T1	T1h.
B/1	R	S
B/1,5	R	R
B/4	S	S
K-12	S	S
Y40		
W400.	R	R

$\therefore$   $V_{ia}^R$  in K-12 is not entirely homologous with B/1 either with respect to tryptophane requirement or to sensitivity to T1h.

T1h ( $10^9$ ) plated with ca  $10^8$  W400 +  $10^8$  K-12. Uniform growth of bacteria - 1 possible plaque (v. small) - streak back on W400. No plaques.

June 15, 1948.

Variations in concentration, in nutrient agar + 1% lactose.

per ml	K-12	S-20.
50r	faint red	Borders of streak + i.c. stained.
100r	beginning red	more thoroughly stained
200r	W.I.C. deeply stained.	"
500r	" v. " "	" " " " " "

50r + Brilliant Green 25r. — sharply inhibited. A few red resistant.

Variation in nutrient medium - 50r T2/ml. Agar 1.5% Lactose 1%  
 K-12 S-20.

- |                  |                         |  |
|------------------|-------------------------|--|
| 1. Peptone 1%    | WIC faint red.          | WIC deep red                                     |
| 2. " 1/2%        | Some large colonies red | Some WIC deep red.                               |
| 3. N2 Case 1%    | faint red.              | All IC deep red; borders of streaks are stained. |
| 4. Casein Hc 1%  | All -, except near 50r. | All colonies uniformly deep red.                 |
| 5. N2 Tare 1%    | —————>                  | Intermediate between 4 and 3.                    |
| 6. N2 Amine B 1% | Well isolated faint red | W.I.C. deep red.                                 |
| 7. " " A 1%      | All colorless           | All colorless.                                   |

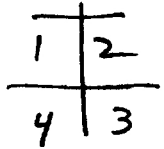
④ is the most satisfactory medium here encountered, giving a uniform intense red reaction. 50r may be optimal level. Expt. 5 variations in T2 concentration, pH of medium + addition of Brilliant Green.

T2 Reagent for enteric pathogens.

227a

June 17, 1948.

Make up lactose agar with Casamino acids 1%, Yeast Extract .1%  
 Streak out ① K-12 ② *Shigella flexneri* ③ 753 ④ S-20. P17.



N18: ① ② ③ ④  
 50r T2. faint red near Host colonies are inhibited but Many large up colonies.  
 - Y.Cx. ④ *A. flexneri* small, deep red. deep red Entire growth red.  
 white.

50r T2 + Y.Cx. As above. K-12 a little redder in their parts of the plate, near S20.  
*Shigella* much larger.

Mucose. All white. faint pink in spots.  
 Maltose. ④ All red. 1 & 2 are faint red in certain colonies (all *Salmonella*?).  
 Galactose. All ~~red~~ All white.

2% Casamino acids. K-12 colonies near S20 are red. Y-53 most inhibited, but red.  
 Brilliant Green 25r All inhibited except S20 - good red colonies.  
 " " 10r All but S20 inhibited.  
 T2 10r. *Shigella* red & white colonies. 753 spotty red streaks. S-20 Uniform light dirty red.  
 T2 25r. As 10, more intense.  
 T2 50r, preautoclaved. Like standard.

See EMB. ① large white ② inhibited ③ + ④ large white.

Grow Y2 broth cultures of: *shelae overnight.*

S20

S21

Numerous plaques.

S22

S23

S39

S40

S43

S46

Numerous plaques. (maybe confused with S22 serum).

S56.

Sediment most of the cells + heat supernatant 30 m. @ 57° to kill cells.  
Spread S36 (*Gallinarum*) as N.A. and inc.  $\bar{c}$  log<sub>10</sub> of supernatant  
to test for lysozymicity.

Use S21 as the standard for possible studies on lysozymicity.  
(Mutants can be used on synthetic plates).

Add 2 ml supernatant + 1 drop S36 culture + shake overnight.  
Sediment, add supernatant to fresh S36 culture, shake 6 h., sediment  
+ filter. = S55.

Salmonella - irradiation for mutants on T2. 229

June 19, 1948.

spread SY7 + SY8 on galactose T2, + a few plates each of  
glucose, mannitol, + gluconate T2.

# Cross tests of *Salmonella* phages.

June 17, 1948.

On bac T2 plates, spread 1 drop of  $\phi$  + 1 drop bacteria.

S20

S21.

~~S20~~

Sp-1  $10^3 - 10^4$  tiny plaques, but no confluent lysis.

numerous plaques, obscured by smearing of resistant?

Sp-2 Confluent lysis + a few dozen large and resistant colonies.

A few plaques noted. See above?

Sp-3 ? Smear areas of lysis.

Confluent lysis obscured by smearing.

All plaques are quite small when noted. Recover large

plaque phage from original streakings from tube phage.

Cross-streak on T2 agar: Sp-1, Sp-2, + Sp-3 + Sp-4.

Sp-5, smear on S36 shows no plaques (smearing?) but when streaked exhibits numerous plaques.

S-20

S-20

S-20/2

S-20/2

S-20/2

S36

} No lysis.

} lysed only by Sp-5.

June 16, 1948.

Plate 1 drop Y10 + 1 drop (ca  $10^9$ ) phage on EMBLac. (-NaCl!)

- T1 Uniform lysis. Ca 100 ~~to~~ resistant. Test these on T5, T1h.  
 T2 v. numerous small plaques peripherally; cleared area centrally.  
 T6 ditto.  
 T7. Uniform lysis. Ca 100 resistant.  
 T1+T7. No survivors.  
 T1+T2. Edges of some colonies irregular. Otherwise like T1 only.  
 T1+T6. Numerous (ca 50-100) resistant, many with plaques in them.

Omission of salt may have prejudiced these results. Repeat the series + check sensitivity to phages.

100 Y10/1 were tested on T1h and T5. 99 were resistant to both  
 1 was T1h<sup>R</sup>; T5<sup>S</sup>.  
 Subculture as W-401  
 = Y10 V<sub>1a</sub><sup>R</sup>.

June 18, 1948.

Plate 1 drop (= ca  $10^8$ ) Y10 + 1 drop (ca  $10^9$ ) phage on nutrient 16 layers.

T1. Uniform lysis. Ca. 300 resistant.

T2. Uniform lysis. Ca. 10-12 (mucoid?) resistant.

T3k. U.L. Ca. 10-20 resistant.

T4. Uniform lysis. 2 mucoid resistant.

T5. U.L. Ca 300 resistant.

T6. U.L. Ca 100 resistant.

T7. U.L. Ca 200 R. (spreading contaminant).

T1+T2. Ca 10-12 R. (Some nibbled).

T1+T3 1 nibbled resistant

T1+T4. 2 mucoid resistant.

T1+T6. 1 mucoid; 1 "non"-mucoid resistant (rest?)

T5+T6 10 mucoid resistant.

T1+T7 1 tiny colony, probably cont.

T5+T7 No resistant

grew out as mucoid lact.  
"purify" as W-402.  
pulsas 231-1.

pulsas 231-2  
did not grow out on lac E M13

[ Compare with E. coli B where, according to Demerec + Lucia, the combinations 1,4 ; 1,5,4 ; 2,3,4,7 ; 1,2,3,4,6,7 occur with some frequency. (1,6) combinations should be studied more extensively, also using coli B. ]



June 19, 1948.

Test Y10/1 m. (on nutrient salt agar).

T1h	T5	
S	S	4
R	R	96.

Y10/5 m T1 51 all R  
Y10

Y10/6 m T2, T4.

5 tested, T2<sup>s</sup> T4<sup>s</sup>. 16 more tested. T2<sup>s</sup>.

∴ 21/21 T6<sup>R</sup> are T2<sup>s</sup>. This differs from (B).

→ Purify as 231b 1-4. Check for T1 resistance.

Test on nutrient NaCl Agar (NSA):

	T1	T1h.
W400	R	R
W401	R	S?
-1	R	S *
-2	S	S *
-3	R	S *
-4.	R	S *

(T1-sensitive —)

\* These streaks show a heavy underlying layer of growth which may also be indented with plaques. This makes scoring somewhat uncertain. -2 showed complete lysis in the same region. Streak out this growth as 231b-1A etc.

Tests repeated at room temperature show 231-1 to be completely resistant to T1h ~~also~~, but sensitive to T5, while W400 scores T5<sup>R</sup>. Repeat all tests with once purified colonies.

Revised scoring of K/1 as T1h resistant may have been due to absence of NaCl in the medium.

Streak out the substratum in the streaks of 231-b-3 & 4, T16.

(3) shows considerable lysis in both broad streaks, and superimposed development of some mucoid resistant. (4) streaks out well. Purify 4 further & test isolated colonies against T16 and T5.

231b - ~~2~~ 41 etc.

Test 5 colonies.

T16	T5.
S	S
S	S
R	R
R	R
S	S.

This background is, therefore, for the most part sensitive although lysis may be delayed.

Do not pursue further.

Perhaps plaque formation should be studied quantitatively?

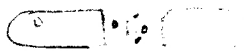
June 21, 1948.

231b-1, 3+4 form rather small colonies on nutrient agar. Continue purifying them to establish stocks. Retest isolates on USA

	T1	T1h	T5	
W400	R	R	R	Has been misclassified.
W400	R	S	S	
Y100	R	S*	S	} W -
-1	R	S	S	
-2	S	S	S	
-3	R	S	S	
-4	R	S	S	
-1*	R	S	S	

\* from T1 original test streak.

\* incomplete lysis.



Plaque ~~formation~~ appears in the "resistant" section. This may be due entirely to incomplete absorption of virus.

Check infection of -1, -3 + -4.

	T(0)	+TLB <sub>1</sub>	+TLB <sub>2</sub> +T <sub>4</sub> p.
1.	-	+++	+++
3.	-	+++	+++
4.	-	+++	++ <del>---</del>

June 19, 1948.

Irradiate Y10 + Y40 on Lac-T2.

Y10 a) 4 seconds 5 pl. x ca 2000 = 10,000. Plates very crowded.  
 21. ① all+

b 5 sec. 10 pl x ca. 600 = 6000.

- |     |   |  |            |
|-----|---|--|------------|
| 11. | • | All+, rather small cols.                     |            |
| 12. | ○ | All+   |            |
| 13. | ⊙ | W406. slow+                                  |            |
| 14. | • | W407 -                                       |            |
| 15. | ⊙ | + and slow+ , do not recover.                |            |
| 16. | ● | All slow+                                    |            |
| 17. | ⊙ | <del>Mostly +, a single - ? noted W408</del> | No mutant. |
| 18. | " | slightly slow. do not recover.               |            |
| 19. | ⊙ | all+   |            |
| 20. | ⊙ | All+   |            |

Y40 a) 4 secs. 4 plates x ca 1000/plate. = 4000

- |   |    |   |                  |
|---|----|---|------------------|
| 8 | 1. | ⊙ | Apparently all+. |
| 7 | 2. | • | W403             |
| 6 | 3. | • | 2+ colonies.     |

b) 5 secs. 9 plates x ca. 500/plate 4500.

- |   |    |   |                             |                       |
|---|----|---|-----------------------------|-----------------------|
| 5 | 4. | • | <del>W409</del> W408        | 4 mutants (8 tested). |
| 4 | 5. | • | W405.                       |                       |
| 3 | 6. | ⊙ | A few + colonies.           |                       |
| 2 | 7. | ⊙ | Apparently all+, some slow? |                       |
| 1 | 8. | ⊙ | W404 slow fermenter         |                       |

Streak out on EMBS to find possible mutants.

June 18, 1948.

Plate 820 + 821  $\bar{c}$  1s-1, 2-3.

	Sp-1	Sp-2	Sp-3.
S-20	$\approx 10^3$ sm. plaques. Uniform lysis, moderate sm. plaques.		No plaques.

S-21.	<u>No plaques.</u>	<u>No lysis.</u>	<u>A few large plaques.</u>
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Phages are therefore specific for S-20 & S-21. Regrow them!

Cross test; streak on plates

	Sp-1	Sp-2	Sp-3	Sp-5
S-20	R	fairly ev. lysis.	R	R
S-21	R	R	R	R
S-26	R	R	R	S
S-20/2	R	R	R	R
"	✓	✓	✓	✓

Phages Sp 1-5 grown again on specific hosts in #2 Y2 both N-P 19.

Spread carefully  $\bar{c}$  sp. host on NSA to get estimate of titer.

Sp 1 - S20. confluent lysis + resistant colonies.

Sp 2 - S20 ditto

Sp 3 - S21 A few dozen large plaques,  $\bar{c}$  concentric rings; titer clearly very low. A few very large plaques, with indefinite margins.

~~Sp 4 - S26~~

Sp 5 - S36 Patchy areas of complete lysis.

See over:

Spread S36 on (1) N.A.

(2) T(B<sub>1</sub>).

Suppose:

N.A.

1. B-P-5

Indefinite zone of lysis & halo clear.

2. S20

3. S21

4. SW7

5. SW8

6. SW10

Good growth; sharp margins (lytic halo??)

T(B<sub>1</sub>) SP5: Central area of growth; wide halo on margins (3-5 num).

S20 Good growth. No marginal halo

S21 Marginal halo (ca. 5 num) discernible.

SW7 No growth; definite marginal lysis, best observed around pinpoint inoculation.

June 20, 1948.

On NSA, plate "1 drop" each of bacteria + phage.

- ① Y40 + T5
- ② Y40 + K-12 + T5
- ③ K-12 + T5.

A21. ① Uniform growth.

③ Uniform lysis + resistant colonies, ca 200.

② 2 plates - Uniform growth.

No virus mutants of T5 active on Y40 were noted.

lysogenicity of S-21 mutants.

June 20, 1948.

From irradiated T<sub>2</sub> plates, pick single colonies of SW-7 and SW-10, in attempt to find non lysogenic colonies. Streak growth directly on a) Lac EMBS and b) ~~SW~~ T(10,1) smeared with SY-36, ~~and~~ look for lytic areas on b).

- Plate 1. SW7. 42 colonies tested. 42 lytic areas
2. SW7. 42 tested. 41 lytic areas. 1 untested (out of bacterial smear)
3. SW10 28 tested 25 lytic areas.  
3 not clear, retest.

~~Retest SW1-3~~

June 22, 1948. Irradiate 10 secs. on Lac EMBS plates. Repeat above procedure.

1. SW7. 62 tested. Carbons lytic. Lytic zones usually somewhat turbid. Occasional clear plaques, probably virus mutants.
2. SW8 8 colonies which grew on minimal agar. These are barely distinguishable on lac agar. All but one is not lytic. Isolate 1 active, 1 inactive & test for Salmonella. With these exceptions, all of 63 tested are lytic.
3. SW10. 65 tested. All lytic.
4. SW11. None lytic of 65 tested. [ Is SW-11 a mutant of SW7? ]

Note Note small possible plaque-like areas in the streaks of S21 deus. Streaked on + lysing SB6. (LSS36 lysogenic?)



June 27, 1948.

Repeat expt. on SW8, plates incubated 20 and 30 secs.

Some tests were made by puncturing agar with inoculating needle rather than making a short streak.

109 tests. Each survivor carried intact phage!

June 19, 1948.

Use T2 500/ml + Casamino 1%, Y. Exh. 1% Sugar 1%.

Irradiate all cultures 5 secs. ~~500~~

Y40. Glucose ca. 500/plate. Most colonies deep red! Occasional wh. cols!

glucanate. 1 plate: central spreading zone of pink colonies; start at thinnest part of plate

1 plate: uniform white colonies; 1 (2) found. - slow on Yna  
W409

Galactose Some plates sl. smeared. Occ. red colonies

10 plates x > 600 cols. too crowded to read well 1 picked to Gal EMB.

Y10 Glucose. 2 pl. x 800 cols. 2 likely mutants. No!

Galactose 10 pl. x 800.

SW7. Yna. Many cols rather deep pink. Pick deepest one.

Gal. As above

Mannitol Many colonies bright red!

No other mutants

SW10 }  
Sua }  
Gal } As SW7.  
Mannitol }

June 21, 1948.

Plate Y10 with T1h. Test resistant to T1 and T5.  
70 tested. All were resistant both to T1 and to T5.

58-161 with T1h. Test on T1 and T5.

60 tested. 57 resistant to both; 3 show some action of T5 but not of T1.

T1h T5.

= 237-1

Plaque ridden; must be sensitive

W-413 237-2

R

S

W-414 237-3

R.

S

} shows a substrate of unlysed cells  
similar to that of  $\frac{1}{2}$  on T1H.

Y10 with T5. Test on a mixture of T1 + T1h.

68 tested All resistant.

Y10 with T2. 1 plate shows half dozen moderately large colonies and 1-200 rather small.

Y10 - T6.

Y10 - T1 + T2 20-30 good sized rough colonies. Several mucoid ~~rough~~ radiate colonies also noted. Pick + test.

T1 + T6. Several mucoids per plate, only.

W401 plated with T2h. June 26, 1948.

75 resistant colonies picked and tested for T1-resistance.  
All 75 colonies were resistant to T1 (cf. Benia's report that B1/2h was sensitive).

Salmonella cross-

238.

SW3 x SW10.

June 19, 1948.

Grow up cultures, wash + spread on T(0) agar.

P21. Pick colonies and streak on Acetone + ~~Acetone~~ Xylose  
EMB.

SW3 - numerous colonies. 11 picked X+A+

SW10. 5 picked all X-A-

SW3+SW10. 22 picked. 19 X+A+ 2 X-A- 1 ? (maybe A-X+).

Streak out on acetone + xylose = 237-1. : Mixture of  
A-X- and A+X+.  
No Recombination.

	1	2	3	4	5	6	7	
SW11	0	AA3 + 0	H.C.	Y.Cx.	U <sub>1</sub> ts.	MCV	Rhamnose.	
	- ✓	- ✓	+++ -	++ ++++	- -	+++ ✓	- ✓	H.C.

	1	2	3	4	5	6	7	
SW5	0	MC	V	MCV	Y.Cx.	X-1	X-3	Y.Cx.
	-	-	-	-	+++	-	-	!

<del>SW5</del> W93 Valine +:	0	+	-	±	-	-	-	?
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Y132 Arginine +: 20h.	0	+++	-	+++	++	-	-	<u>MC.</u>
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SW11. Grew on A3 + A5 or A3 + EA. ∴ Requires either histidine or threonine.

SW11.	A3 + H	M	A3
	A3 + Th.	Th.	-

June 23, 1948.

1. W-183 x W-401.
2. Y87 x W-401.

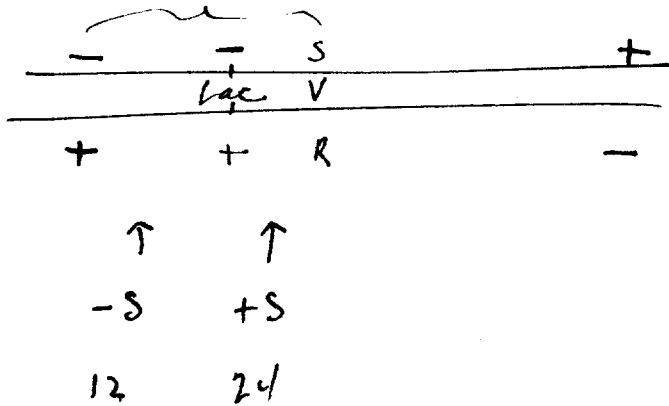
Slide P26 and test summary on T1; EMS Lac.

①

-R	-S	+R	+S.	$\frac{R}{S}$	$\frac{31}{18}$	. 49
3	4	15	5			
4	2	9	7	$\frac{+}{-}$	$\frac{36}{13}$	. 49
7	6	24	12			

B-M-lac-V<sup>S</sup> x B+M+T-L-lac+V<sup>R</sup>      Lac = 72%, linked to BM.  
 V<sub>1a</sub> = 60+% also linked to BM.

Actual order:



But there are not linked to each other! V<sub>1a</sub> may, then, be to the left of B<sub>1</sub>!

(1) W401 x W183. T1:

\* T(B<sub>i</sub>)

-R	-S	+R	+S.
24	17	36	10.

Note: 46+ : 41-

14+ : 18-

---

60 : 59

T(O)!

4	10	17	1
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Ratio should be 80+ : 40- !

(2) W401 x Y87.

T(B<sub>i</sub>)

	T <sub>1</sub> T <sub>5</sub> <sup>R</sup>	V <sub>1</sub> <sup>S</sup>	T <sub>1</sub> <sup>R</sup> T <sub>5</sub> <sup>S</sup>	T <sub>1</sub> <sup>S</sup> T <sub>5</sub> <sup>S</sup>
lac-	15	9	6	3
lac+	27	20	7	13

24

lact+ =

117:54 ok.

V<sub>1</sub><sup>R</sup> V<sub>1</sub><sup>S</sup>:

42:29 ok.

---

71

\* Nutrition of W401 needs to be rechecked!

June 28, et seq.

① 58-161 / T1. 100 tested 98 Resistant to T1h and T5  
 2. Sensitivity .....  
 presumably  $V_{1a}^R$ .

Purify as w-413 + w-414.

② 58-161 / T1h. Test on T1+T5. 56 tested.  
 15 = 241-2

③ w183 / T1h 28 tested. " 15. = 241-1.

④ ~~w-401~~ / T1h. Slow absorption but lysis finally complete.  
 Test on T1+T5. 55 tested.  
 Many milled struts. 195. (241-3-12).

	T1h	T5	T1		W-415	415	
w183.	R	S	R				1
58-161	R	S	R	plaque ridden	416	416	2
w-401	R	S	R		417	417	3
3	R	S	R	plaque ridden	418	418	4
4	R	S	R		419	419	5
5	R	S	R				
6	Lysed	S	R		418	420	7
7	R	S	R		419	421	9
8	Lysed	S	R		420	422	11
9.	R	S	R.	A few plaques.			
10.	Lysed	S	R.				
11.	Mucoid	S	R.	but T5 S!			
12.	Mucoid, T5 <sup>R</sup>	S	R.	lysed....			



V<sub>1a</sub><sup>R</sup> crosses.

242.

~~June 27, 1948. Et Seq. July 4, 1948.~~

Ant (B.) unless indicated.

(1) W401 x W-183

(2) W401 x 487

(3) ~~4100 x 58161.~~  
494 x W-314.

July 6, 1948.

- ① W-183 x W-401    4+ : 3-    All T<sub>1</sub>-S!
- ② W-415 x W-401    See below.
- ③ W-415 x 464.

-R    -S    +R    +S.  
 8    1    10    1    ∴ not allelic to V<sub>1a</sub>

~~③. all = 19R 3S    ∴ not allelic to V<sub>1</sub>  
 Call the resistance factor carried by W-415 V<sub>1c</sub><sup>R</sup>. Its phenotype  
 is T<sub>1</sub>R    T<sub>1</sub>hR    T<sub>5</sub>S.  
 May be allelic to V<sub>1</sub><sup>R</sup>~~

③. All T<sub>1</sub>R. - Some are T<sub>5</sub>S.

1. =  $\phi\text{ONa}$  2. =  $\phi\text{OH}$  3. =  $\phi\text{OGal.}$

July 9, 1948. Beckman Spectroph.

M/5000 o-nitrophenols.

$\lambda$	S.W. (mm)	1	2	3
350	.3	.270	.706	.325
340		.217	.669	.431
330	.3	.205	.583	.519
320		.269	.513	.564
310	.32	.418	.571	.559
300	"	.662	.843	.541
290	.37	.930	1.232	.589
280	.4	1.010	1.445	.749
270	.43	.860	1.324	.980
260	.48	.881	.978	1.045
250	.54	1.166 1.157	.570 .571	.860 .857
240	.63	1.84	1.570	.819
230	.84	+4	.870	1.158
220	1.2	<del>0</del>	1.446	1.600
215	.9	<del>0</del>	2.25	2.4
210	1.3			
290	.2	.922 .930	1.188 1.173	.590
288		.975	1.238	.611
286		.979	1.290	.630
284		.998	1.331	.658
282		1.005	1.370	.696
280		1.00+	1.394 1.403	.735

264 = max

280

1. =  $\phi\text{ONa}$  2. =  $\phi\text{OH}$  3. =  $\phi\text{OCal.}$

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270	.43	.860	1.324	.980
260	.48	.881	.978	1.045
250	.54	1.166 1.157	.570 .571	.860 .857
240	.63	1.84	1.570	.819
230	.84	+4	.820	1.158
220	1.2	<del>0</del>	1.446	1.600
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286		.979	1.290	.630
284		.998	1.331	.658
282		1.005	1.370	.696
280		1.00+	1.394 1.403	.735

264 = max

(280)

		1	2	3
278	.2	.990	1.390	.780
276		<.96	1.379	.821
274		.935	1.345	.880
272				.920
270				.970
264				1.045
260				1.037
262				1.040
264				1.044
265				1.038
266				1.027
263				1.040

Tungsten Lamp

340	.18	.226	.672	.447
350	.27	.300	.638	.326
360	.11	.372	.616	.217
370	.09	.509	.530	.130
380	"	.664	.410	.068
390	"	.818	.293	.034
400	.15	.980	.185	.015
410	.05	.979	.180	.011
470	.04	1.088	.053	<0
440	.03	.925	.015	0
460	.03	.603	.03	-

V. ...  
 ...  
 ...

480 ,13

500 ,02

~~530~~

575

,316

,134

,050

0

1039

0

~~475 + 1%~~ T2 Medium.

(14)

To NA-lac-T2, add: / 50 ml.

①. 5ml M/10 buffer pH 7.0	Y10	S20	Y87
②. 1 ml "	-	+++ uniform!	+++ exc. bi. st.
③. .5 ml "	-	+±	+±
④. Sodium lactate 50% .5 ml	reqs. all cult.		
⑤. CaCO <sub>3</sub> g.s. .1%	-	± occ +	± occ +
⑥. Sodium succinate 1g.	±	±	±
⑦. Asparagin .2g	mits. g.		
⑧. Na formate g.s. .5%			
⑨. Methylene blue	-	+	+
⑩. Control.	-	OK w/ col.	++ occ col.

Repeat critical members. Some numbers rubbed off flask during autoclaving + maybe confused. Buffering seems to be the "lead"

July 10, 1948.

N.L.A. + (50ml.)

1. Buffer pH 7 1ml 14/10.

2. Sodium lactate .1 ml 50%

3. Asparagin .2g

4. Sod. succinate .5g

5. Sod. formate 10% 1cc

6. —

7. Buffer 14/10 pH 6.0 1ml

8. " 14/5 pH 6.6 .5ml

446 487 520.

- sl. vials. <sup>unif.</sup> ++± ++ - not h. sh.- <sup>all but finest</sup> +++ +++

- ++± +++

- <sup>lyt. red</sup> + <sup>lyt. red</sup> +

- vials. ± +

~~+~~ - faded +++- <sup>in some colonies</sup> faded faded.

- ± faded

addition of sodium lactate seems to be helpful.



July 7, 1948.

Cultivate overnight in YB:

SW-7, SW-12, SW-7 + SW-12.

Wash and plate on T(B<sub>1</sub>):

1. SW-7
2. SW-12
3. SW7+SW12.
4. (1) + (2).

No colonies (except for obvious contaminants) on any plates. 7/10/48.

July 8, 1948.

Test by cross-streaking.	SD-2	SD-6.	Growth in broth.
S-20	S	R	R
SW-7	R	S.	S
SW-12.	S	R	R.

∴ 16 is sensitive to SP 2, suggesting that we have here smooth + rough phages, as confirmed by growth habits.

---

Plate #21 c SW12. No plaques noted (e.g. SP 3).

SW3 / Sp 4 ultimately gave a fairly dense secondary growth, limited at first to a few colonies.

SW7 / Sp 6 gave a large proportion of resistant, ticks + purify.  
(possibly because taken from a roll culture).

SW3 / Sp 2 gave a few colonies at magnis which are probably sensitive

July 10, 1948.

(1) SW7 x SW12. Grown separately overnight in YB and plated  
on T1B, J.:

July 12: colonies noted on X and SW12 plates.

SW12<sup>R</sup>. 10 tests all Ar+ Sp6<sup>R</sup>. SW12 is supposedly Ar-!  
These tests n.g.

# Mapping the V loci.

249

July 10, 1948.

w-112 (Lac- $V^S$ ) X.

1. w-413 ( $V_{1a}^R$ )!!! No yield! on T( $B_1$ ). 17+ : 99- on Lac EMS!  
Sensitive!!!

2. w-416 ( $V_{1c}^R$ ) excellent yield. Test from T(0) + T( $B_1$ ) to EMS & EMA. 7/12

3. ~~487 ( $V_1^R$ )~~

①: - : 72 S      1? R  
+ : 14 S      0 R.

249-1 →

w112    S!  
w413    S!  
S! from T(0) All S : 1+ : 6-

kryple's very close linkage of  $V_{1a}$  to  $B_1$ . Check parents:

②. from T(0):

	-R	-S	+R	+S
	11	16	0*9	9.
T( $B_1$ )	55	83	35	10.

\* 9+ colonies (not otherwise scored) were "incompletely" lysed by T1 but supported definite plaques.


streak out some streaks for further identification:

many colonies show "partial" lysis.

- (2) Many "R" streaks show some regions of lysis within the streak.  
The following is offered:

~~-R -S +R +S.~~

S.O:

1. "-R"
2. "
3. "
4. "
5. + 

Test 5 colonies derived from each.

1. 5 - cultures show *fuzzy* lysis, some individual plaques, *same for all 2 + 3 cultures*  
*fuzzy*

∴ these crosses could not be scored. (use ~~these~~)

recombinants originate from W416 (Vic<sup>R</sup>) which is  
T1<sup>R</sup> T14<sup>R</sup> T5<sup>S</sup>.

Compare 24961 with W416 and 58-161 *m*, T1, T14, T5.

EMBLac

EMS + TLB<sub>1</sub>

NSA.

July 10, 1948.

Nutr. Lac Agar + 50 r/ml T2 - + :

	W413	W112	SW 7.
1. —	—	++	++
2. Na lactate .01 ml	—	++	±
3. .05	—	++ <sup>is.</sup>	+++
4. .10	±	+++	+++
5. .50	+ max:	+++	+++
6. 1.0.	inhi -	inhi	inhi.

11 etc. .1 ml lactate

11. —	±	+++	+++
12. + .1 ml M/5 NaOH	—	—	— no inhibition?
14. M/10 buffer pH 6.0 1 ml	±	+++	+++
15. " pH 7.0 1 ml smeared.			





Mutation test: irradiate 58-101 on medium #1. 27 plates.

On many plates, all colonies have red centers.

Pick up those with most intense reaction.

ca 150/plate.  
4000 colonies.

This may be in part an effect of radiation

1.		→ —	W425	R
2.		→ + and -	W425	S
3.		→ all +		
4.		→ —	W427	R.

T1: probably contains T.O.