Mapping Lac_4 -

May 21, 1948.

W-67 × Y64/ $Lac_4 - V_1^S \times Lac_2 - V_1^R$.

Among 28 plates carrying ca 100 good-sized colonies each, only 7 + colonies were noted (2 uncertain). Ca 1/400 + : - .
Score +'s for phage resistance

Lac+ : (only 3 rapid +). . All R.

Lac - :	R^2	S
	10	0
	20	0
	18	0
	48.	0

Sensitives are again missing.

3 hypotheses :

① Lac_4 - is a lethal in sexual progeny

② Lac_4 - is linked to a "lethal" which may be a nutritional requirement.

③ Lac_4 + are not produced in these crosses due to chromosome aberrations or related phenomena.

① Check nutrition of W-67

② Cross W-67 and Y64 on glucose medium

③ If an "inversion" what are the limits of its action.

Lac_3 ; Lac_4

May 24, 1948.

On EMS-lac-13,

(1) W-108 × Y 4/0. Cross n.g. W-108 + heir streaky very large ly lac+. (ca 1:4)

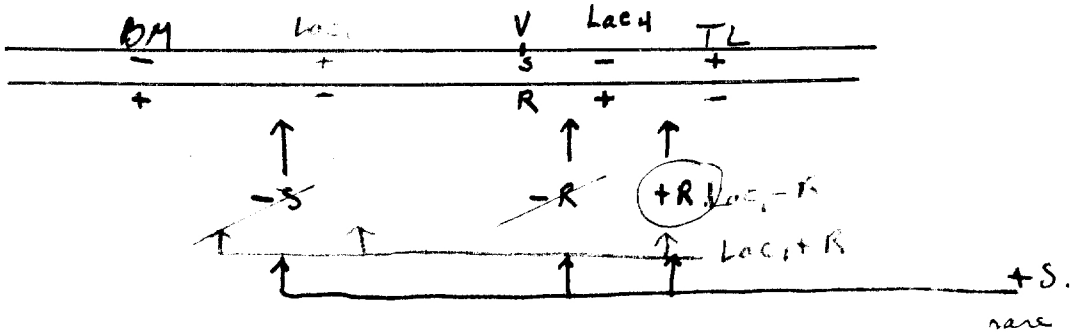
(2) W-67 × Y 4/6. Only one occasional + colony. None on 3 glucose plates.

5+ tested HCl V_1^R.

What linkage relationships are indicated if the Lac_4- are merely not recovered? The combinations are:

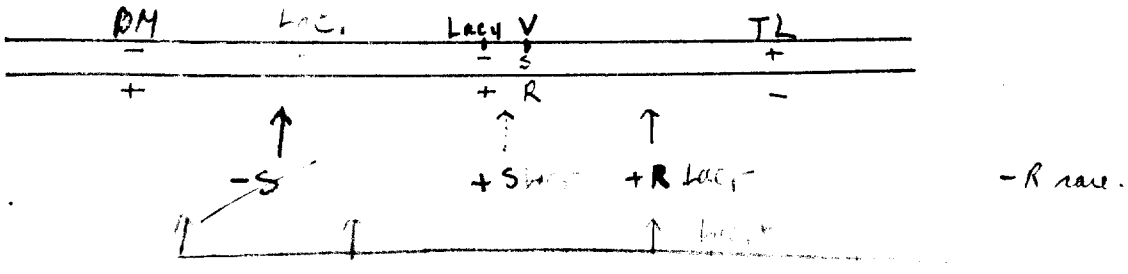
BM Lac+ V_1^R TL. × Lac- V_1^S. Lac_4 may simply be closely linked to V_1 or situated so that a triple interchange is required to give a Lac+ V_1^S combination, e.g.

I.



or.

II



Crossing Media

May 24, 1948.

Basic salts + EMB +:
lactose series + TLB, BM L.
glucose series + B_1. G.

1. Na succinate 1%
2. " " .5%
3. Asparagine 1% Designate EMA. (cost > $1/liter)!
4. " .5%
5. Na acetate 1%
6. " " .5%
7. Na glutamate 1%
8. " " .5%

(A). Cross W-108 × Y40 on a plate each of series G. 1P24.

(W-108 is ca 1/4 Lac +. ∴ ratios cannot be convincing.)

(B). streaks out on a plate each of series L.

3P

(A). 1+2. No. protoplasts colonies. Pinpoint background. (poss. a few v. sm.)

3.+4. Numerous protoplasts > 1 mm. diameter, many already showing Lac+ or -. 4/a little larger than 3, but uncertain.

5. Pinpoints

6. like 5

7. Pinpoint background.

8. << 7.

Asparagine, so far, is the ~~most~~ superior supplement.

8:30P.

1, 2, 7, 8 pinpoint background.

3, 4. (asparagine) 3: v. well developed colonies, especially Lac+. Numerous - colonies not so large but more numerous.

4: do. Lac+ more accentuated Lac- possibly slightly smaller.

5, 6 (aspartate). 5: Fewer colonies, Lac+ only

6. Ditto.

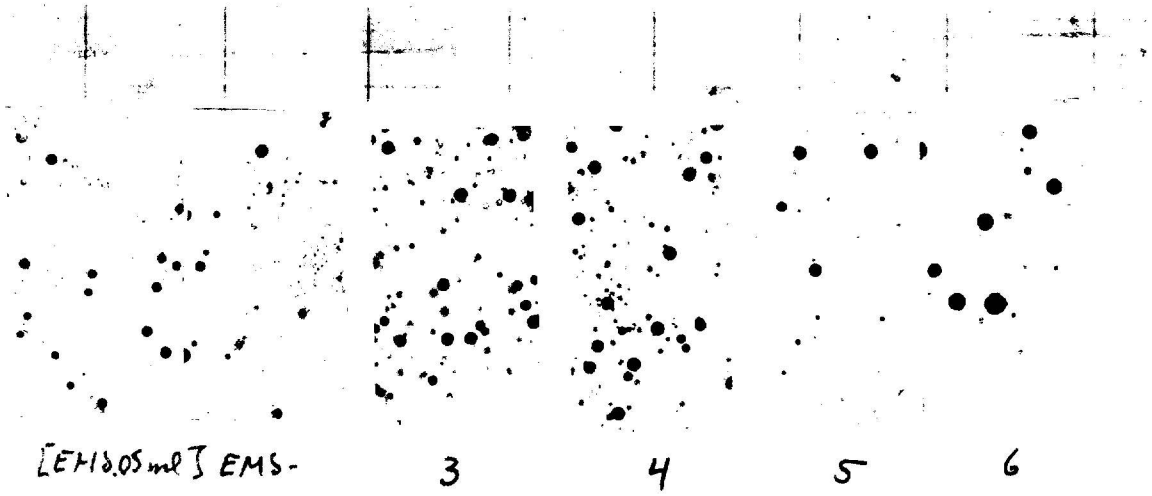
9A 26.

1, 2, 7, 8. Pinpoints, v. s.

3 shows slightly higher yield than 4, which permits less crowding on 4. Lac+/- character is perhaps more distinct on 4. Background is satisfactory - probably less marked on 3.

5. Yield about 1/5 of 3. Lac- tend to be smaller than Lac+, but not unsatisfactory

6. Like 5. EMS standard: Excessive background; yield poor + variable in size


[EMS 0.05 ml] EMS-
3
4
5
6

Map Lot 2 + Lot 3. 104

May 26, 1948.

on Lac EMA':

(1) Y46 x W45

(2) W108 x W45

(1) No yield.

(2).

	−	+
4	◆	o
3		o
2		o
1		o
2		o
	12.	0

Yields too low.

May 27, 1948.

On Lac + Glu A:

(3) W-67 x Y46 No yield (1 colony / 4 plates.) 5 + V_1^R. No -

(4) W-67 x Y64.

A29: Yield much higher in W-67 x Y46 than in W67 x Y64.

All - (many started to pepillate - probably Y64).

Test on T1.

(4): 33 - all R. No +.

May 28, 1918

(1). W-145 x Y4/0^R. Lac

(2). W-337 x Y4/0^R ⟵ Lac B.
Lac O
Lac B
Mal B.

(3) W-145 x Y87.

(4) W-45 x W-145

(5) W-337 x Y87.

(6). W-337 x W45. Lac B.

A31:

3:

+	−
1	9
1	3
0	23
2	35 | 37.
1	18

3+ : 53− / 56.

∴ ~~Lac~~ Lac$_5$ ≠ Lac$_1$−

2: L_B.

0	0
2	0
0	0

M_{13} (0 3plates).

L_0

0	0
0	0
4	0

L_{B_1} 2Plates ~~co~~ smeared + and −

1:

+	−	
9	16	
8	14	
2	4	
~~12~~ 5	12	
0	5	
3	6	
0	3	/ 27 60 / 87

4:

+	−
0	
12	2
0	0
0	1

Yields too low for satisfaction

(5) On Lac B$_1$.

1	0
0	1
0	0
1	0
2	1

Background rather heavy, but not smeared.

(6). On Lac B$_1$.

12	0
12	0

dense background.
Many small petologaphs.
1 plate only satisfactory. Hole!

(7). Colonies picked exhaustively + tested on Lac EMA + T1.

−R	−S	+R	+S.
11	4	3	1
13	4	3	4
11	3	5	0
15	3	0	1
50	14	11	6

81.

64 17

61 20

June 2.

③ (W·145 × Y87)

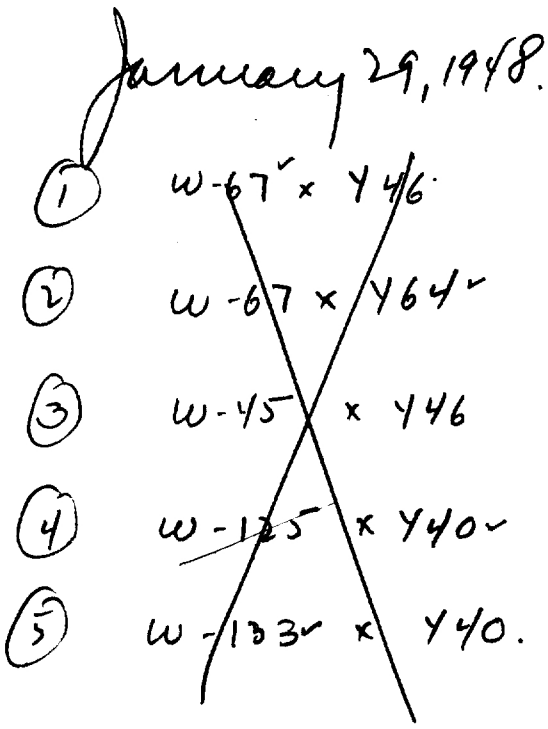
+	−
6	25
4	29
3	4
4	20
17	78

] 95

⑥ W-45 × W-337. Plates crowded. About ½% + colonies!

Brace repetitions!

January 29, 1948.



1. W-67 x Y46
2. W-133 x Y40.
3. W-67 x W-133

A31.

(1) Yield (0). (Glucose EMB) 10 plates & 3 Lac plates.

(2). ON: − >> +. (linked to BM).

(3). 2 plates. Streak to Lac B,.

June 3.

2: On Lo, 14 +: 2 −

on Lo,, many plates show more − than +. Many mucoid colonies are papillate or have turned color.

On smears:

+	−	
9	12	
14	18	
8	15	
31	45	76.

$\chi^2_1 = 10.9$ $p = .001$

2

2 - streaked to Lac B, A., T_1.

	$-R$	$-S$	$+R$	$+S$
L_0 plate	10	3	0	2

"+" of previous page ~~mean~~ may not be truly so.

L_{B_1}.

16	12	10	0
13	8	2	0
29	20	12	0
	49	12	

These plates are truly to old for accurate study.

3: 13 all -

May 29, 1948.

Irradiate suspensions of S-20, and 21 as follows.

Grown (6 h.) suspensions of S--- in YZ-glucose, shaken, resuspended in H_2O.

S-20 exposed tomHanovia output at aperture of lamp in quartz flask shaken by hand. 5 ml. suspension added, .5 ml removed at stated intervals to 10 ml. tubes of YZ glu cose shaken at 37.

S-21 exposed in 1 ml. lots in 10 cm Petri Plates, exposed at table level (ca 12 cm) .5 ml samples removed from each plate.

S-20: 10, 30, 60, 120 and 180 secs. Samples 10+30 +++ 60+ +++.

S-21 2, 5, 10, 20, 30 and 60 secs. Samples 2-10 +++ 20-60 +++.

Dilute S-20, 10 second and S-21 5 second exposures 10^{-7} and plate in minimal layered agar, 2P30.
For ufemice, S-20 = ~~S-1~~ S.W-1 and S-~~21~~ = SW-2.
Ca 30 plates each, and 10,000 colonies.

11 picked, 9 grew up in series S-~~21~~
23 " , 21 " " series S-20.

Numbers 1-21 are S-20; 22-30 are S-29.

Mutants SW-3 and SW-4 ( ) from S-20

SW-5-8 ( ) from S-21.

Test putative Salmonella mutants.

	T(o)	HC	Vits	Y. Ex.	Lac EMB	Glu EMB.	
1	++						
2	++						
3	++						
4	++						
5	++						
6	++						
7	++						
8	++						
9	++						
10	— +	++	— +	++			SW-3
11							
12							
13							
14							
15							
16							
17							
18							
19	— +	++	— +	++			SW-4
20							
21							
22	—	—	—	++			SW-5 —
23							
24	—	—	± ++	+++			SW-6
25	—	++	—	++			SW-7
26							
27							
28							
29							
30	—	++	—	++			SW-8

Lac EMB: All – and fairly typical except 24 which is thin with – papilla

Glu EMB: All + and typical exc. 24 which is thin.

(SW-6, SW-7:) S.O. on glucose agar.

(margin, beside 2–21:) V. rough.

	① lys+arg	② meth+cys	③ leuc, isol, val.	④ φal, tyros, tryp't.	⑤ ..., threo, ..., prol.	⑥ serine glycine alanine hydroxypro	O	HC	Individual AA:
SW-3	±	±	±	±	+++	±	±	+++	
4	±	±	±	±	+++	±	±	+++	
7	—	—	+++	—	—	—	—	++	I-V ±
8	—	—	—	+++	—	—	—	++	
S-21									

	YNA	Y. Grth.	N2 Case	Pur + Pyr	H.C. + Vits.	O
5	–	++++	–	–	–	–
6.	–	+±	+	–	++	
S-21.	+++	+++	+++	+	+++	+++

Vits. not V.

(margin:) ... +V.

6. 10 vits, K, V, + – :

(deficient)

Plate a mixture of W-108 and T1 on Lac EMB. Select 8 surviving colonies and streak out 3 times. Test these 8 and W-108 on T1 and on T5:

	T1	T5	
1	R	R	W-399
2	R	S	W-400
3	R	R	
4	R	R	
5	R	R	
6	R	S	
7	R	R	
8	R	R.	
W.108	S	S	

The R,R types are presumably V_1^R and the R,S V_{1a}^R. Select 1 and 2

This is an unusual preponderance of V_{1a}^R: (2/8).

1 → See 231c. W-400 is $T5^R$.

Lac$_{2,3,4,5}$ and V$_1$, V$_{1a}^R$ Crosses.

May 31, 1948.

On Lac + Glu EMA'.

(1) 58-161 x W399

(2) 58-161 x W400

(3) W45 x W399

(4) W45 x W400

(5). W67 x Y10

(6) W67 x Y64

(7) W67 x Y46

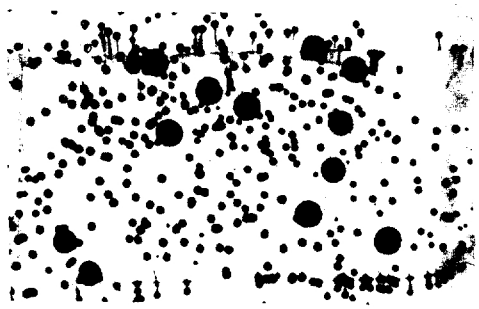
(8) W145 x ~~Y46~~ 58-161.

~~(9) W145 x Y266~~

(9) W-145 x W45

Jun. 3.

1. Color faded. Pick colonies at random for test ± Lac, T1.
2. ditto.
3. No yield (1 col/3 plates).
4. All – 138 colonies, probably not faded. Close linkage of Lac$_2$ to Lac$_3$ confirmed. Streak to Maltose. All out of 53 are Mal – with heavy + contamination.
5. Yield OK. > on glucose than on lactose. Perhaps 10 or 20% of colonies on Lac are –.
6. Two colonies just starting
7. No yield, on glucose or on lactose
8. Like 1. Pick at random to lactose.
9. 1 plate. 1+, 3 or 4 – colonies.



210 – 1

① 2 classes of colonies. large spreading, probably +
and small compact, −?

Frequencies: "+" "−"
20 578 | 598. Ca. 3.3% + (in agreement with best previous observation

Test "+" and "−" separately on EMA-Lac B,.

	−K	+R	−S	+S.
"+"	27	0	0	0
"−"	53	0	1	0
	80	0	1	0	/81.

② Same as ① in appearance & pigmentation of +.

"+"	30	0	0	1
"−"	53	0	0	0
	83	0	0	1	/84

Altogether, only about 2/~~8~~165 or ca. 1.5%

5g. Picks from glu EMA to lactose EMB.

53 picked. 4 lac −. Streaks out on Lac EMB.

210 - 5g (1-4).

Nutrition of W-67. 21

May 25, 1948.

Tests on: (cell + BM) P25 - A26.

(1). T(m) + 1% succinate

(2). " " + Y. Ex.

(3). " " N2 Case

(4) Vits.

T(o) [glucose-asparagine].

(11). —

(12). Y. Ex.

(13). N2 Case.

(14). Vits.

W-67 is not nutritionally distinguishable from 58-161.

	A W-67		B 58-161.	
1	±	++	±	++.
2	+		++	
3	+++		+++	
11	+++		++	
12	+++		++++	
13	+++		+++	
4	—		—	
14.	+++		+++	

P26 + A27.

Streak out on Lac A + BM. etc. P26.

11 A. A27 Lac A. Glu A. Lac E MB.
pinpoints. ++ v. small

11 B. +++ +++ +++

Lacy – should be produced without pyruvate on Glu A plates.

B. subtilis recombination

May 30, 1948.

Incubate + shake in Y2-glucose tubes, overnight,
① ② ③.
Bs 16, Bs 164x and Marburg = Bs+.

Wash + resuspend cells in = vol. ~~cit~~ citrate saline buffer.

Spread 1 drop each of ① + ② together and separately on T(0) plates. Also inoc Y2 with .5 ml inocula together + separately + shake. Also carry along ③.

June 2, 1948.

① 1 colony, 1 Slight background

② 0, 0. Practically no background.

(1+2). 11, 6 background rather heavier than with ① only.
(Need other markers).

Also plate suspensions from above: Read 4 V:

① 0, 0

② 0, 1

① + ② (inc. separately) 4, 9. } (odd!)

(1+2. inc. together). 1, 0.

The possibility of recombination is not ruled out by these experiments.

Drug resistant mutants of B. subtilis

June 2, 1948.

P. Spread .1 ml of suspensions of p. 212 on Nutrient Agar plates containing indicated u/ml of penicillin + streptomycin:

① B_s 16 (tryptophandeas) ② B_s 164x (lysinedeas).

① P1. Scattered colonies in thicker portions of plate
P5 ca 20 colonies destruct; some smearing confuses count
P10 5 destruct colonies.
S1 Almost confluent background, with papillae
S5 ca 200 destruct colonies, no background
S10. ca 100 destruct colonies " "
N.A. Heavy smear.

② P1. ca 12 destruct, v. large colonies (smearing).
P5. 2 colonies, quite large
P10. No colonies.
S1. As ①.
S5. (plate rather dried). Ca. 500 colonies (smeared?).
S10. Several hundred colonies.
NA Heavy smear.

Keep Highest plates for purification on N.H. $\bar{5}$ + $\bar{5}$ drug.

Stock out. Test 5 single colonies each on P10, S10 and NA. → apparently not resistant.

	NA	P10	S10
16/P10	++++	−	−
16/S10	++++	−	++++
164/P10	++++	−	−
164/S10	++++	−	++++

very sharp destructions on streptomycin agar.

P3. Inoc Y2-glucose ē /P10 and /S10 to obtain cultures for higher step mutants. A4 Spread 1 drop each culture on NA ē : Read A5.

	P5	P10	P50	P100	S10	S50	S100	S500
16/P10	v. numerous scattered.	v. numerous scattered.	v.n.s.	large many smeared + variable	1-200	7 large 20 small.	0 later 6-10 small.	0

↙ See above not resistant

16/S10	almost smeared.	ca 100 scattered	ca 100 scattered colonies.	30 resistant	smeared.	carbam.	(carbam. smeared.)	ca 100 scattered colonies

164x/P10	100	200	20-30	~~0~~ 2.	1-200	2 large 15 small	0	0

164x/S10.	numerous sm. colonies almost a smear.	500 colonies (small).	200 small cols.	100 v. small colonies.	Smeared	40	6	0

Test the following, as indicated.

S500 S100 P10 P100 S10

16/S10

16/S10/S100

16 S10 /P100

See next page.

64S10

Test colonies from the following plates & cultures.

	P10	P100	S10	S100	S500.	D.A.
" 16 S10 "	S	S	R	R	S	
" 16 P10 "	S	S	S[R]	S	S	
" 164 S10 "	S	S	R	S	S	
" 164 P10 "	S	S	S	S	S	
16 S10 / S500 [1]	S	S	R	R	R[S]	
164 S10 / S100 [2]	S	S	R	R	R[S]	
16 · P100 [3]	S	S	S	S	S	
164 · P100 [4]	S	S	S	S	S	
16 · S10 · P100 [5]	S	S	R	R[S]	S	
164 · S10 · P100 [6]	S	S	R	S	S	

Streptomycin resistants are OK, sharp destinction between the 10 and 500 unit levels. No penicillin resistants so far noted.

Streak out, on NA, the cultures 213B-1 and 213B-2

June 3, 1948.

(1) W-337 x W-45

(2). W-145 x Y40

(3) W-126 x Y40.

Simultaneously, streak out W-45 and Y40 on Lac A +(B+).

P4. W-45 + Y40 are well grown on the synthetic medium, but none of the cross plates show any colonies of significant size.

P5. 1: No colonies on Lac A + B1.

2: No colonies on Lac A.
Some plates of T(B1) have colonies, singularly scattered

3: No colonies on T(B1) or Lac A + B1.

P6. 1. No Colonies.
2. Pick colonies from T(B1) to Lac T1.
3. 1 + colony on plate.

June 4, 1948

W-133 YY/4/0. on

A) T(B,)

B) Lac A(0)

C) Lac A(B)

D) Lac A(B,)

P6. Colonies appearing on D, apron C. Ca 6/plate on A.

P8.

A. ca 6/plate

B. 2+/5 plates

C. Ca 100/plate 1:1 +:- (Heavy background.) 59+:51-

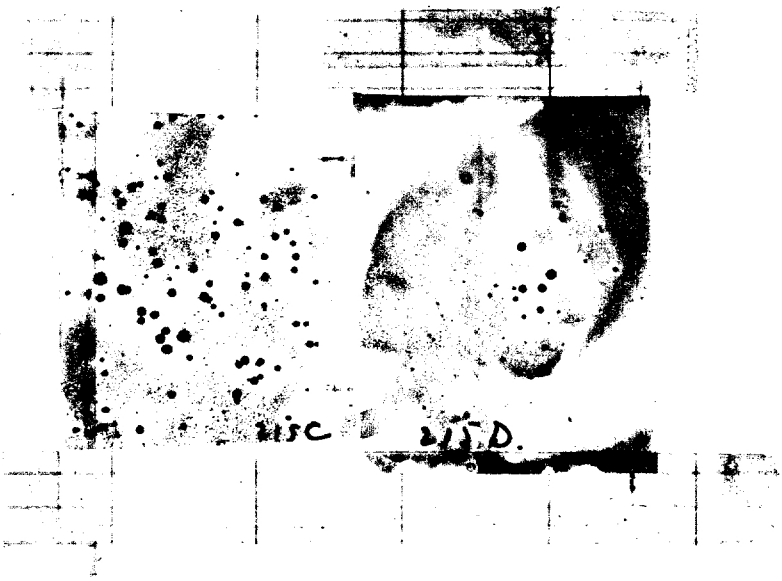
D. Ca 50/plate 26+:16-

A. Pulse to water + test suspensions on T1 on Lac EMB. – Background too heavy All Lac+ vR.

B. –

C. & D. Picks + and – separately.

		R	S
C.	+	24	1
	+		
	–	20	6
	–		
		45.	7
D.	+		
	+	17	0
	–		
		11	1
		28	1



Salmonella Irradiation
double mutants, "crosses".

#16

June 4, 1948,

Irradiate washed 8 h. suppensions of SW-3, SW-7, SW-8 and S-21, in 1 ml. lots in open Petri plates. Recover ½ ml samples to NZ-glucose broth, and shake overnight. In S-21 series, plate .05 ml sample from the initially inoculated cultures to estimate killing rate. 5,10, 20, and 30 seconds under Hanovia lamp.

Assuming inoculum of $.5 \times 2 \times 10^8 \times .005 = \cancel{5 \times 10^6}$ 5×10^6, the killing's can be estimated.

Secs.	S. ca. 5000	pS. 3
(10	239	4.3)
20	8	6
30.	10.	6.

These suspensions were inadvertently autoclaved.

— S21

Irradiate the above washed suspensions, 10 secs, as above, dilute as indicated and plate directly into detection plates. SW-3 suspensions not available

— S21 | 10P6, 36h. Cover ē NZCase-Yeast extract-Agar.
— SW7 | SW7 series not yet grown. Do not cover.
= SW8.

Mix on T(0) plates single drops of SW-3, -7, & -8 as indicated.

3	Colonies P6. 3,2	P7 ca. 50.
7	~~2~~,1 0 (+ contam.)	,0.
8		0., 0., other plate heavily cont. ē Aspergillus.
3×7	2,1	Numerous plaques noted (lysogenesis?)
3×8	2,1	
7×8.	heavily cont 1 or 2 colonies.	

See 217.

SW7 series formed small colonies only on June 9. Throughout plates. L-IL-V supplement isobarically not optimal in the proportions used.

S21 and SW8 series. About 20% of S21 and 10% of SW8 are small colonies. Either mutant or contaminant. Pick + test about 100 in each set. Pick colonies to sm. tubes YE. With loop, streak on EMBLac and put residual inoculum from loop into T(O)+ tryptophane. Most were – in small tubes; the following were +:

S21: 19, 29, 39, 59, 79, 89, 99, 100.

9th row tubes were more elevated. Could this acct. for + 's among them? (Heavier aeration?).

SW8. (delay scoring).

Test S21: 1–3 and SW8 1–2 on T(T+) large tubes.
All +++.
Small tube tests are inaccurate. T.O. expt.

---

217. Plate SW-3 & SW-7 on N.A. in 10⁻ dilutions indicated.

SW-3	SW-7	
10^{-1}		~~Lawn~~ Confl. growth
10^{-7}	10^{-1}	Isolated colonies (ca 1000)
	10^{-7}	do.
10^{-7}	10^{-1}	Confluent growth. No plaques.
10^{-1}	10^{-7}	
10^{-1}	10^{-1}	

No evidence of lysogenesis on nutrient agar.

June 5, 1948.

sw-6. (pab.)

	0	Vits.	pab.	HC	pab+HC	pab, HC, P.P.
	−	−	−	−	−	−
after 48-72h.	−	+	+	−	+±	++

sw-7 (leuc, isol, val).
d S-21 control.

	0	HC	L	IL	V	L·IL	L·V	IL·V	L·IL·V
-7	−	+++	−	−	−	−	−	±.	++
					leucine - isoleucine - valine				
+ (Y)S -21	+++	+++	+++	+++	+++	+++	+++	+++	+++

sw-8. (trypt.)

	0	trp.	indole	anthran.	nicotinic
18h.	−	+++	±	−	−
later			+++		

Medium for crosses.,
Lac$_1$, , Lac$_3$. Effect of shaking on crosses.

June 8, 1948.

Grow Y53, Y40+ W108 in Y2... (glucose or gluconic)

A - shaken B - unshaken. Mix = volumes + plate

1 drop each on lac A+B, and lac S + B, ., T(B$_1$).

1. Y53 × Y40
2. W108 × Y40.

{ B suspensions are, of course, much less dense than A. }

A 16.

1A. 27, 23, 34 on T(B$_1$).
(2 - 1+), 0. on lac A.
7, 4, 11 tiny colonies on lac S.

1B. 1, 0 on T(B$_1$).
>100, [redacted], lac A. on S. medium colonies.
16+, 22 −, 15+ 24 −, lac S. Better definition of +/− but not yet quite easily.

2A. 5 on T(B$_1$).
4, 5, 7 on lac A. All −
4, 4 on lac A −

2B. 52 − 2+, lac A. } +/− definition good, somewhat better than on S.
97 − 6+ lac A. }

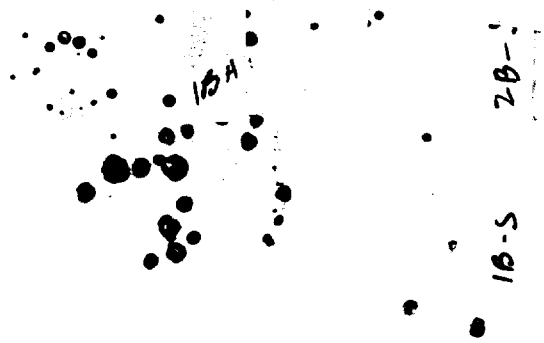
30 − 1+ lac S. }
21. 3+ } Conclude: Shaking is certainly deleterious to crosses!

P11.

1B. (Lac S.) 34+ : 31 −
[Too many + J.

Lac A. 9+ : 15 −

2B.



Nutrition of S-W 7.

To 10 ml T(0) add:
18h.

A. 0.2 mg dl-isoleucine and:

	valine	
1	0	±
2	.02	⋮
3	.05	⋮
4	.10	⋮
5	.20	⋮ ±

B.

1	0.2 mg. dl-valine and 0 isoleucine	−
2	.02	±
3	.05	±
4	.10	++
6.	.20.	±

(Try adding leucine to this!)
Cf. 70:30 used by Bonner et al for 16117.

C. ~~.17 dl-isoleucine~~
~~.25~~

.12 dl-isoleucine
.02 l-leucine
.03 dl-valine.
(optimal for Neurospora).

D. H.C. +++.

Ca 2:1 valine : isoleucine is best so far.

SW-5. Tween 80, Intact RNA, Oleic acid, Conyzmase All −
V. Arth. +++

Phenolphthalein Phosphate.

Prepare plates of NA to which Na Phenolphthalein Phosphate (Paul-Lewis; sterile filtered) is added.
Streak out A. (SW-7) B. (K-12) & C (B. subtilis 16).

After 24 hours growth, expose plates to NH_3 vapor.

A + B. show no change in color at any zone.

C: 100 r No change
300 r colonies became light pink
1 mg. colonies became a dirty pink.

Class:	SW 3	SW 7
Dulcitol	v. weak +	v. weak +
Rhamnose	++	—
Cellobiose	— alk.	—
Salicin	— *	— *
Inositol	—	— pap. (S.V.)

blue tinge to colonies not hitherto noted

Note: very weak + fermentation of rhamnose & of inositol can be secured by selecting papillae of SW 7.

These are extremely weak.

Fermentation mutants of Salmonella.
" Reactions.

June 10, 1948.

Irradiate SW-7 and -8, 1 ml in open petri dish, 10 secs. dilute ½ ml/10 broth, and ~~plate~~ spread 1 drop per plate of xylose + arabinose EMB.

(1) SW-7 on arabinose; SW-8 on xylose.

(2) Also, about 10 plates each., 1 drop whole culture spread on plate and irradiated directly, 5 secs.
SW-3 / arabinose SW-7 / xylose.

164. SW-7 and SW-8 are xylose-negative, to surprise!

SW-7 treatment on arabinose was excessive + only a few dozen colonies per plate. No mutants.

Suggests selecting for Xyl + mutants. ✓ Papillae observed in colonies after 3 days. Reversions easily selected + purified.

Check fermentation reactions on -EMB:

	SW-3	SW-7
Xyl	++ ✓	- ✓
Ara	++ ✓	++. ✓
Glu	+++ ✓	++ ✓
Gal	++ ✓	++ ✓
Sma	++ ✓	- in thin part of plate; ++ elsewhere
Mal	++ ✓	++ ✓
Sorb	+ +I ✓	Needs to be checked. + +±
Mannitol	++ ✓	++ ✓

Correlation between glucose and xylose??

Salmonella fermentations are much slower than coli!!

is ++ except in crowded areas.

Tetrazolium Reagent.

June 11, 1948.

Incorporate 50 r/ml TZ Reagent into agar + 1% lactose as indicated.

A. N2 Broth ($PO_4^{\equiv}$ buffer) = N2L
B. " + .1% Na formate N2LF
C. Nutrient broth NBL
D. " " + Formate NBLF.

A.11. Streak out, on each plate:
K = K-12
S = B. subtilis 16 Inhibited on each plate.
SW = SW-7
W = W-400 (Lac_3^-).

A. K: Colonies colorless or faint pink. 1 large dark red colony (223-1 → (223-2

SW Isolated colonies dark red.
W: colonies dark red.

B. As A. K more to red but not intense.
SW red & white colonies in the colorless zone.
W all colonies dark red; definition somewhat better than A.

C. K nearly colorless; All colonies of W & SW show up very well.

D. About the same as C. K more pink. S+SW somewhat more intense.

Test 223-1 & -2 on homologous media & on Lac-EMB.
1 is Lac- 2 is Lac+ (probably colony from SW-7).

See over:

Mix K, + W and streak on NL., EMB Lac

+ and - easily scored in each other's presence provided the plate is not too crowded, whereupon one finds the - 's score as colorless. The method shows considerable promise for the detection of non-fermenters.

Different bases should be tried in an attempt to obtain uniform coloration of lac -, even in crowded areas, which would facilitate their detection.

Nutrition of SW-5, -7.

June 11, 1948.

SW-5

	Y. Ext.		⇌ L. Bulgaricus factor.
1	5 mg	+++	−
2	1 mg	+	−
3	500 γ	± later +++ (sw.)	−
4	100 γ	−	−
5.	20 γ	−	−

} not L. Bulgaricus factor.

SW-7. Valine 0.2 mg/tube.

Isoleucine

1: 1.0

2. 1.2

3. 1.4

4. 1.6

5. 1.8

6. 2.0

~~7~~. Ditto + .2 mg l-leucine.

11

[redacted]

13

[redacted]

[redacted]

[redacted]

Salmonella phage.

June 14, 1948.

Cultivate S-20 + S-21 in ½ overnight, i shaking.
Centrifuge raw Madison sewage & filter supernatant. (Sewage Filtrate)
Add 1 ml SF + .5 ml S-20 or S-21 to 10 ml broth.
Incubate 6-8 hours. Both are thoroughly turbid cultures.
(225-20, -21). Sediment bacteria. Test supernatant for phage by ① 1 drop "phage" + 1 drop bacteria ② streak out phage & bacterial smear.

225-20: ① ② } large plaques noted in both. (May correspond to the phage attacking resistant bacteria? – small plaque phage also noted.

225-21 ① Pattern of resistant colonies.

② small plaque phage noted along streak.

Suspend plaques in water and streak out on homologous bacterial smears. [Crude phage suspension should be filtered.]

After several streakings, pick from single plaques to broth cultures + recover phages. These may not be pure.

Sp-1 S20 ~~large~~ small plaque
Sp-2 S20 small "
Sp-3 S21 small "

June 14, 1948.

Test, on T1 + T1h (raced from North):

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

∴ V_{1a}^{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 out on O on W400. No plaques.

Tetrazolium

June 15/1948.

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

per ml	K-12	S-20.
50r	faint rust	Borders of streak + I.C. stained.
100r	beginning red	more thoroughly stained
200r	W.I.C. deeply stained.	"
500r	" v. " "	" " " "
50r + Brilliant Green 25r.	—	Mostly inhibited. A few red resistants.

Variation in nutrient medium — 50r T2/ml. Agar 1.5% Lactose 1%

	K-12	S-20.
1. Peptone 1%	WIC faint rust.	WIC deep red
2. " ½%	Some large colonies rust	Some WIC deep red.
3. N2Case 1%	faint rust.	All IC deep red; borders of streaks are stained.
4. Casamino Ac. 1%	All −, except near S20.	All colonies uniformly deep red.
5. N2Tone 1%	⟶	Intermediate between 4 and 3.
6. N2 Amine B 1%	Well isolated faint rust	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. re variations in T2 concentration, pH of medium + addition of Brilliant Green.

T2 Reagent for enteric pathogens.

June 17, 1948.

Make up lactose agar with Casamino acids 1%, Yeast Extract .1%
Streak out ① K-12 ② Shigella flexneri ③ Y53 ④ SY-20. P17.

1	2
4	3

N18:	①	②	③	④
50r T2.	faint rust mar	Host colonies are	Inhibited but	Many large ugly colonies.
– Y.Ex.	④ Albumin	small, depgd.	depgd	Entire growth red.
	white.			

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

Glucose. All white. faint pink in spots.

Maltose. ④ All red. 1 & 2 are faint red in certain colonies (alkaligenes?).

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. Y53 spotty red streak. S-20 Uniform light dirty red.

T2 25r. As 10, more intense.

T2 50r, precautoclaved. Like standard.

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

Salmonella phage — lysogeni.

Grow Y2 broth cultures of: shake overnight.

S20

S21 Numerous plaques.

S22

S23

S39

S40

S43

S46 Numerous plaques. (maybe confused with S22 smear).

S56.

Sediment most of the cells + heat supernatant 30 m. @ 57° to kill cells. Spread S 36 (Gallinarum) as N.A. and mix. ± loopful of supernatant to test for lysogenicity.

Use S21 as the standard for possible studies on lysogenicity. (+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 6h., sediment + filter. = ϕS_5.

June 19, 1958.

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

Cross tests of Salmonella phages.

June 17, 1948.

On hac T2 plates, spread 1 drop of φ + 1 drop bacteria.

	S20	S21.
~~S20~~		
Sp-1	10^3 - 10^4 turbid plaques, but no confluent lysis!	numerous plaques, obscured by smearing of resistants?
Sp-2	Confluent lysis ± a few dozen large red resistant colonies.	A few plaques noted. See above?
Sp-3	? Smeared areas of lysis. All plaques are quite small when noted. Recover large plaqued phage from original streakings from Crude phage.	Confluent lysis obscured by smearing.

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 } No lysis.
S-20/2 }
S-20/2 }
S36 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 resistants. Test these on T5, T1h.
T2 v. numerous small plaques peripherally; cleared area centrally.
T6 Ditto.
T7. Uniform lysis. Ca 100 resistants.
T1+T7. No survivors.
T1+T2. Edges of some colonies irregular. Otherwise like T1 only.
T1+T6. Numerous (ca 50-100) resistants, many with plaques in them.

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

100 Y10/1 were tested on T1h and T5. 99 were resistant to both
1 was $T1h^R$; $T5^S$.
Subculture as W-401
= Y10 V_{1a}^R.

Phage mutants of K-12.

June 18, 1948.

Plate 1 drop (= ca 10^8+) Y10 + 1 drop (ca 10^9) phage on nutrient Ub plates.

T1. Uniform lysis. Ca. 300 resistants.

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

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

T4. Uniform lysis. 2 mucoid resistants.

T5. U.L. Ca 300 resistants.

T6. U.L. Ca 100 resistants.

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 resistants.	grew out as mucoid bac+. "Purify" as W-402.
T1 + T6.	1 mucoid; 1 "non-mucoid resistant (cent?)	picked as 231-1.
T5 + T6	10 mucoid resistants.	
T1 + T7	1 tiny colony, probably cont.	picked as 231-2
T5 + T7	No resistants	did not grow out on bac EMB

[Compare with E. coli B where, according to Demerec + Luria, the combinations 1,4 ; 1,5,4 ; 2,3,4,7 ; 1, 2,3,4,6,7 occur with some frequency. I. (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 on T1 51 all R
Y10 S.

Y10/6 on T2, T4.

5 tested, $T2^S$ $T4^S$. 16 more tested. $T2^S$.

∴ 21/21 $T6^R$ are $T2^S$. 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 scored $T5^R$. Repeat all tests with once purified colonies.

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

Streaks out the substratum in the streaks of 231-b-3 + 4, T1h.

(3) shows considerable lysis in ~~both~~ broad streaks, and superimposed ~~heavy~~ development of some mucoid resistants. (4) streaks out well. Purify 4 further & test isolated ~~s~~ colonies against T1h and T5.

2316 - ~~2~~ 41 etc.

Test 5 colonies.

T1h	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 gel. Continue purifying them to establish strains. Retest isolates on USA

	T1	T1h	T5	
W400	R	R	R	Has been misclassified.
W401	R	S	S	
Y100	R	S*	S	
-1	R	S	S	W-
-2	S	S	S	W-
-3	R	S	S	W-
-4	R	S	S	W-
-1*	R	S	S	

*from T1 original test streak.

* Incomplete lysis.

Plaques appear in the "resistant" section. This may be due entirely to incomplete absorption of virus.

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

	T(0)	+ TLB,	+ TLB, + Trypt.
1.	-	+++	+++
3.	-	+++	+++
4.	-	+++	+++

Tetrazolium — bacmutation sum.

June 19, 1948.

Irradiate Y10 + Y40 on Lac-T2.

Y10 a) 4 seconds — 5 pl. × ca 2000 = 10,000. Plates very crowded.

21. ⊙ all +

b 5 sec. — 10 pl × 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. ◉ Mostly + ; a single − ? noted W408~~ No mutant.
18. " Slightly slow. do not recover.
19. ⊙ all +
20. ⊙ All +.

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

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

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

5 4. • ~~W409~~ W408
4 5. • W405.
3 6. ⊙ 1 few + colonies.
2 7. ◉ Apparently all +; some slow?
1 8. ✺ W404 slow fermenter

4 mutants / 8 tested.

Streak out on EMB to find possible mutants.

June 18, 1918.

Plate S20 + S21 & Ps-1, 2-3.

	Sp-1	Sp-2	Sp-3.
S-20	Ca 10^3 sm. plaques.	Uniform lysis, moderate size plaques.	No plaques.
S-21.	No plaques.	No lysis.	A few large plaques.

Phages are thus far specific for S-20 & S-21. Regrow them!

Cross test; streak on plates

	Sp-1	Sp-2	Sp-3	Sp-5
S-20	R	faint cl. lys.	R	R
S-21	R	R	R	R
S-36	R	R	R	S
S-20/2	R	R	R	R
"	"	"	"	"

Phages Sp 1–5 grown again on specific hosts in ~~H2~~ Y2 broth N-P 19.

Spread loopful & 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 & concentric rings: Titer clearly very low.

Sp 4 – S20 A few very large plaques, with indefinite margins.

Sp 5 – S36 Patchy areas of complete lysis.

See over:

Spread S36 on (1) N.A.

(2). T(B,).

Superimpose:

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,) SP5 Central area of growth; wide halo on margins (3-5 mm).

S20 Good growth. No marginal halo

S21 Marginal halo (ca. 5 mm) discernible.

SSW7 No growth; definite marginal lysis, best observed around periphant inoculation.

T5 h.

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 T2 plates, pick single colonies of SW-7 and SW-10, in attempt to find non lysogenic colonies. Streak growth directly on a) Lac EMB and b) T(B,) smeared with SY-36, 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 EMB plates.
Repeat above procedure.

1. SW7. 62 tested. Each one lytic. Lytic zones usually somewhat turbid. Occasional clear plaques, probably vir 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 small possible plaque-like areas in the streaks of S21 dens. streaked on + lysing SB6. (Is S36 lysogenic ?)

June 27, 1948.

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

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

109 tests. Each survivor carried intact phage!

Tetrazolium: use in mutation rsn.

June 19, 1948.

Use T2 50γ/ml + Casamino 1%, Y. Extr. 0.1% Sugar 1%.

Irradiate all cultures 5 secs. ~~+500~~

Y40. Glucose ca. 500/plate Most colonies dup'ed! Occasional wh. cols!

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

1 plate: uniform white colonies; l. (●) formd. – slow org rsn

W409

Galactose Some plates sl. smeared. Occ. red colonies

10 plates × >600 cols. too crowded to read well 1 picked to GalEMB.

Y10 Gluconic. 2 pl. × 800 cols. 2 likely mutants. No!

Galactose 10 pl. × 800

SW7. Sucr. Many cols rather dup pink. Picks dupe at one.

Gal. As above

Mannitol Many colonies bright red!

No other mutants

SW10 Sucr. } As SW7.
Gal
Mannitol

June 21, 1948.

Plate Y10 with T1h. Test resistants on 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	} show a substrate of unlysed cells similar to that of V_{10}^{R} on T1H.
W-414	237-3	R.	S	

Y10 with T5. Test on a mixture of T1 + T1h.
68 tested All resistant.

Y10 with T2. 1 plate shows half a dozen moderately large colonies and 1-200 rather small.
Y10 - T6.
Y10 - T1 + T2 20-30 good sized rough colonies. Several mucoid ~~radiate~~ 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. Luria's report that B/1/2h was sensitive.

Salmonella cross.

SW3 x SW10.

June 19, 1948.

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

P21. Pick colonies and streak on Arabinose + ~~Arabinose~~ 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+).

Streaked on arabinose + xylose = 237-1. : Mixture of A- X- and A+ X+.

No Recombination.

Nutrition: SW5, SW11, Y132, W93.

	1	2	3	4	5	6	7	
		AA3 + ...						
SW11	0	0	H.C.	Y.Cx.	Vits.	HCV	Rhamnose.	
	−	−	++	++	−	+++	−	H.C.
	✓	✓	✓	++++	−	✓	✓	

	1	2	3	4	5	6	7	
SW5.	0.	HC	V	HCV	Y.Cx.	X-1	X-3	Y. Cx.
	−	−	−	−	++	−	−	

~~SW~~ W93	0	+	−	±	−	−	−	?
Valine +:								

Y132	0	+++	−	+++	++	−	−	HC.

Arginine +: 20h.

SW11. Grows on A3 + A5 or A3 + EA. ∴ Requires either histidine or threonine.

SW11.	A3 + H	H	A3
	A3 + Th.	Th.	−

V_{1a}^{R} Crosses.

June 23, 1958.

1. W-183 × W-401.
2. Y87 × W-401.

Pick P26 and test smears on T1; EMS Lac.

(1).

−R	−S	+R	+S.
3	4	15	5
4	2	9	7
7	6	24	12

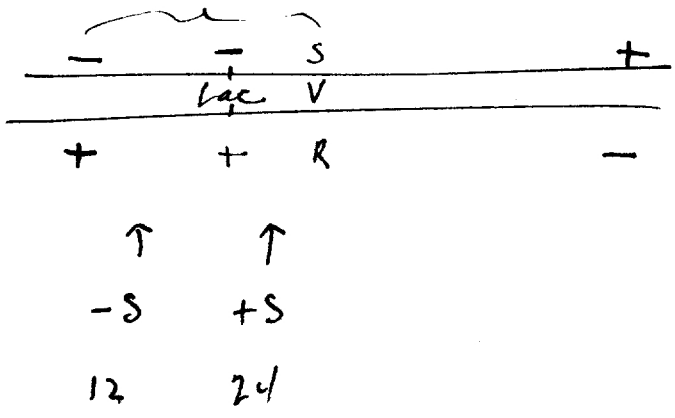
$\frac{R}{S}$ $\frac{31}{18}$. 49

$\frac{+}{-}$ $\frac{36}{13}$. 49

B-M-Lac-V^S × B+M+T-L-Lac+V^R Lac = 72%, linked to BM.

V_{1a} = 60+% also linked to BM.

Indicated order:



But these are not linked to each other! V_{1a} may, then, be to the left of B_1!

2 yoa

(1). W401 × W183. T1:

Note: 46+ : 41−
14+ : 18−
60 : 59

* T(B1)	−R	−S	+R	+S.
	2Y	17	36	10.
T(0)!	4	10	17	1

Ratio should be 80+ : 40− !

(2) W401 × Y87.

T(B1)	$T_1T_5{}^R$	$V_1{}^S$	$T_1{}^RT_5{}^S$	$T_1{}^ST_5{}^S$	
Lac−	15	9	6	3	2Y
Lac+	27	20.	7	13.	47
					71

Lac+ : −
117:34 o.f.

$V_1{}^R$: $V_1{}^S$:
42 : 29 o.f.

* Nutrition of W401 needs to be rescheduled!

June 28, et. seq.

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

Purify as W-413 + W-414.

(2). 58-161 / T1h. Test on T1 + T5. 56 tested.
1S = 241-2

(3) W183 / T1h 28 tested. " 1S. = 241-1.

(4). W[redacted] / T1h. Slow absorption but lysis finally complete.
Test on T1 + T5. 55 tested.
Many nibbled streaks. 1QS. (241-3-12).

		[redacted]	T5	T1		W-415	415 1
W183.	1	R	S	R			416 2
58-161	2	R	S	R	plaque ridden	416	417 3
W-401	3	R	S	R			418 4
	4	R	S	R	plaque ridden	417	419 5
	5	R	S	R			
	6	Lysed				418	420 7
	7	R	S	R			
	8	Lysed				419	421 9
	9.	R	S	R.	A few plaques.		
	10.	Lysed.				420	422 11
	11.	Mucoid			but T5 S!		
	12.	Mucoid, T5[R], lysed....					

V_{1a}^{R} crosses.

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

An T(S.) unless indicated.

(1) W401 x W-183

(2). W401 x Y87

(3) ~~Y100 x 58 161.~~
Y94 x W-314.

July 6, 1948.

(1) W-183 x W-401 4+ : 3- All T_1- S. !

(2) W-415 x W-401 See below.

(3) W-415 x Y641.

-R	-S	+R	+S.	
8	1	10	1	∴ not allelic to V_{1a}

~~(3). all - 19R 3S ; not allelic to V_1 -~~

~~Call the resistance factor carried by W-415 V_{1c}^R. Its phenotype is T1R T1hR T5 S.~~

May be allelic to V_1^R

(3). All T1R.- Some are T5S.

1. = ΦONa 2. = ΦOH 3 = ΦOGal.

July 9, 1948. Beckman spectroph.

M/5000 o-nitrophenols.

λ	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	.928	1.045	264 = max
250	.54	1.166 1.157	.570 .571	.860 .857	
240	.63	1.84	.570	.819	
230	.84	+4	.820	1.158	
220	1.2	∞	1.446	1.600	
215	.9	∞	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 (280)	.735	

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282		1.005	1.370	.696	
280		1.00+	1.394 1.403 (280)	.735	

		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	.276	.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
420	.04	1.088	.053	<0
440	.03	.925	.015	0
460	.03	.603	.03	—

480	.13	.316		
500	.102	.134		
520		.050		
525		0	.039	0

~~Y15 + 1%~~ T2 Medium. 1Y3

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

		Y10	S20	Y87
(1). 5 ml M/10 buffer pH 7.0		-	-	±
(2) 1 ml	"	-	+++ uniform!	+++ exc. br. st.
(3) .5 ml	"		+±	+±
(4) Sodium lactate 50%	.5 ml	Inh. all cult.		
(5). $CaCO_3$ g.s.	.1%	-	± occ +	± occ +
(6). Sodium succinate	1g.	±	±	±
(7). Asparagin	.2g	inh. gr.		
(8) Na formate g.s.	.5%			
(9). Methylene blue		-	+	+
(10) Cartiol.		-	OK usl. col.	++occ col.

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

T2 Medium. 241a

July 10, 1948.

N.L.A. + (50 ml.)	Y46	Y87	S20.
1. Buffer pH7 1 ml M/10.	- sl. inh.	uniform ++	++ - not th. sh.
2. Sodium lactate .1 ml 50%	—	all not tiniest +++	+++
3. Asparagin .2g	-	++±	+++
4. Sod. Succinate .5g	—	lgit red +	lgit red +
5. Sod. formate 10% 1 cc	- inh.	±	+
6. —	~~+~~	- faded in some colonies	+++
7. Buffer M/10 pH6.0 1 ml	-	faded	faded.
8. " M/5 pH6.6 .5 ml	-	±	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,):

1. SW-7
2. SW-12
3. SW 7+SW 12.
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 SP2, suggesting that we have true smooth + rough phages, as confirmed by growth habits.

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

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

SW7 / Sp 6 gave a large proportion of resistants (possibly because taken from a old culture) Pick + purify.

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

July 10, 1948.

SW7 x SW12. Grown separately overnight in YB and plated on T1B, J.

July 12: colonies noted on X and SW12 plates.

SW12^R. 10 tests all Sr+ Sp6^R. SW12 is supposedly Sr−!
These tests n.g.

July 10, 1948.

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

1. w-413 (U_{1a}^R) !!! No yield! on T(B,). 17+ : 99− on lacEMS' Sensitive!!!

2. w-416 (V_{1c}^R) Excellent yield. Test from T(0) + T(B,) to EMS & EMA'. 7/12

3. ~~Y87 (V_1^R)~~

(1): −: 72 S 1?R
+: 14 S 0 R.

kuplee's very close linkage of V_{1a} to BM. Check parents:

	W112	S!
	W413	S!
249-1	→	S! from T(0) ALL S : 1+ : 6−

(2) from T(0):

	−R	−S	+R	+S
	11	16	0 *9	9.
T(B,)	55	83	35	10.

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

many colonies show "partial" lysis.

streaks out some streaks for further identification:

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

~~R S + R + S.~~

S.0:

1. "– R"
2. "
3. "
4. "
5. +

Test 5 colonies derived from each.

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

∴ these crosses could not be scored. Recomb^nts originate from W4/6 (V_{1c}^{R}) which is $T1^{R}\ T1h^{R}\ T5^{s}$.

Compare 2496 1 with W4/6 and 58-161 re , T1, T1h, T5.

EMB Lac

EMS + TLB,

USA.

July 10, 1948.

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

		W413	W112	SW7.
1.	—	—	++	++
2. Na lactate	.01 ml	-	++	++
3.	.05	—	++ +++ vs.	+++
4.	.10	±	+++	+++
5.	.50	+ max:	+++	+++
6.	1.0.	uni -	uni	uni.

11str. .1ml 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 run: Inoculate 58-101 on medium #1. 27 plates.
On many plates, all colonies have red centers. ca 150/plate. 4000 colonies.
Pick up those with most intense reaction. → This may be in part an effect of radiation

T1:

1. ● → — W425= R probably contam. T.O.
2. ◉ → + and - W425 S
3. ◉ → all +
4. ● → — W427 R.

V mapping. 251.

July 12, 1948.

(1) W413 x Y64 413 mg. Good yield.

(2) W416 x Y64 on B_1 T(0). ok!

(3) Y87 x W401.

(4) W415 x Y10.

2. taken from B_1. Pick large colonies to water; small cols. to EMS! 66 small: 82 large noted. Test T1, T5.

large: EMS. -R -S +R +S.

small: 8-: 40+.

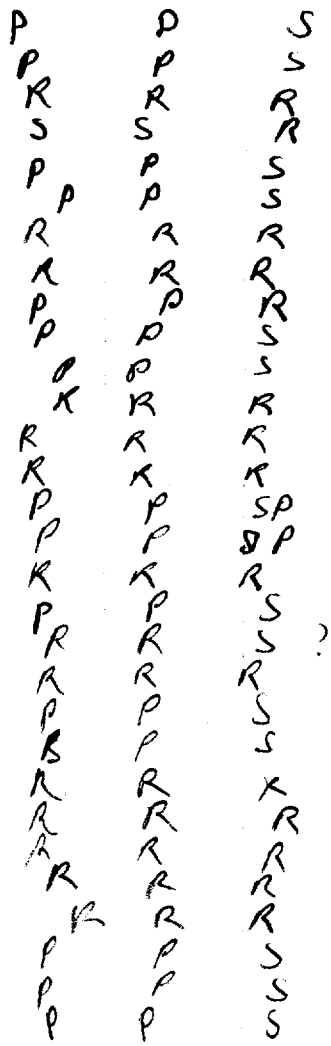
	T1h	T5	T1
-:			
+:			

EMS ~~EC~~ routes to T.O.

	T1	T1h	T5
vac t:	R	R	R
	P	R	S
	R	R	R
	P	R	S
	P	P	P
	R	R	R

R, S, + P

—:



?

?

251-1: many colonies were radially sectored, suggesting segregation. On first subseq. streaking, both +, −, and radial streakings were noted. Supplete; test + and − both a + and − were T. (S.

Restreak from broad streak of 1st plate:

251-2.

25/c.

EMS' isolates to EMS'.
mostly Lac−

T1	T5		T1	T5		T1	T5
R	S		R	R		P	B
P	S		P	S	Lac+ →	R P	S
P	S		R	S	Lac+ →	R P	S
P	S		R	R		P	S
P	S		R	R		R	S
R	S		R	S		R	S
P	S		R	S		R	S
P	S		P	S		R	R
R	S		R	R		R	P
P	S		R	R		R	R
R	S		P	S	Lac+ →	R	R P
R	S		R	R		R	R
R	S		R	S	Lac+ →	P	P
B	S		R	R		R	R
D	S		R	S		R	R
R	R		R	R		R	R
R	S		R	R		R	S
R	R		R	R		R	K
R	R		P	S	Lac+ →	B	S
R	R		R	R			
R	S	~~Lac+~~	R	S P			
P	S		R	R			
R	P		R	R			
R	S		R	S			
R	S		R	S	Lac+		
S	S		R	R	+		
R	R		P R	S			
R	R		R	R			
R	S		R	R			
			R	R			
			R	R			
P	S	Lac+					
R	R		R	S			
R	S		R	R			
R	R	Lac+	R	R			
R	S		R	R			
R	K		R	S			
R	R	lac	P	S			
R	S		P	S			
P?	S		R	R			
R	R		R	S			
			R	R			

251-1 definite ! (S)
purify + recheck.

Possibly diauxic?

251aa

July 21, 1948.

See 251b-c.

From streak-out plate of "251-1" chose 9+ and 10− colonies and 1 mixture for phage test:

Lac	T1	T5	Lac	T1	T5
+	P	S	−	R	R
+	P	S	−	P	S (251-3)
+	P	S	−	R	R
+	P	S	−	R	R
+	P	S	−	R	R
+	P	S	−	R	R
+	P	S	−	R	R
+	P	S	−	R	R
+	P	S	−	R	R

I +S −R

Note: parents were W416 and Y64.

58-161 V_{1C}^{R} ~~58-161~~ V_{1}^{R} T-L-B_1- Lac −

Except for 251-3, the culture seems to have "decomposed" into parental combinations. Check nutrition!

Restreak from gross mixtures: +, −, and mixed cols. seen.
(from 251-1:) 251a had only + and −

July 13, 1948

58-161 37 plates 6 sec. cotton smeared but estimate ca. 7000 tested.

Nutrient Agar + 1% lactose + 50 mg/l. T2. Autoclave together

1.  +++ and slow W-426
2.  + and - 427
3.  + and - 428
4.  + and - 429
5.  + and - (faintly slow). 430

July 15, 1948. T2 Glen cum.

100 plates x ca. 150 cols./plate = 15 000 tested.

4 mutants recovered + tested to be V_1^s!, Lac- (? for 433)

False Partially "typed"
section of 24961 from t 1151ac / T1.
and S.O. 254-1.
" partial tapes " in this section.

(A). Phosphine "GNR" received from Amer. Cyan. Co. Made up to 1 mg/ml and filtered through paper. Add to Nutr Broth + autoclave. Add to maize cane. indicated in γ/ml:

SW7:	10	No appreciable growth inhibition noted. Use 100γ level for further expts.
	20	
	30	
	50	
	80	
	100.	
	0.	

B7:1 — Turbidity may be due to dye. Use 10γ level.

SW10. do. B10:1

(B). Potassium arsenite, Merck, made up to M/100 (as $KAsO_2$)

SW7 1:100 some inhibition B7:1

1:50 appreciable "

SW10. 1:50 " " B10:1

Use M/10,000 = 1:100 in further expts.

Wash cells for all transfers.

(A) 7:1 is first tube recorded as 2:3, etc.

P15: Transfer from :2 to :3, bagged transfers.

A10-5.

10 tested 9 camphage.

1? Repeat test.

A7-5.

16 cultures tested on S436. + SW-10

all still camphage.

> [illegible] phage.

Check new phage ~~strains~~ resistants

			T1	T1h	T5	
1		401	R	R P.L.	S	
2		402	Mucoid R	R	R	
3	4	410	S	S	S	
4	3	411	R	R	S	
5		412	R	R	S	
6	5	413	2 plaques S]	S	S	Sensitive n.g.
7		414	R	R	S	
8		415	R	R	S P.L.	
9	8	416	R	R	S	
10	10	417	R	R	S	
11	11	418	R	R	S	
12	12	419	R	R	R	T5 R ! !
13	13	420	R	R	R	" ! !
14	14	421	R	R	S	
15	15	422	R	R	S	
16	6	58-161	S	S	S	
17	7	~~Y80~~	R	R	R	
	8					

Salmonella:

	Sp 2	Sp 6	
SW3	S	R	
SW7	R	S	
" SW3/2 "	S	R	
"	S	R	
" SW7/6 "	R	S	not true resistant!
"	R	S.	" " "

July 17. Recheck:

		T1	T1h	T5	
LacEMB:	W417	R	R	S	partial lysis not clearly seen with W417 especially on media where its growth is deficient.
	Y10	S	S	S	
	249-61	P	P	S	
LacEMS+TLB,	W417	R	R	R	
	Y10	S	S	S	
	249-61	P	P	S	
LacNATZ	W417	R	R	SP	
	Y10	S	S	S	
	249-61	P	P	S	

July 16, 1948.

Grow W-252 and W-327 in Gna broth overnight.
(Test first on Lac + Mal EMB, T2).

	EMB/Glu	EMB Lac	EMB Mal	T2 Lac	T2 Mal	
252	−	++ (1-noted)	−	+++	+++*	all white!
327.	−	−	±+	±	+++	

purify + restreak. ~~Inoculate~~ 10 plates each of T2 Lac + T2 Mal ~~with 252 + 327 respectively~~

Inoculate suspension of 252 Lac+ on EMB + T2, four plates each.
Controls: EMB: all ~~red~~ +++.
T2 " "

EMB:
1. Small − ? large + small S.O. on EMB. all +.
2. ◉ + and − W436

T2
3. ◑ + and slow
4. ⊙ slow +
5. ● all − W437
6. ◎ + and slow
7. − colony noted on original streaking of CO-252. = W431

19. Streaked gives colonies with a strong -) reaction on T2. Purify and keep as W-462.

4 stations in all ; ca 200 strains. 6.

July 19, 1948.

Inoculate W252, purified, 6 secs. on a) ~~EMB Lac 45 plates~~
b) T2 Lac 45 plates.
ca 200 ps = 9,000.

D.G. fecit) W327 " 6 secs. on a) EMB Mal } 45 plates.
b) T2 Mal } 200 ca. = 18,000.

W252). b). S.O. from T2 to EMB Lac.

1 plate
1. slow
2. slow
3. slow
4. slow
5. + and - W-438
6. slow.

W327). b).
1. - or slow. W439.
2. + and slow
3. + and - as. W440
4. mostly -. W441
5. all +
6. + and slow
7. + and slow 442
8. all +.
9. +, -, and slow 443 - 444 3 445 +
10. + and slow
11. + and - 446
12. + and slow 447
13. + and - 448.
14. all - 449
15. all +
16. all - 450.
17. + and slow +
18. + and - 451
19. all + S.O. on T2.
20. + and - 452.
21. o. slow + small
22. o " "
23. mostly -; some +
24. o. slow + small
25. o (temperature?) all +
26. o - (slow ±?) 453.
27. o all - 454
28. o - occ. + 455
29. o + and - 456.
30. o + and - 457
31. + and - 458
32. + and - 459
33. mostly - 460
34. o mostly - 461

EMB. →

All cultures listed : use Lac 1 / 5 / 1

July 16, 1948.

Prepare N.A. plates ± 2% sucrose + 50 r/ml T 2 + varying Tergitol 7 ~~(in 1 tube)~~ in ml/50 of .1% solution:
N: − sucrose S: + sucrose.

P18: Tergitol	N	S.
.2	Mod growth ½ plate	heavy growth + conidiation
.5	"	"
.7	no growth	Sl. mic. growth + conidiation
1.0	1 cm. thin growth	Moderate growth to edge of plate
1.4	< "	No growth

No plates showed colored mycelia.

Next day: growth similar + advanced Neocolor.

July 20 ff.

SW7/6 purified from 254 uridine following induced colonies. High mutation rate from R → S apparent.

July 19. S.O. SW7/6. Test 20 colonies on Sp 6.

19 R
1 S.

1R inoculum for cross

July 22, 1948. SW7/6 × SW10

on T(0):
SW7
SW10 = Tr− Ar+ $Sp6^S$ R.M. → Tr+ Ar− $Sp6^S$ on NSA to check stability.
SW7/6. 1L− Ar+ $Sp6^{R\to S}$

also S.O. parental suspensions

July 25, 1948.

SW7.	No cols	/2 pl.	
SW7/6	"	/2 pl	
SW10	2 cols	/2 pl.	→
10 × 7/6	9 cols	/2-3 pl.	Test → 9 cultures.

#5 Ar+ $Sp6^R$
#1-4, 6-9. Ar− $Sp6^R$.

Repeat phage tests on T(0) C
S71 control. Checks on fermentation of Mal, Lac + Gal.

all sensitive!

Contn. 251.

Test five "±" colonies from 251a for mutation

		0	BM	TLB,	BM TLB,
±	1.	++++	++++	++++	++++
	2.	"	"	"	"
	3.	"	"	"	"
	4.	"	"	"	"
	5.	++	"	++	"

Lac+	1.	—	+++	—	+++	BM!
	2.	—	"	—	"	BM.

MTH-W472 to solar transfer.

Lac 259-6	1.	—	—	—	++	TLB, BM.?
	2.	—	—	++	+++	TLB,.

T5 S!

When first streaked, with single exceptions, was T-L-B,. Needs for a biotin requirement.

"+" colonies seem to be prototrophic, and are splitting off numerous recombination types. Streak out tubes of I/BM TLB, and test colonies for additional markers and phage characters available.

P24. (1 - (2 streaked out from BM TLB, is Lac E M B. Test mutation for single + and a single − from each:

		0	BM	TLB,	Cm.	TS	
2	1 −			+++	+++	R	
2	2 −			+++	+++	R	
2	3 −		+++		+++	S	✓ is Lac −
2	4 −	—	+++	—	+++	S	Note! If Lac −, a
2	5 −			+++	+++	R	recombinant.
3	1+		+++			S	
3	2+		+++			S	↓ W-4/66
3	3+		+++			S	
3	4+	+	+++	+	+++	SR	
3	5+		+++		+++	S	

-S +R.

July 23, 1948.

(A) S47 / Galactose EMB. 6 secs. Hanovia Lamp.
31 x 300+ readable plates (many others smeared). ca 19,700.
11 possibles tested. 260-A:111. 1 Gal- found SW-13.
Check on Sp-6.

(B) ∅ 161 / Glucose T2, EMB. 45 } x ca. 300 each.
45 } many smeared.
T2. 3 tested. 1 + and -
260-1. Restreak and test on Lac, T1.
Lac - T1[S] W-467

July 23, 1948.

S.O. from 251-a-1 to EMS. Predominantly lac+ prototrophes (1:100 or -). Pick 28 of these and streak out on lac EMB, P24. Same suspensions. Designate mosaic + as M. Write types in relative order of frequency. ( ) v. rare.

P25.

1. M − +
2. M − +
3. M + (−).
4. M (−) (+)
5. M
6. M −
7. M (−) (+)
9. M −
10. M + −
11. M − (+)
12. M − (+)
13. M + (−)
14. M (−) (+)
15. M (−) (+)
16. M.
17. M (+)
18. M (−)
19. M
20. M (−) (+)
21. M − (+)
22. M + (−)
23. M − +
24. M −
25. M − (+)
26. M − +
27. M − +
28. All −.

Streak out on ~~EMB~~ EMS.
a) M colonies
b) equally dense mixtures of − and +

Streak out on EMB: M colonies.

Test for sensitivity.

Suspensions 1-9 were tested with T1 and T5 for sensitivity to T1+T5 or T(0). Each culture was sensitive to both phages. From this T(0) plate, inoculate T(0) slants as W465: 1-9. (H for Hutchinson).

July 25, 1948.

PLAN: Streak out in series 465-1, -2, -3, -4. → EMS. → EMS; → EMB. → EMB..... to indicate whether 465 can be "purified".

P25. Streak out -1, -2, -3, -4. (from T(0) phage test plate: see 261.)

A. EMS' Numerous colonies, all +. on all 4

EMB. +, -, and M colonies predominating.

A27. S.O. 4 Colonies from A1.

EMS 4 cols from B1. ⇒

B.

EMB. +, - and M predominant.

C. EMS. All four are +

P28 EMB. + and -; too thin to determine whether they are mosaic.

Take 1 col. each from C for D +.

P30. D: EMB. P31. Most colonies still mosaic.

EMS. (A1) ① 1 + colony with - sector. Others (—) ⇒ E(1-4).

② all +.

③ all +

④ 1% - ; others +.

P31. + colony to T(0) liquid. grow overnight. Streak out on EMB dr 262 - D11 ca 60% Variegated. Numerous + colonies.

P1. E. EMS. 1, 3, 4 all + 2 1:100, - : +

EMB. All preponderantly variegated. Select four colonies from "4" for

F↓

Aug. 12, 1948.

J. EMS: 1-4 All +

EMB: 1, 2, 3, 4 } mostly Var.

EMB + Na. nucleate 1/2 0/0, 1/10 0/0 } variegation unscreable due to modification

EMS. All +. EMB. All Var.

K. EMB

P14. EMS 1-3. All + 4. 1 -. 2 cols suspended for M ↓

L. EMB All V.

P16 (M). EMS 1,2 All + // EMB Varieg. Store in refr.

P23+ (N). do. Store in refr. P28 +.

9/10 co. 0. do. from EMB plate to EMS for P, 9/20/48.

Verify colonies on EMS + transfer to T(0) agar as W465 282 P

persistence of heterogenic.

August 3+, 1948.

F. EMS. All 4: all +. 4 cols. from ①

EMB. all predominantly variegated.

A7.

G. EMS All +.

EMB. 1. Predom. Var.
2. " "
3. Partially Var. Many full + or sl. variegated colonies.
4. Predomin. Variegated.

Select 4 colonies from EMS-1 as H: 1-4.

" " " EMS-3 as H: 5-8

A8: 1-8 tested as T1, T5. on EMS; EMB. All 8 were +S on T1, T5 EMS.

H. On EMB, all showed ± resistants in varies regards, T1 + T5 illustrating the segregants.

EMB: 1-8 all prominently variegated.

EMS (A9) 1: appreciable — (1-2)

2-8 All +. For I, choose 2 cols. from 3 and 2 cols from 5. (3-4)

A9. EMS
EMB.

EMB.

I 1 Var.
2 Var.
3 Var. } colonies tend to look uniformly dark when crowded.
4 Var. }

EMS. All, all +. ~~2 from~~ 4 from ③ → J P10.

P10.

J

July 26, 1948.

Sec:	261-	Lac.	O	BM	TLB,	BMTLB,	Lac	T1	T5	Recheck lat. reading
1	7	−			+++	+++	−	~~R~~	~~R~~	
2	7	+.		+++		+++				
3	8	−	−	−	−	+++	−	R	R	MTLB, ✓
4	9	−			+++	+++				
5	10	−	−++	−++	−+++	++++	−	R	R	
6	10	+				++++		−		
7	23	−	−	−	−	++++	−	R	R	MTL ✓
8	24	−			+++	+++				
9	26	−	+	++−	++−	+++	−	$P^{R?}$	$R^{S?}$	mixed?
10	26	+		++−		++++	+	P	S.	Parental.

~~#~~ 259-6. MTL

~~K~~

~~B~~..

Test for phage and streak on bac E M B from BM TLB, tube.
Repeat nutrition of 3 + 7 directly.

263: Test - segregants.

R: MTL - 15 TL - ~~13~~ 14. Prototroph. - 1

T - 2 M-1 ML 2 MT 1.

S. M 6.

O 2.

+ :

R. TLB, (M?) 1.

S. M 8.

M is definitely not segregating properly, being in marked excess both in Lac - and Lac + categories. Is it segregating properly? However, this may not be a random sample.
B + B, certainly are not.

Save as (HFS)

W-472.	M-T-L-	Lac-R.	= 259-6.
473	M-	Lac-R	
474	M-L-	Lac-R	
475	M-T-	Lac-R	
476	T-	Lac-R.	
477	T-L-B,-	Lac-R.	} (for further crosses).
478	M-	Lac+ S	

Retest single colon

		-T BMTL-B.	-B BMLB,	-M LTB.	-L BTLB,	+ BMTB,	BMTLB,	
W463	3	—	—	+++	—	—	+++	MTLB,
W467	7	±	—	+++	—	—	+++.	MTL(B,?)

	O	BM	TLB,	BMTLB,
5a	—	—	—	+++
5b	—	—	—	+++
9a	—	++	—	+++
9b	—	++	—	+++
10a	—	+++	—	+++
10b.	—	+++	—	+++

but Lac −
~~parental~~. Clear
plaque. ~~Undetectable~~

parental in all respects.
i.e., BM Lac+ $V_5^S \cdot V_{1C}^R$

Pick 45 prototrophs at random from EMS. ←
and test for phage sensitivity to T5.

Lac− (4 colonies) 4 S 0 R.

Lac+ (41 ") . 37 S. 4 (?) R

Rechecked of 4 S's. all were S.

all + prototrophs → primarily M colonies, with poorly demarcated sectors. Also occasional + and −

(the plating of 261-1 [L→] is again the most sharply sectored colonies noted so far).

Search for Syncaryon:
W-1 × Y40.

July 27, 1948.

Cross heavy suspensions of W-1 and Y40 on EMA/O) Malyon.

Replate,

P28:	−	+
	26	2
	17	2
	13	5
	15	0
	16	1
	8	0
	8	0
	11	1
	18	1 +1?
	15	1
	17	2
	5	1 SEC.
	14	1
	11	0
	22	3
	226	21

Pick all +'s and a) streak out on Lac EMB b) test with T1 on EMS.
4 +R 6 -S 6 -R. No +S (possible heterozygote).

A29. New crop of Mal+ colonies (some rather hazy). Pick + test on lac, T1.
14 tested with lac, T1.
5 -S 7 -R 1 ?+S Streak out on Lac S + lac EMB.
264-1. pure Lac+.

July 26, 1948.

Grow 261-1 in T(0) 24h. Dilute out and plate carefully on EMB, EMS.

		Total.			
1. EMB.	1	14.	3−	2+	9M.
	2	12	1−	3+	8M
	3	13	4−	1+	8M
	4	10	1−	2+	7M
	5	12.	3−	1+	7M.
		61	12−	9+	39 M.

2. EMB.	21.	4	2
	28	4	2
	17	1	1
	21	4	3+
+'s very large.	27	3	4
		16.	12
3.	32	1	4
	33	1	1
	37	4	4.
	35	2	2
	45	6	7

	Total.	#	−	#+	M.
4:	19.		3	1	
smeared.	31		0	2	
	25		2	0	
	37		9	0	
	22		2.	3	

Collect +, −, and clearly sectored colonies from these plates.

O = +

S.

O = #− Test on EMBLac / TS.

Sectored colonies were chosen for complete analysis if they appeared to have segregated early in colony formation.

Pick 4 colonies (A-D) from each set of plates (1-4). + S.O. on Lac EMB

		+	−	Total	Mean + prototrophs
EMS:	1.	7	0	7	
		12	0	12	11
		14	0	14.	
	2.	15	0	15	
		20	0	20	17
		15	1	16	
	3.	35	0	35	
		19	0	19	27
		34	2 ?	36.	
	4.	23	0	23	
		22	0	22	27.
		42	1	43.	

fida – colonies more or less randomly from 265 plates x test E
T5. Parental Comb. = Lac – $T5^R$; Lac + $T5^S$. (Latter diff. by M)

Lac+: ~~9R · 1S~~.
9S : 1R

	R	S		
Lac–	9	1		
	16	4		
	15	4		
	40	9	49.	Ca 20% of the Lac– segregants are non-parental.
	9	1		
	49	9	58.	Ca 10% of the Lac+ segr. are non parental.

July 29, 1948.

1A: 1-9 Lac− 10 Lac+

1B: 11-7 Lac− 8-10 Lac+

1C: 21-25 Lac− 26-30 +

1D. 31-35 − 36-40 +

2A: 41− −50

2B 51− 60

2C 61− −70

2D 71− −80

B− and B_1− have been scoring v. poorly sided + should be omitted from consideration

parents were M− Lac+ V_5^S

T−L− Lac− V_5^R.

Test sensitivity to T5:

	1A		1B	1C	1D	2A	2B	2C	2D.
	0		10	20	30	40	50	60	70.
1	R		R	R	S	R	R	S	Lac− R +++
2	R		R	R	S	R	R	S	R
3	R		R	R	S	R	R	S	R
4	R		R	R	S	R	R	S	R
5	R		R		S	R	R	S	S
6	R		R	S	S	S	S	S	R
7	R	−	R	S	S	S	S	R	S
8	R	+	S	S	S	S	S	R	R
9	− R		R	S	S	S	S	RS	R
10	+ S		R	S	S	S	S	S	Lac+ R. TL.

Nutrition: 1 (MTL B_1 M. TL M MTL TL(B_1) L +++

10. M +++ M (T?) · M · TL. T/L) TL. TL

10 Lac+ 11 scored as T+ completely sided, 21, 51, 10, 50, 60 = 5 were parental (i.e., had pro… M… TL.

W-471.

July 30, 1948.

Retest cultures 71-80 nutritionally and for lac; phage, from phage test plates. Prepare 2D mixture on slant as 265-20.

	Repeat phage	Lac	T5	Nutr.			Lac	T5	Nutr.	
71.	−R	−	R	MTL	✓	+++ G	61. −S		M	✓
72	−R	−	R	TL	✓	M	−S		M	✓
73	−R	−	R	MTL	✓	+++	−S		+++	✓
74	−R	−	R	TL(B,)	✓	M	−S		L	✓
75	−S	−	S	M	✓	TL	−S		L	✓
76	+S; −R	+	R	+++	✓		+ S		+ S	M ✓
77	+S	±	S R	M	✓		+S; −R		+ R	+++ ✓
78	+S; −R	+	R	+++	✓		+S; −R		+ R	+++ (+++) ✓
79	+S; −R	+	R	+++	✓		+S; −R		+ RS	+++
80.	+S; −R.	+	R.	+++	✓		+S.		+S.	M

heterozygotic

Do. 61-70. Phage tests n.g. Repeat phage tests. Repeat!!

Many of the Lac+ recombinants are apparently +S: H− still heterozygotic in these plates, especially if prototrophic. Perhaps they have a lower segregation frequency. Streak out #78 and #80 on EMS Lac

See 271

These colonies obviously have more than 4 kinds of recombinants

July 28, 1948.

Grow SW10 (Tr- Ar-) and SW13 (IL- Gal-) in YB overnight, wash + plate conc. suspensions on T(0) plates.

P28. 10: (3 plates). No cols.

13: 3 plates No cols.

X: 7 plates. Syntrophic background & a scattering of tiny colonies. Pick some of these out on T(0).

1.
2.
3. 3 tested on gal; arab. No exchanges.

1: Gal- Ar+

7. Gal+ Ar-

A29. Pick 9 further cols + test:

9 tests: all Gal+ Ar-

Summary: 16 Gal+ Ar-
1 Gal- Ar+

segregation from 1046S.

From Exp. 265, pick variegated colonies, streak out + recover 1+ and 1− from each variegated. Align as far as possible (some plates had no well coi-lated +'s so that the −'s are unpaired). a − b +.

		Lac	T5	Nutr. (Liq.)	Agar.
1	a	—	R	~~M~~ +++	B, +
	b	+	S	M	B, +
2	a	—	R	M T L [illegible]	B, −
	b	+	S	M	B, +
3	a	—	R	M	B, −
	b	+	S	M	B, +
4	a	—	S	[illegible] TL [illegible] M	B, −
	b	+	S	[illegible] M	B, +
5	a	−	R	v.g.	B, +
	b	+	S	M	B, +
6	a	n.g.		M (L)	B, +
	b	+		M	B, +
7	a	—	R	M [illegible]	B, −
	b	+	S	M [illegible]	B, +
8	a	−	S	TL [illegible] M	B, −
	b	+	S	[illegible] M	B, +
9	a	—	R	TL	B, +
	b	+	S	M [illegible]	B, +
10	a	—	R	TL	B, −
	b.	+	S	M [illegible]	B, +

1-20 are from earlier streakings.

M+ } 5
M− } 14.

R+ } 7
S− } 11

T+ } [illegible] 14
T− } 5

L+ } 13.
L− } 6

In this series, liquid nutritional tests covered only MTL due to the failure of B + B, to score & present washing facilities.

Every + in this series is M− Lac+ V_5^S

The "−"'s are: −S: 2 −R:, with a variety of nutr. requirements.

Preserve (2a).

	A.		B.
	Lac	TS	ML
21.	+	S	± R.
2	−	R	spaces +
3	−	R	+ S
4	−	R	+ S
5	−	R	+ S
6	−	R	+ S
7	−	R	+ S
8	−	R	+ S
9	−	R	+ S
30	−	R.	+ S

	A		B
31.	−	R	+ S
2	−	R	+ S
3	−	R	+ S
4	−	R	+ S
5	−	R	+ S
6	−	S	M + S
7	−	S	M + S
8	−	S	M + S
9	−	R	+ S
40	−	R.	+ S

	A	B.
41.	− R	+ R
2		
3		
4		
5		
6		
7		
8		
9		
50.		

phage ?↑

51.	−	R	+ S
52	−	R	+ S
53	−	R	+ S
54	−	R	+ S
55	−	R	+ S
56	−	R	+ S
57	−	R	+ S
58	−	R	+ S
59	−	R	+ S
60.	−	R	+ S.

61	−	R	+ S.
2	−	R	+ S
3	−	R	+ S
4	−	R	+ S
5	−	R	+ S
6	−	R	+ S
7	−	R	+ S
8	−	R	M + S + R
9	−	R.	+ S
70	−	R	+ S.

11	−	R	+ S
2	−	R	+ S
3	−	R	+ S
4	−	R	+ S
5	−	R	+ S
6	−	R	+ S
7	−	R	+ S
8	−	R	+ S
9	−	R	+ S
20 M	−	S	+ S

Of 100. acceptable tests, 5 recombinations between lac and TS.

	A	B
71	− R	+ S
72	− R	
73	− R	
74	− R	
75	− S	M
76	− R	?
77	− R	
78	− R	
79	− R	
80	− S	M ↓

	A	B
81	− R	+ S
2	"	"
3	"	"
4	"	"
5	"	"
6	"	"
7	"	"
8	"	"
9	"	"
90	"	"

	A	B
91	R	+ S
2	− R	+ S
3	− R	+ S
4	− R	+ S
5	− R	+ S
6	− S	+ S
7	− S	+ S
~~8~~		
~~9~~		
~~100~~		

	A	B
101	− R	+ S
2	− R	+ S
3	R	+ S
4	− S	+ S
5	− R	+ S
6	− S	+ S
7	− R	+ S
8	− R	+ S
9	− R	+ S
110	− R	~~+ S~~ + R

	A	B
111	− R	+ S
2	− R	+ S
3		+ S
4		+ S
5		+ S
6	− R	+ S
7	− R	+ S
8	− R	+ S
9	− R	+ S
120	− R	+ S

	A	B
121	R	+ S
2	− R	+ S
3	R	+ S
4	R	+ S
5	− R	+ S
6	− R	+ S
7	− R	+ S
8	− R	+ S
9	R	+ S
130	− R	+ S

	A	B
~~130~~		
131	− R	+ S
2	− S	+ S
3	R	+ S
4	− R	+ S
5	− S	+ S
6	− R	+ S
7	R	+ S
8	− R	+ S
9	− R	+ S
140	− R	+ S

Total: among ca 135 { lac − ~~14~~ recombinants. (−8)
135 { lac + 2 recombinants (+R)

Many of the - cultures of the preceding series are somewhat densely papillate, suggesting they may be mycoi. Purify the following as Lac,- V, recombinations.

4a, 8a, 21a, 36, 37, 38, ~~39~~, 75, 80, 96, 97, 104, 106, 110, 113, 132, 135 (a).

68, 110, (b).

Nutritional Tests.

On liquid:

W447	TLB,
W448	M.
W-1/1	TLB,
W21.	TM! ?

		Lac	T5	Nutr. (liquid). ✓	
	132a	−	S	M	
	113a	−	S	M	
	37a	−	S	M	
	38	−	S	M	
A.	20	−	S	M	
	106	−	S	M	M
	133	−	S	M	M
	96	−	S	M	M
	80	−	S	M	M
	75	−	S	M	M
	W·478	+	S	M	M−
	110B	+	R	TLM	TLM (B, B, ?)
	68B	+	S	M	M−
	36a	−	S	M	M−
	21	−	R	M L	M-L-
	8	−	S	M	M−
B.	4	−	S	M	M−
	110	−	R	T L	T-L-
	104	−	S	M	M−
	97	−	S	TLM.	M−
	W-21.			M−	

See 274.

~~Today~~ August 1, 1998.

Cross, heavily, W477 x 478 on EMS Lac agar (- thiamin) for Lac+ combinations.

A4: Occasional + colonies; no - noted at this time Ca 2-3/plates.

29 + tested all TS[S.] on EMS. However, all but "8" are apparently pure + when streaked out on EMB. 267-8 shows marked variegation. S.O. on EMB, EMS + transfer to T(0) as W - ~~477~~ 479

A.) Single colonies from 1-29 were picked and streaked for test on TS on EMB + EMS. These plates were inadvertently refrigerated until P.M. when they were incubated.

B) Streaks from A4 TS-test plate were picked for ~~septal~~ retesting on TS, EMB + EMS.

A: EMB: +S. No - residue suggesting segregation.

B. ditto. All seem to be stable +S. This is incredible in terms of linkage hypothesis. Save 1-5 as 267:1-5 for further study later.

D.'s tests on W-470.

August 2-3, 1948.

	W-470		W-108		58-161	
Glu	++	A+G	−	A+G	+++	A+G
Lac	−	−	−	−	"	A+G
Mal	−	−	−	−	"	A+G
Tre	−	−	−	−	++	A+G
Gal	⊕	−	+	A+G	+++	A+G
Sna	+	A+G	+	A+G	"	A+G
Arab	+	A+G	+	A+G	"	A+G
Xyl.	+	A+G	+	A+G	"	A+G
Fruc	+	A+G	−	A+G	"	A+G
Mann.	+	A+G	−	A+G	"	A+G
Rham		A+G		A		A+G

Tests 16h. fermentation tubes.

M3/1 " "

August 3, 1948.

P2. 1 colony from 262E (synth.) inoculated in T/O. Shake overnight.

10 A3. Transfer .5 and 1.0 ml to 10ml fresh T/O and shake.

9 pulled by Dr. McCoy to a tryptone broth; None grew. Expt N.G.

Chemical control of respiration.

Phosphate and nucleate

22

August 3, 1948.

Use same inoculum as in 269. (Washed)

Incr. .5 ml into each of following: (additions / 10 ml tube) Hill media

		Turbidity 8P4:	etc. TLB, BM.
1. Basal (see infra) — phosphate		18	22
2. "	+.05 ml "	29	42
3. "	0.1 " "	35	45+
4. "	.5 " "	48	75
5. "	1.0 " "	43	96
6. "	+ .5 ml P. + 5% Na nucleate	3 (deposit on bottom)	9 sterile
7. "	" 2%	11	(colored.) 15
8. "	" 1%	21	57
9. "	" .5%	27	63
10. T/O)		60	87 (colored).
11. Permeasay broth.			
12.		H_2O 4.	2-
		Incr. 14	14
		Standard A. = 100.	

Basal = /l. See Columbia p. 104 ff.

KNO_3	1
Na AcO (Na acetate)	.5
Na citrate	.2
Amm Sulf.	2
$MgSO_4 6$	.1
~~CaCl~~	~~+~~
$CaCl_2$	+
Glucose	5

phosphate solution: Class:

30 g K_2HPO_4

10 g KH_2PO_4 / l. ≐ 10 mg P/cc.

Streak out cultures from: (1); (3), (5), and (9), (10), (11).

	1	3	5	9	10	11
V	26	21	5		1	
+	4	6	11	mostly	6	
~~+~~	5	~~4~~	15	rr +	4	could not find rod!

Aug. 1-3, 1948.

Ref. 265c.

265-68 and 265-78 are derived from single, apparently pure, + colonies which behaved a) prototrophically and b) in lac T5 broke up into +S and -R. Streaked out on A) Lac EMB and B) Lac EMS.

A). Pick single + colonies and test on T5 on EMB and EMS.

EMB: 10 cols. -68 all +R. Retest!
-78 "

EMS: none grew.

B) Scattering of + prototrophes & rare -. Pick +'s and a) streak out on EMB b) test on EMS·T5 c) on EMB T5.

b & c: b. all one +S. c) all reacted +R.

a) AY: seems to be segregating typically +, - and Many pseudo-int

Aug. 6, 1948.

① 477 × 478 — Lac EMS.

② 477 × W-21

③ 478 × W-1/1 (on Mal EMS) 3M + 4M n.g. background too heavy

④ W21 × W-1/1. (on Mal EMS)

P.8. ① 9 plates. ca 8+ : 4 −.
Pick + cols. + test for T5 resistance on EMS Lac'. Also, S.O. on EMB.

② 9 plates Lac EMS. ca 7- No +! Pick one possible slow + on Lac + Mal EMB → is (−) on Lac S, and slow after + on Lac EMS. no Maltose.

③ 8 Lac S plates.

+	−	+	−
9	10	10	5
3	4	22	15
3	10	8	11
4	5	6	5
		4	2
19.	29		
		50	38 / 88.

Test on Lac S for T5 and S.O on Mal EMB!

---

① 2 n.g. 1, 3-7 tested: are Lac+, V_5^S on Lac EMS!
None of these show signs of variegation when streaked out on EMB Lac!
~~A9~~ → 6 additional + and 1 −.

③ 19 tested: 17 is −S; All +'s are T_5^S! Streak out on Mal EMB: #1 is Mal+! others are Mal−. Streak out #1 on Lac EMS ~~and~~ #4, & #7 as possibly Lac ± from appearance of phage plate.
1. [illegible] EMB, all +S #4 [illegible] 212.
P.O. #7 is distinctly variegated. S.O. on Mal & Lac EMB.

W482 - 483. 212b.

Aug. 11, 1948.

See 272 last IP.

W482 } colonies on MalEMB: all —
W483 }

on LacEMB: Most colonies were + or —, occ. Var.

482: 1
2
3
4.

483 - showed more frequent variants.

Take Lac+ photographs from 8/9 plate on LacS 273-3-4
and 273-3-1.

482: EMB { 1. +, - and V
2. Mostly V.
3. + - and V.
4. Mostly V.

Pick to T(0) as W482. from EMS.

483. 1. +, - and V
2. Mostly V. → W483.
3. Mostly V
4. (EMB) — .

(3). 51 additional Lac+ tested on Mal EMB – T5.

A10.

8 were appreciably Mal+. All apparently $T5^R$; Streak these out as 272a 1–8. Parents were checked: & OK.

W21	Mal –	V^S
W477	Mal+	V^R
W478	Mal+	V^S
W480	Mal –	V^R

40 Lac– tested: 3 possible Mal+ noted. $2^S : 1^R$.

S.O. as 272a 9-11.

9. Pure Mal+
10. Mal– and +; no sectored cols. } on Mal EMB.
11. Pure Mal+.

On Lac EMB.

1. Occ. Var. colonies. Streak to Mal EMB, lac EMB+ rac EMS as W484.
2. + and –
3. Pure +
4. + and –
5. + and –
6. + and –
7. Pure +
8. – and Var. As (1) W485.

484 – Pure Mal+. Lac + and –. and Var. Lac S not yet ready.

485 – Pure Mal+ Lac +, – and Var. " "

486 – Mal+ or ± Var, + and Lac – "

Aug. 13-14.

Isolate + check W482- W486.

482. 1. Mostly V. 2. + and V. 3. V. 4 V, +.

483. 1. large V 2. V, +.

3 + V } all +!

484. 1. V. 2 V. 3 V. 4 V.

485. 1 V. 2 V, +. (3 V.) 4 V.

486. (1 V. 2. V, +, − 3. V, V.

272-1 colonies. 5+ 5-(6-10)

1. Mostly -, some + No V.
2. " " "
3. All +
4. All +.
5. +, - and Var. Pick as W486 to Lac S, Lac B, Mal B.
6. Mostly -, some + No V.
7. "
8. "
9. "
10. "

Phage tests on T5 Lac S

	Lac	T5
1	−	R
2	−	S
3	+	S
4	+	S
5	+	S
6	−	R
7	−	R
8	−	R
9	−	R
10.	−	R.

, no residual lysis characteristic of V_{1C}^{R}

Chemical control of segregation.

August 7, 1948.

Basal medium of 270. + 1.5% agar. Adjust upwards to 7.3 before adding buffer.

1. + M/500 phosphate, pH 7.0. T(0).
2. + " " + BMTLB,
3. + M/50 " T(0).
4. " " "
5. " " " + ½% Na nucleate.

P7. Streak out a colony from 262-51 as some of heterozygotes. Also, suspensions of W-477 + W-478.

51 grew rather well on all media. 477 + 478 did not grow on 1 or 3. W478 did very well on the other media, and 477 moderately

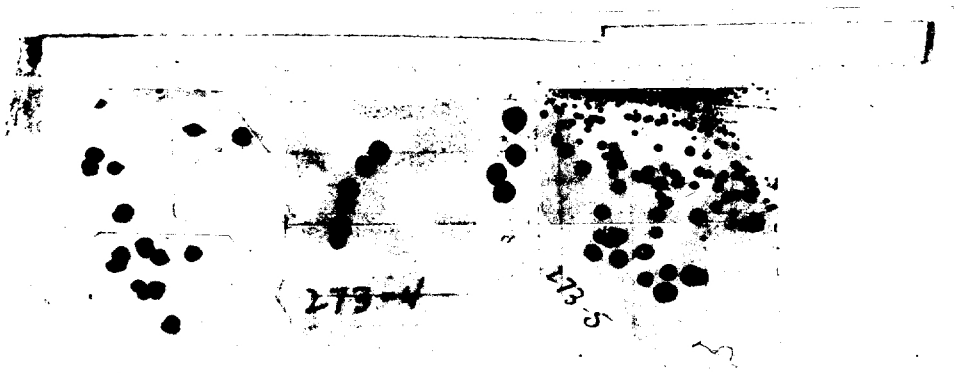
A9. well! Pick 10 colonies each from 2, 4, & 5 + S.O. on Loc EMB.

A10. (1). 1 V. 2V. 3V. 4V. 5V. 6V. 7V. 8.V. 9V. 10V. Predominantly variegated.

(2). 1-3. V pudan. 4 T, V. 5. V. 6. V. 7.V., 8.V. 9-10 unisolatable

(4). 1-4 largely + and −, occasionally variegated. 5-8 same. 7-10 same.

(5).



273-1

$PO_4^{=}$ M/500 $PO_4^{=}$ M/50 $PO_4^{=}$ M/50
Na nucleate .6%

Segregates from W-465 continued.

August 8, 1948.

S.O. to purify: ~~1-10 (Repeat!)~~ 121-130.

	Lac	T5	A.		Lac	T5	B.
121	−	R	TLB,		+	S	M
2	−	R	M		"		M
3	−	R	M		"		M
4	−	R	TLB,		"		M
5	−	R	M		"		M
6	−	S	ML		"		M
7	−	R	MLB, −		"		M
8	−	S	TLB, ✓		"		M
9	−	S	TLB, ✓		"		M
130	−	R	ML		"		M

6, 8, and 9
These were streaked out as Lac and individual colonies tested.
10 colo. each, all are Lac− V_5^R! Cf. growth in + tubes!

8/11-12-24

	Lac + cult.	−B	−L	−M	−B₁	−T	+	All lac +. Vs	Nutr.	Nutr. lac-par.
(75)	8a	+	+	−	+	±	+	S	M	M− ✓
	25a	+	+	−	+	+	+	R	M	M− ✓
	37a	+	+	−	+	+	+	S	M	M− ✓
	38a	+	+	−	+	− +	+	S	TM	M− ✓
	96a	+	+	−	+	− +	+		TM	M ✓
	97a	− +	− +	− −	− +	− −	+		ΔC −	M− TM−
	20a	+	+	−	+	− +	+	S	TM	M− ✓
	104a	− −	− +	− −	− −	− −	− +		TMB.	M
	113a	−	−	−	−	−	−		mang.	M− ✓

* B, 4, 20, 21, 37, 80 : Vs-S

\# 75 Vs-R Recheck: S.

104 is of special interest.

Test W465 segregation monoplex 275

Aug. 9.

(A). Pick lac+ papillae from 266d test plates and s.o. on lac EMB. 2/streak.

(B). Plate 132a, 113a, + 37a suspensions from BHTLB. tubes ~ T5 and T6. to pick up resistants.

			Isolated + T5	Nutrition.
(A10).	21a.	Clear + and −. No variegation (V).	S	
	20a.	Do.	S	M−.
	97a.	Do.	S	M−
	4a.	Do.	S	
	38a.	Do.	S	M−
	37a	Do.	S	M−
	113a.	Do.	S	M−
	132a.	Do.	S	
	80a.	Do.	S	
	96a.	Do.	S	M−
	133a	Do.	S	
	104a	Do. (1 papilla)	S	TMB, − ! ✓
	110a.	Do.	(R).	104 Lac − M −
	106a	Do.	S	
	8a	Do.	S	M−
	75a	Do.	S	M−

Study intensively papillae of (104) (110). Streak − and + to NA slants.

Selective media for ferm. controls.

Steak Plate out on nutrient lactose agar + K_2HPO_4 2g/l +:
lact ind −.

		+	−	
putrephenolat	1%.	—	—	48 hours.
	.1%	—	—	
	.05%	—	—	
	.01%	+++	+++	v. differential inhibition!
	.005%	+++	+++	" " "

No Buffer:
Sod. sulfite ½% +++ ++

Na Benzoate 1% — — Agar v. soft
.1% ± ± growth only in heavy streak.

Na Salicylate 1%
.1% ± ±

Neutral Red .04%. +++ +++ Background of − changed to yellow.
Colonies, especially + take up fair amounts of dye.

Janus Green .04% ++ ++ Sl. inhibition; − cells somewhat reduced compared to background. + cells same color as background.

B = phosphate buffer M/50 7.0

Acid Fuchsin.	+		−	
		Red		Red.
.4%	+		+	decol.
.2%	+++	Red.	+++	decol.
.1	+++	Red.	+++	decol.
.05	+++	Red.	++	dec.
.02	+++	Red	++	dec
.01	+++	Red	+++	dec

	+		−	
		Red		decol.
.4%	+++	Red	++	
.2%	+++	Red.	++±	dec.
.1	+++	Red	++−	
.05	+++	Red	++	
.02	+++	Red	++	dec.
.01	+++	Red	dec.	+++.

+ colonies generally took up some dye; − did not but decolorized the dye, presumably due to alkaline shift.

Crosses on low P media.

August 12, 1948.

W-251 × W-480 B-M-Ar-

T-L-B$_1$-Mal$_1$-Lac$_1$-V$_1$^{R}

Grow very heavily on a low phosphate EMS:

EMS – Phosphate

+ K_2HPO_4 M/500

+ Ethylenediamine citrate buffer pH 7.5 M/100. (= Medium 277).

Cf. EMS normal.

No colonies found at all, either on P media or on EMS.

August 17, 1918.

Streaks out 262-J. as: (BHTLB added).

EMS: methyl mangathyl.

1. [EMS-P] + M/100 Phosph. buffer pH8.
2. " M/500 + citrate M/100
3. " M/1000 + " M/100.
4. [EMS] + ARSENATE M/1000
5. " " M/200
6. " " M/100
7. " " M/50.
8. " BARBITURATE M/500
9. " " M/100
10. " " M/50.
11. EMS + H.C. + BENZIMIDAZOLE M/1000 = 118 γ/ml
12. " " M/2000 =
13. " " M/5000.

a) Growth of + and -.

VY46 Y87.

1. limited. +, - not scored.
2.
3. Growth very poor.
4. Growth moderate; fermentation inhibited.
5. ditto
6. ditto
7. " , growth may be sl. inhibited.
8. mod. gr., no ferm.
9. Slow. inh.
10. No growth.
11. Growth & ferm. OK.
12. OK.
13. OK.

b) I: Too soon to read.

o growth + - ferm.

N15: (A) Growth of Y46(+) and Y87(-).

1 G+ F ±
2
3 G ±
4 G ++± F −
5 Growth moderate. Considerable rate of fermentation.
6 G(++) F(±)
7 G(++) F(±).
8 G(+) F(+).
9 G(±)
10 G(±)
11 G(+++) F(++)
12 G(+++) F(++)
13. G(+++) F(+++).

(B). (3). G(++−) F(++). V(0).
(2).
(1). G(+++) F(+++). + and − colonies, but no visible variegation!
4. G(++) F(+++) + and − ", no visible variegation.
5. " " " "
6. G(++±) " +, ± some variegation?
7. " " " " !
8. G +++. F+++. variegation possible, but not easily read.
9. ++ +++ " "
10. +± ±
11. +++ +++ Variegation +++.
12. " " "
13. " " Like 8.

EMS does not show satisfactory working of variegation.

Do W-278 manoplate.

August 11, 1947.

(1) W-478 × Y-46 on Lac 5'.

(2) Plate with T6 on Lac EMB: 107 Resistants observed: all lac+.
Purify for W278/6 stock to use in crosses.
Plate c T5 : ca 100 resistants all lac+

89 tested; 1 - colony noted [279-1]. Pick + test for T6, resistance T7.
on Maltose EMS!
Mal−, $T1^R$ V_6^R V_7^R. ∴ contamination.

August 10 – 1948.

W478 × W480 on MalS + LacS.

(A). On MalEMS (noB,):

N12: 182 – : 16+] 198. Ca 12:1 (Cf 100:1 for standard)

1-15 FullMal+ 16 is sectored + and –. Test on LacS-T1 and S.O. on LacEMB.

(B). LacEMS (noB,) 15+ : 41–] 56.

(C). "Slow" or indefinite Mal+. Test for T1 on EMS-Lac and for Mal segn. on EMBMal

	(A). Mal+:	LacEMS-T1	MalEMBLac.	∴ None of these are Lac segregating.
1		+ P	+	
2		– R	–	
3		– P	–	
4		– P	–	
5		– R	–	
6			–, +	
7		– R	–	
8		– P	–, +	
9		+ P	+	
10		– R	–	
11		– P	–	
12		+ P	+	
13		– R	–	
14		– R	–	
15		– R	–	
16		– R.	–	+ and – on Mal EMS.

(C). Mostly Lac –. ∴ 8 : +P. None segregating on MalEMB

(B).

↓

August 13, 1948.

B. Lac+:

	Mal S	T1	Lac EMB.
1	−	?	++
2	−	P	++
3	−	P	++
4	−	R	++
5	−	?	+, V?
6	+	P	++
7	+	P	++
8	−	P	++
9	+	P	V, −
10	±	S	+
maybedupl. ← #11.	−	P	++
12	−	R	++
13	−	R.	+.

Retest 5, 9, 10 and 11 from Mal S plate.

A. Streak from Lac S to Mal EMB.

1-5 pure + 6 + and − No V. 7-16 All +. No Mal variegation!

C. Ditto: 1. − 2 − 3 −, + 4. − 5 S +, − No V.

9-11 +, − No V. 12. + 13-16 +, − No V. 17-18 +, − No V.

B.

	Lac S	Lac EMB.	Mal EMB.
5:	All +.	All +.	All − No V.
9.	All + —	→	
10.	+ Lower than other pictographs.	Variegated.	All + ?? * maybe variegated. = W487.
11.	+.	+	−

colonies were possibly variegated, but could not be definitely scored.
Streaks out from Mal and from Lac on Lac + Mal + Cf.

Segregation of W481.

484
482

280b.

10 colonies from Lac EMB streaked out. 1 - (A) and 1+ (B) from each. B- not scored. Exc. where indicated

A.			B.		
	1	B_1 - B+		1	B_1 - B+
	2	TLB_1 -		2	B_1 - B+
	3	TLM -		3	B_1 - B+
	4	TLM -		4	B_1 - B+
	5	TL		5	B_1 - B+
	6	TLB_1		6	B_1 - B+
	7	TLM		7	B_1 - B+
	8	TLM		8	B_1 - B+
	9	TLB_1		9	B_1 - B+
	10	B_1 - B+		10	B_1 - B+

All segregants were Mal+ and ($T5^R$.) Remark: all B's show signs of ~~same~~ sensitivity to T5, as does A10 and possibly A1. Not sharp!

W482 (6 pairs). All Mal - $T5^R$.

A				B.		
		484.	484.			
	1.	TLM	TLM		M	M
	2.		N.G.		M	M
	3.	TLM	TLM		M	M
	4.	TLM	TLM		M	M
	5.	LM	✓ = W491	*	M(+)	++
	6.	TLM	✓ = W492		M.	M Kupas W-~~481~~ W-493

W484 6 pairs. Mal T5

A.		24h.			40h.		
	1	TLB_1	+	R		M	+ R
	2	TLB_1(4)	+	R	TLB_1 *	M	+ R
	3	TL M	+	R	M *	Later+M * TMB.	+ ?
	4	TLB_1(M)	+	R	TLB_1	* ++(TM)	+ R
	5	TLM	+	S	✓	M	+ R
	6	TM	+	R	M	M	+ R

* Streaked and picked colonies.

Aug. 14 –

1-5 from Mal 6-10 from Lac. Segregating colonies to EMB.

	Lac	Mal.
1.	Mostly –, some + and V.	Mostly + and a diffuse "i"
2.	" "	" "
3.	Many + and –, also V.	All +
4.	Many – and + " V.	"
5.	Mostly –, +. " V.	All +.
6.	+ and – ; a few V.	All +.
7.	–, + ; " "	Mostly diffuse +, some sm. sharp +
8.	–, + many V.	" "
9	–, + several V	All +
10.	–, +, "	All +.

Pick 10 – and + (A, B). and test on Lac EMB for phage rec. from Lac EMB.

Pick 10 Mal + & test on Lac for T5-K.

Aug. 12. 1948.

Droz. fresh 58 hrs into F(O). +.

	A 13	P 14.
O	O	±
B	+++	+++
M	±	+
T	±	−
MT	++	+++
L		±
ML	±	+++
B_1		−
MB_1	±	+++
TL	±	++±
TB_1	−	++
LB_1	−	+
MTL	++	+++
MTB_1	++	+++
TLB_1	±	++
$MTLB_1$	++	+++
MLB_1	±	+++

MT especially have considerable activity, possibly in excess of that shown separately.

August 16, 1948.

Prepare washed cultures of A 58-161 and B-W-1 from Penassay Bx. Dilute to gave A/B 1:1000 and B/A 1:1000. Inoc. .2 ml each into tubes indicated. Assay for original content at 10^7 dilution, and add 30000 u Penicillin G / 10 ml tube = 300 u/ml: 2PM. 6:15 PM, assay at dilutions equivalent to 10x (A) and 100x (-) original content, allowing for 90-99% total killing. Also, streak out each culture on Lac EMB.

O: A/B 764 ♦: 1+ Total Count = 1.53×10^9

O B/A. 528+: 3- " " = 1.08×10^9

1. B/A T(Lm) BMTLB, Lac.	All+	T.C.^{9} =	.24	pS. = .65 .65
2. B/A T(BMTLB,).	All+		.25	.36 .64
3. B/A T(BM)	All+		.22	.30 .70
4. B/A T(TLB,)	All+		.30	.44 .56
5. B/A T(Q).	All+		.35	.51 .49
6. A/B - (1)	All-		.6	.73 .27
7. " (2)	All-		.09	~~1.08~~
8. " (3)	All-		.7	.19
9. " (4)	All-		.7	.19
10. " (5).	All-		.09	~~1.08~~

Note: This run was made with cells grown overnight which had been washed and refrigerated in saline for several hours. The killing has been much less altogether than in Zinder's expts. It is likely that very fresh cells have to be used!

		−	+
A/B	0.	135	0
		169	0
sci.		156	1
		161	0
		143	0
		764	1

m = 153.

		+	−
B/A.	0.	135	2
		68	0
		107	0
		100	1
		118	0
		528.	3

m = 106

1. Crowded+ 7−

1A. 256+ 1−

2. Crowded+ 7−
A. 247+

3. Crowded+ 11−
A. 220+

4. Crowded+ 3−
A. 298+ 1−

5. Cr. + (A 349+) 14−

6. Cr − 0+
A. ca. 600 −

7. Cr (sm. col. rnd.) No+
A. 90 −

8. Cr (−) 0+
A. ca. 700 −

9 0+
4 (A) ca. 700 · 0+

10. ~~2+~~
10A 89 − 2+

Penicillin Radiation Kinn
for glucose - mutants.

Aug. 16, 1948.

Irradiate 4 ml 58-161 suspension 5 secs. in small gem dish under Harrowia lamp. Recover 3 ml and incubate 1 ml each in 1/2 gma. (2 used).

A 17. Wash thoroughly. ~~HA~~ N17. Inoc. 1/2 ml into

1. A. T(BM). 2 B. T(m) Glucose + BMTLB,. 2 C. T(m) Lac + BMTLB,

2 D T(BM). Add 300 u/ml Penicillin G and shake for 4 hours.

Plate out in Lac + Glu EMB at cumulative dilutions of
2.5×10^{-7} (5), .. $\times 10^{-6}$ (4), .. $\times 10^{-5}$ (3), $\times 10^{-4}$ (2).

A. (5). 149, otherwise. $\rho S \doteq .3$

(2) smeared)

B. (5) 78, 57, 69, 92, 22, 81. $m = \frac{399}{6} = 66$ $\rho S \doteq .58$

C. (5). 94, 88, .. $\rho S \doteq .43$

2 smeared.

N2 assented D. (5) 296. $\rho S \doteq 0.$
4
3
2 Smeared.

Do not attempt to assay for biochemical mutants. Fermentation mutants were looked for on the (3) (4) and (5) dilutions.

Aug. 16, 1948.

W-478 × 480 on var. medig. see 64.

NB. – (A). Lac EMS. B, 40 + cols. } No controls did 1: · ≥ 1. on Lac EMB.
(B). Lac EMS. 40 + cols. }

(C). Mal EMS B, 32 sectored colonies, relatively isolated, picked to water and streaked on Mal S.

(D) Mal EMS. B, 40 "pure" Mal+ streaks direct on Mal EMB

A). All pure + & occasional –. No variegation.

B. Not accurately readable A19. ~~37 × 38 may be heterozygous~~ A20 No variegated cols.

(C). On Mal S. ~~—~~ (17-20 also inadvertently on Lac EMB (No var.)

(D). 40 tested. No variag. possibly excepting # 15. Retest.

1. Mostly + 1 –. 5. +, – 9. +, –
2. " " 6. All + ~~10. +~~, = poor growth.
3. All + 7. +, – 11. Mostly + 1 –
4. + and – 8. –, + 17. " " 25. +, – unusual.
13. +, – unreadable 17. All + 21. +, – 26. –
14. +, – 18. +, – 22 All – 27. +, – unusual.
15. +, – 19. – unusual. 23 +, – 28. +, 1 –
16. All + 20. +, – 24. +, – 29. +, –
30. +, – 31. +, – 32 + –

C. Tests of purified Mal+ and Mal- prototrophs on Lac EMB. Lac recorded.

	Mal+	Mal-
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	—	+
32	—	—

Totals

	Mal-	Mal+	
Lac-	20	21	41
Lac+	7	8	15
	27	29	56

Correlations:

Mal+ ↓ / Mal- →	Lac-	Lac+
Lac-	15	1
Lac+	3	2

Lac and Mal are independent.

	M- L-	M- L+	M+ L-	M+ L+	
F =	15	1	3	2	21
Exp =	12	3+	3+	1+	

① W478 × W480.

② 58-161 × W480.

A.) Lac B, B) Lac (o) C). Mal (o).

1A. 108 + colonies picked and streaked out on Lac EMB. #109 is a Lac sectored colony.
#72, 88, #30, #12, #56 appear possibly heterozygous. Restreak on Lac EMS, EMB to check.

1B. 14 possible "+" colonies. 1. ++ 2. − 3. ++ 4. −
5. − 6. ++ 7. ++ 8. ++
9. ++ 10. Var? 11. ++ 12. ++
13. ++ 14. ++.

1C. 44 picked; not readable P23. P24 No Var.

2A. 70 picked. No variegated.

2B. 18 picked. "

2C. 37 picked not readable P24. No Var. Streak susp. on Lac EMS; pick 4 colonies & S.O. on Lac EMB to test heterozygosis.

72A1 None hetero.
88A1 #1 hetero. #3 is not.
30A1 4+: all varieg. 4 Lac −.
12A1 4+. ~~Contains~~ not. het. 5 hetero. 6,7 Lac −.
56A1 4+ none pure → 1− W503
10B1. All 4 hetero. → W502.

5-8 on Lac S to select + papillae.
see 287.

72 and 56 have to be tested again;

88-1 (Lac±) = W494 88-3 (Lac+) = W495 12-5 = W498 12-1 = W499 12 7 = W500
30-1 " = W496 -5 (Lac−) = W497 10B1-1 = W501

Aug. 28, 1948.

Redrock 56 × 72. 10 cols. each from EMS treated plants, s.o. on EMB.

No variegations seen. : Not heterozygotic.

Mal reversions of W482.

Aug 20+, 1948.

Several attempts were made to recover Mal+ papillae from W482 which, still segregated for Lac, to determine whether Mal heterozygotes could be obtained. A series of + colonies was picked from

W482 in T(m) Maltose → Mal EMS. to EMB/Mal and EMB/Lac.

of 8 colonies, #1, 6, +8 were probably segregating for Lac, and all the others probably so. It could not be clearly ascertained whether there were any Mal- colonies or sectors. Transfer to T(0) slants.

Isolary each, vaugated, from Lac EMB of 286-1, 6, +8 streaked out on Lac and on Mal EMB. On Lac, predominantly + and - ± some ± colonies. On Mal, exclusively Mal+, suggesting heteroploidy between the Mal and Lac loci.

Rep (8) as W-504.

Lac - mutants of ~~E~~ co-hypo.

~~Exp~~ 287

Aug. 26, 1948 ff.
Dep. 285.

30 A1 (5-8) more striated on EMSlac N27 many papillae, pealed.
4 pulled from each colony & S.O. on Lac EMB + EMS as 287-1-1....
2 -
3 -
4 - ....

12 A1 - (6, 7) showed no marked papillae at this time on
EMS although beautifully papillate on EMB.

+(1): 1. + and - Var? 2. +, - 3. +, - 4 +, -

(2). 1-4 +, - (3).

(3) 3, +, - Var? 1-2, ~~4~~ +, - (4) 1-4 +, -.

Recheck + colonies from EMS of 1-1 and 3-3

No variegation.

Aug. 30, 1948.

Résumé: see 275.

(1). 104 Lac- is M- but a Lac+ papilla was M-T-B_1-. This segregant is, conceivably, M- Lac- T+ B_1+ , and in the course of purification of the papillae, a new segregant may have been obtained.
M- Lac- T- B_1-

(2). 110 Lac- is TS_S; 110 Lac+ TS_R.

Streak out 275- ~~-1~~ = 288-1 104 Lac- and 110 Lac- = 288-2. on L from NA slants.

a). Lac EMB. b) Lac EMS ± methionine.

Test mult. 5 cultures each of -1 and -2 from EMB Lac plates.

-1	-2
1. BMT	MTL
2. BMTB,	MTL
3. M-	----
4. (BMTLB, ?)	(B)MTL
5. "	MTL

~~Results~~: When heavy inocula are taken to EMS Lac, M, -2 gave no growth whatever while (1) gave rather scattered colonies. If the original M- cultures had been heterozygous, they are now thoroughly segregated. ~~However 288-1-3 (or 288-3) may still be useful. Transfer from L to a T(Meth) strain.~~ Terminate Expt!.

Aug. 30, 1948.

A. Y87 × W255 Gal S + B_1.

B. W488 × W480 Lac S B1 are Lac- or EMS!

C. W488 × W255 Gal helicoz.? ~~Lac S~~ Gal S

D. W491 × W255, add leucine & mixtures. Gal S

A. (B,) 194 − : 17 +] 211 = 8% Gal +
(o) 71 − : 6 +] 77 = " "

∴ should be between B_1 and V_6, left of Lac 0

check Gal + for lac, T1. ~~Could~~ Were not accurately scored for lac or Lac S. Gal + may introfere?

	Lac	−R	−S	+R	+S
Ao	Gal + :	3	0	2	1
	Gal −	6	4	0	0
AB_1	Gal +	15	2	0	1
	Gal −	16	4	0	0

Total for Gal and V_1.

		R	S
+		20	4
−		22	8

B. 105 + prototrophs picked by DG and S.O. on Lac EMB, saving suspensions.
9
The following were definitely segregating for lac:

	Colony	Mixture
7	Mal −	—
32	Mal −	—
51	Mal −	—
52	Mal −	—
56	Mal +	+, −?
~~78~~	Mal −	—
78	Mal −	—
94	Mal +	+
100.	Mal +	+
70.	Mal −	—

+ 6 Mal − : 3 Mal +

season, not Gal or lac (probably was Lac+ v)

#70 and ~~three~~ uncertain at first reading.

S.O. 56 on Mal EMS.

C. 44 Gal + S.O. on Gal EMB. All pure +.

D. 11 Gal + S.O. On Gal EMB " " .

E. 40 − cultures streaked out on EMS lac.

See 293

Sept. 4, 1948.

>89 cultures SO Lac EMS. Picks 4 + cultures from each (+ only found) and a) SO Lac EMB b) Streak to Mal EMS.

NB. 56 maybe Mal+/Mal−

94 maybe $V_6^{R/S}$

± = Variegated.

	EMS Lac 1	2	3	4.	Mal EMS .1	2	3	4	
7.	±	±	±	±	−	−	−	−	W5 [illegible]
32	±	±	±	±	−	−	−	−	W5 3
51	±	±	±	±	−	−	−	−	W5 4
52	±	±	±	±	−	−	−	−	W5 5
56	+	+	+	+	+	+	−	+	None variegated!
77	±	±	±	±	−	−	−	−	W5 6
78	±	±	±	±	−	−	−	−	W5 1
94	[illegible]	[illegible]	±	±	+	+	+	+	W5 [illegible]
100	±	±	±	±	+	+	+	+	5 1

~~70. Mutant.~~

70.	+	+	cols! +	−	+	−	−	−	−
	+	+	... +	−	+	−	−	−	−
	+	±, +, −					−	−	

W5 3

70 segregates much less frequently than the typical heterozygotes!

5 colonies on EMS Lac picked to Mal EMS + scored as + and −.
1-15 Mal− and 21-36 Mal+ SO Lac EMB to find any heterozygotes.

~~94 colonies (incl. 1-4) picked (1-9) and tested for V_6^R / T5 on Lac EMS [ref. 293]~~

None of these 31 colonies show Lac heterozygosis. When streaked out on Mal EMS, 56 showed + colonies and + uncertain. Test these on Mal EMB. → Mal−.

Original Slant of 56 S.O. on Lac EMB shows pure Lac+ and a single (1:7100) Lac− colony. Maybe "70" type!

A number of Gal- cultures were tested for Lac+ on Lac S.
21 + cultures picked + S.O. on Lac EMB.
19 were pure lac +. 2 were predominantly - but may have heterozygous components. (# 11 + 22). Repeat tests on EMB and EMS Lac (L) with these suspensions.

Single colonies picked and tested for T5, T6 resistance on EMB, EMS Lac. All were T6^S exc. indicated.

	TS	EMB	EMS.	EMS pigment to EMB.	EMB cols. A	Nutr.	B	A	B	...
1	R		R	+	+	+				
2	R		R	+	+	+				
* (3)	R		S	+	±	+	S	S	S...	
4	..	too thin		+	+	+				
5	S		S	+	+	=				
6	R$^+$	too thin		Lac+	=	=	Pure Mal+ ↓			*
7	S		S	+	+	+				
8	R?		S	+	+	+	Both pure+ and ± prototrophs			
9	R		R	T6^R; ✓ +	+	+	1-6, 8± 7+ = w581			
10	R		R	+	+	+	= ws80			
11	R		R?	+	+	±				
12	R		R	+	+	+				
(13)	R		S	+	+	+	S	S	S...	
14	R		TT	+	+	+				
15	R		R	+ *	+	+				
16	R		—	—	+	+				
17	R		TT	+	+	+				
(18)	R		S	+	+	−	R	R	(6R 1S.)	= = *
(19)	R		S	+	+	+				
20	R		R	+	+	+				
21	R		R	+	+	+				
22	R		R	+	±	+				
23	R		R	+	+	+				
24	R		R	+	+	+				
(25)	R		S	+ (v?)	+	+	S	S	S...	
26	R		S	+	+	+				
27	R		TT	+	+	+				
28	R		R	+ (v?)	+	+				
29	R		R	+	+	+				
30	R		—	—						
31	R		—	+ ?	+	±				
32	—		—	—	+	+				
33	R		R	+	+	+				
34	R		R	+	+	+				
(35)	R		S	+	+	+	S	S	S...	
(36)	R		S	+	+	+	S	S	S...	
37	R		R	+	+	+				
(38)	R		S	+	+	+	S	S	S...	
39	R		R	+	+	+				
(40)	4 cannd.		S	+	+	+	S	S	S...	

↓ See 294

Streaked out from EMB ♀ testers to Lac EMS to obtain segregants. ⊙ should be checked exhaustively for ♀^{R} segregation. ♀ includes reteste

Rebuilds

Sept. 5, 1948

Papillae picked from EMS streaks of 289E and S.O. on LacEMS + EMB.
#'s: 6, 11, 13, 14, 16, 17, 19, 20, 27, 30, 31, 32 — could give no papillae. Hold plates
See 293 for tabulated results.

Sept 2, 1948.

	O	V	AA	VAA.	V (-AA) series.	
SY19.	++	+++	+++	+++	+++	prototroph

SY58	−	+	-AA series. -4, -6 +	others −	36h. -1 + ~~others~~ faint ± AA only +.

					HC	HCV
SY71.	−	+++	++	+++	+	+++

SY70.	−	Cyst +++	M +++	Homocystine +	No_3 1/1000 +++		PARATHIOTROPH.

	O	B_1	Thiazole	Pyrimi.	Py+Th.	
SY36.	−	+++	+++	−	+++	Thiazoleless!

SY56. none grown as yet. B Typ A Typ Alcohol [illegible]
36 hours − − ++ ++ − ++ [illegible]

SY71. Vitamin Series. Single ~~additions~~ omissions. − B_1 shows some diminution?
AA series, " group " .

SY56. HC, V, HCV, AA, AAV, and reinoculate series above.

SY58. HC, V, HCV, AA, AAV.

SY56: − on V, +++ on others. AA stronger response than tyrosine.

	AA	-12	-3	-4	-5	-6	
SY71 -AA.	+++	+	±	+	+	±	(Pantoic reference!)

	HC	V	HCV	AA	AAV	
SY58.	+	−	+++	±	++	Vit. apparently required.

SY71. − B_1 shows slight diminution. Test vs. Vits.
branmic acid set, AA is higher than any single exogen. omissions.
Test omission series from AA 3 and 6.

SS8: AA – V series: – V_4 is ±. others +. nicotinic Vit K!
– V_{11} –

AA + V	++
AA	–
V	–
0	–

V – AA series: –12 – (cyst, meth) acg, lys. (trans per: val, isol, leuc. work is in /2)
– 3 –
– 4 ++
– 5 +
– 6 ++

Nutrient: AA, + mc, + K, + mc + K.
Vits

SY71	A3	+	Leucine	0	±	Try together & separately.
9/3.	A6	±		B_1	±±	B_1
	A36	+		Pyr	±	
	– L	+		T_2	±	
	– Ile	+		Pyr + T_2	±	
	– Al	+				
	– Gly	+				0, L, B, L+B,
	– Ser	+				
	– IV	++				
	– V	+				

			18h.	
SY56.	B + Tyr. +	0	–	Tyrosine and a component of #12 maybe needed for optimal growth.
		AA	+++	
		–12	+	others +++.
		BAA – Tyr.	+	

Try single omissions + additions with B1 supplement!

SY36. B_1 only.

296b.

	0	BT	BT+ A12	+C	+M	+ Arg	+ Lys.	- C + A12	- M	- Arg	- Lys.
S56.	—	—	+++	++	±	—	—	—	++±	+++	+++

∴ Cystine is required by S56 for prompt growth.

	0	B,	L	LB,	
SY71	—	+++	++	+++	Thiamin!

	0	AH	AA·nic	AA·K	AA·nic+K	AA Vits.	A235 V	V → A23	A25	A35
SY58	—	+±	+±	—	++	++	+++	+	—	—

V → A25: L +, I —, IV —

A35: C —, M —

A23: H —, T —, S —, P —

Complex AA requirements.

[nic required in presence of K!]

SY36. 24h. B, +++ others...

48h. " , T2 +++, Lys+T2 +++, Lys —. vs Thiazole

cf. S. dublin.

Sept. 6, 1948.

SY71.	0	B_1	T_2	Pyr.	T+Pyr.	l-leucine.	
	—	+++	+++	—	+++	++	
	—	+++	+++	—	++	++...	
SY36	0	B_1	l-leucine				
	—	no growth					
SY56	0	BT	BTCys.	TCys.	BCys.	$BTN_{a_2}S$	
	—	+++	+++	+++	—	—	

B, Tyrosine. Cystine replaces biotin and is stimulatory.

SY58.	0	AA	AAVits.	AAV−K.	AAmic	AAmic+R	AA·K.		Vitamins stimulatory.
	—	++	+++	+±	+	++	+		
	—	++	+++	+±	+	++	+		
	Vits + :	A'235	A'25	A'25+L	A'235−L	A135	A135+M	A135+C	A235+Arg	A235+Lys
		++	—	++	—	—	+	+	++	—
		+++	++	++	+++	—	++	++	++	++
		A123	A123−H	123−T	123−Gly	123−Pr.				
		++	++	++	++	++				

Arginine, SM, Leucine, (glutamic) (vits.?)

SY37	0	B_1	T_2	Pyr.	P+T	[redacted].
	—	no growth				
SY3.	TL	TLB_1	TLPyr	TLT_2	TL PyrT_2	
	+	+++	+	+++	+++	

Thiazole

18h

SY58 rather indefinite vitamin requirement: nicotinic.

" " AA requirement. (leucine?, cystine, arginine,

SY71. Thiazole or leucine. Purify!

Sept. 11, 1948.

Streak v. heavy inocula of the following on MalEMS (+B. added). After several days incubation:

W482 Very poor growth ~ numerous papillae. Maybe contam.
W483. No growth!
9/12 W494 Moderate growth; papillae becoming apparent!
W496. " " " " ". Good + in heavy streak!
W498. No growth!

A). Pick papillae from W482 and W496 to 1) [redacted]' and 2) MalEMB. and 3) MalEMS.' 102 papillae Mal−, probably not coli'.

Additional W-496 papillae picked.

B) Streak out cultures of W482, W483, W494, 496 and 498, and 501. on Mal and Lac EMS'. 501 only Grew. Probably B_1 deficient.

291-1. On MalEMB, apparently only ± Mal+ and Mal−.

MalEMS.' + and − colonies. Pick 10+ to water + streak on LacEMB + MalEMB

	1	2	3	4	5	6	7	8	9	10
MalEMB.	All +	+;	−?	++	++	+;	+;	++	+,	++	++
LacEMB	− !	− !									

Malplate rather difficult. Rules fall when kept 1, 2.

−2 +3. 2 cols each picked to MalEMB, pure Mal+ but Lac −

−4 2 cols. #2 maybe Mal+/− ? Recheck. No. Lac −

∴ These papillae are probably segregants, no longer Lac+! Hold 291-1 as such for further study.

September 4, 1948.

1. W491 × W255⁻ on EMS Lac (Leje., Gal.

2. Y87 × W-1 on EMB Lac, Mal

3. " " low P (see 270 etc)

4. W488 × W480 on " Lac, Mal

5. " " low P.

A6. Yield of 2 and 4 much higher on Mal than Lac (as blot. DF on real phen ???)
only 4 Lac+ noted on several plates of (4).

	+	−
~~2M~~	~~6~~ 8	~~3~~
4M	6 + 1[S] = 7	3 3
2M	9	9 5

3). 5M plates. 14 − 2 + 1 sectored. → pure M+. 5 additional M+ colonies P7: 3-5 + 1, 2 sectors (through Mal EMS.
4L plates. 4 − 0 +

5) L ~~6~~ plates 3 −
14 5 plates 1 −

V. poor yields.

4L. 4 colo. + NoV. on Lac EMB

4M. + cols. 5-14 = 10. on Mal EMB. 9, 10 −, (5-8), (11-14) + No Vac

4M − colo. 15-40 = 26 [± 25-28 on Mal by cross Ill+. I. All −
− 4
22 tests.

---

1. Gal. 1-20, All pure +.
Lac 21 − 80. 35 −
No apparent hetero zygotes.
S.O. on EMS Lac to achuls. ←

26?
57?
61?
67

Gal + 60 additional colonies (by DG) All + and −; no variegated.

Lac+.

Sept. 4, 1948.

289B cultures tested on EMS, EMB. Y.

	EMB T5	EMB T6	EMS T5	EMS T6.	
1	~~RS~~ R	RS	S	S	
32	~~RS~~ R	RS	S	S	
51	R	RS	S	S	
52	R	RS	S	S	
Mal+/Mal-? 56	R	RS	S pd.	R	Mal+ and Mal-?
77	R	RS	S	S	
78	R	RS	S	S	
94	R	RS	S	R, S	pure Mal+ ← may have two components.
100	R	RS	S	S	pure Mal+!
70.	R.	RS	S	S	

56. S.O. on Mal S to separate possible components.

94: cols 1-9 tested on Lac EMS/TS. Cf. "10" from Lac EMS.

W-528 1-9 are TS^S $T6^S$; 10 is ^{R,R} and more strongly Lac + than three others

Sept. 7, 1948.

289E-5, 6, 18 & 25+28 merit further study as possible heterozygotes (for nutritional, 4, or "lac" mutable") characters. Pressure on T6 streaks and streakout on EMS Lac for further study.

25, 28 are contaminated. 289E6 is lac+, already heterozygotic = WS64

18 S.O. EMS. a) "F" papillae noted twice S.O. on EMS, EMB Lac.

b) Test 10 - colonies of T5.
All were T5 sensitive both on EMS and EMB.

a) ↓ EMS (1-8) on EMB. 7 and 8 are + and - 1-6 all +.

N11. ↳ EMS, EMB. 8 showed all + on EMB.

7 +, and - ". Test individual ± colonies from EMS. -7. All ++. (on EMS, some are -?)

P12. F5: Pick 8 + colonies of 289F-5 from lac EMS to lac EMB All but 6 were all + (exc. for likely contamination in one plate). F5-6 had appreciable numbers of + and -! Recover from EMS streaks and s.o. on EMS, EMB lac.

6 - on EMS lac. 4 + colonies tested on EMB gave all +

E2 reports that purified prototrophs did not segregate to genetically deficient types.

b)

Sept. 6+, 1948.

P5. S.O. W530, 531 to a) Lac EMB b) EMS.

P6. a) Numerous + colonies, occasional - colonies and colonies with -sectors at edges only. Pick 4 apparently pure + from each → to EMB.

P7. W530: each of 4 showed + and -, no evident sectoring

W531: mostly +. - very occasional.

From W530 sets, pick 4+ and 4- cols (+- in alternating series) for initial testing:

+	1	TMB,	
-	2	MTLB,	
+	3	TB,	
-	4	TMLB,	
+	5	TB,	
-	6	TMLB,	
+	7	TLB,	Lac+!
-	8	TMLB,	Lac+!

(Where is T+?)

b). Pure +. S.O. W530 as EMS and EMB, 4 cols. to carry through purification

9/9/48.

Inoculate heavy suspensions of following into T(m) Mal + Glu. and on EMS Lac + Mal.

		EMS Lac	EMS Mal.
X	482	n.g.	n.g.
X	483	n.g.	n.g.
1	522	+	—
2	523	"	"
3	524	"	"
4	525	"	"
5	526	"	"
6	527	"	"
7	530	"	"
8	531	"	"

At intervals, streak on Mal EMS to recover papillae.

9/10. 526 shows papilla. S.O. Mal EMS to purify; Mal EMB. 2 cols from EMS: pure lac+, pure Mal+!

(Keep on T/O) as 296-1

		to Mal EMS	Mal EMB.	Lac EMB.
9/14. Papillae from:				
522	-1	1 partially isolated +	≥ 1 likely segregated +.	Mostly Var
	-2	Mostly −; 2 real +		" "
→	3.			
523		Mostly −; + ?	Pure −; +	
524		Mostly +	Pure +; −	
526	1	+, −	+, −	
	2	+, −	+, −	
530		+, −	+, −	

9/16. Take "2" well isolated + cols from each of above EMS (exc. 522-3) and S.O. on Mal + Lac EMB.

522	-1	Mal var.	Lac mostly −, Var.
	-2 A	"	
	B		Lac Var.
	-3	Mal+, Var?	+, − & Var.
523		Mal+	Var
524		Mal Var?	Var
526	1	Mal Var?	Var
	2	Mal+	Var
530		+, incr. Var.	+, −, Var, not striking

9/18. Take suspensions to T(o) agar of

↳	as.
522-2B	296-2
-〃	-〃
523	296-3
524	296-4
526-1	296-5
530	296-6

Possibly segregating colonies were taken from these EMS Mal plates to the same agars.

+ 2B' 1-4.

522A'. 1-4 Two types of colony are seen. ① is small and more intensely stained, with a sheen; ② is large, and much less darkly +. No clear-cut mixtures are seen.

523' } Same as above; possible — noted ± 523.
524' }

572-1 4 + colonies: all pure Mal+, Lac −

low phosphate and segregation.

Sept. 7, 1948.

Cross on Lac EMS – P. + is standard Lac S.

1. 487 × W-1
2. W488 × W480

(3)
(4).

+). ~~2.4~~) Low yields, 1-5 cols/plate. Higher on Mal than L!

4M.	5 Mal+ from 6 plates.	1-5	S.O. on homologous EMS + EMS
4L.	5 Lac+ " 5 plates	6-10.	

-P. 2) Yields low.

2M.	11 plates.	2 Mal+	11-12 " "
2L	5 plates.	2 Lac+	13-14

1) 1-10/plate. Mal better. → 15-30 = 16 Mal+.

1M

1L. No Lac+.

3) Yields same as 1. Pick none.

	EMS	EMS
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	++	

Heterozygote recognition.

Sept. 13, 1948

W480 × W488 as lac EMS + Mal EMS Take + prototrophs to homologous medium to purify.

101-110 10 Mal+ from EMS → all pure +
100 lac+ " to EMB.

PM. 1-20 All pure +.

A IS 21-100 Following are heterozygotes, showing +, − and sectored colonies.

	for lac.	A (Mal)	B Mal EMS	
✓ 24 (W480 type)	= H25	—	—	
✓ 31	26	—	—	
32	27	—	++; few −	*
✓ 35	28	++	—	*
✓ 36	29	++	++; few −	*
✓ 43	30	++	" 1 −	*
✓ 61	31	—	—	
✓ 67	32	—	—	
✓ 73	33	++	++	
✓ 76	34	++	++	
✓ 86	35	++	++.	

Pure Mal sep.

Take the single colony suspensions to T(0) slants under W- number. Take up gross streaks from EMS and streak to EMS Mal to look for complementary types (B)

A ⇒ and to EMB Mal

* 32, 35, 36, 43 show discrepancy. Take heavy streaks to T(0) as 298 −, and attempt to separate Mal+ and − prototrophs for separation into complementary types, if such.

See

Heterozygote Test Crosses

9/14/48

(1) W471 × W21. } Lac EMS.

(2) W466 × W33 } → only 12 colonies altogether from (1). All pure +.

to Lac EMS. 100 tested from 2. High yield of heterozygotes apparent.

		1st EMS.	→ Lac EMB	H−	Gal EMS.
1	5?		?		—
2	7 ✓		H	36	—
3	9		H	37	—
4	12?	pure +			—
5	16?		H	38	++
6	18?		H (S30type)	39	—
7	22?		+		+, —
8	24?		+		—
9	29 ✓ +, - prot.		H	40	—
10	34 ✓		H S30type?	41	—
11	36?		H	42	—
12	37		H	43	—
13	38		H	44	—
14	39		+		—
15	40		H	45	—
16	43		H (S30type)	46	—
17	42 ✓		H	47	++
18	46 ✓		H	48	++
19	48??		+?		—
20	65		H	49	—

The above are candidates for further scrutiny: Streak out retained water suspensions on Lac EMS, to Lac EMB + Gal EMS.

9/21. Retest colonies of 5, 12, 22, 24, 39, 48.

299-29 (−). to ~~Lac EMS to pick papillae~~ T'd) for further study.

4 addnl. colo. tested:

5	++
12	++
22	+ some mut?
24	++
34	++
48	+, some +?

Amino Acid Mixes
NK & JL

mixture ½ 1% — as Lugol

A. Non-Essentials: per 50 ml H_2O

				per 100 ml H_2O
	Glycoine	5 mg.	10	20
dl	Alanine	19	38	75
l	Proline	87	174	350
-	HoProline	2	4	8
l	Glutamic	271	(.HCl) 542	1085
dl	Aspartic	60	120	240
dl	Serine	58	116	230
l	Tyrosine	66	132	260
l	Cystine	4	8	15

B. Essentials

l	Argihine	46	.Hcl	92	180
dl	Lysine	79	.HCl	158	315
dl	Tryptophane	12		24	50
dl	Phenylalanine	39		78	155
l	leucine	50		100	200
dl	Valine	79		158	315
l	Histidine	32	.HCL.H_2O	64	129
dl	Methionine	33		66	132
dl	Threonine	40		80	165
dl	Isoleucine	50		100	200

Note: Gelatin differs from Casein:

a) No tryptophane

b) Much more glycine and hydroxyproline

c) No tyrosine?

Sept. 12, 1948.

(DG) W566 - uv 7 secs. -

20 plates X ca 300 colonies → 6,000 scored

September 18, 1948.

(H x N)

(1) W477 x W351 (Lac-, ~~Xylt~~) (2) W477 x W466 (H x H)

(1). No yield! A few lac - only! (2) v. low yield. Pick +'s +

Streaks out on Lac EMB. Following appear to be segregating. S.O. EMS lac.

4 ✓

6 ?

10 ?

12 ?

13 ✓✓

16. ✓✓ = H50

Recover only 16, as the positive is well proven with a single culture.

Also cf. H2 (W479 from W477 x W478).

Complementary heterozygotes
Continue 298.

Sept. 18, 1948.

32, 35, 36, 43

[H27 -, H28 -, H29 -, H30 +]

Pick destructive Mal type to ~~MalEMS~~ MalEMS, LacEMB.

	EMBlac	EMSMal.	
305-43.	++	— occ +	
H30	Segr.	+, occ —	Purify!
H29	Segr.	++	
-36.	++	—	Cosegregant!
H28	Segr	—	
-35	Segr.	—	No diffuence
H-27	Segr.	++	
-32	Segr.	++	No diffuence.

H27+28
Mal reactions
had evidently been
confused

Morphology of H. and N

Sept. 18, '48.

13.61

Streaks out on Lac EMS, varying lac conc. suspensions of 299-[redacted] for normal, and 7, 29, 34 and [redacted] for heterozygotic lac+ prototrophs.

Lac EMS.

1

2 Smooth, flat, uniform surface small margin, not indented

3 sl. rough colony, rough but narrow margin.

51 Fairly colonies like 3; smaller like 2

7 roughish colony, appr. rough margin

29 like ~~2~~ 7, somewhat smoother

34 mixed types like 3 from 3.

65 like 7.

No more distinguishable, everything progressively more faded on lower concentrations of lactose (½%, ¼%, .1%)

No consistent difference can be found between "normal" and heterozygous prototrophs in colony morphology on Lac EMS.

Lac-!

Sept. 21, 1948.

(1) W477 x Y40 m Lac EMS.

(2) (W466 x W483 (5 segm factors heterozygous) dis Lac EMS, also other segs

1. Low yield. Lac+ colonies only noted. 50 colonies from 20 plates pulled for streaking on EMB Lac. 26 tested all pure +.

2. Xyl 1-
5 plates 10 -
2 -
+, -?

1 sharp +! = 30 x 2^-5 Later 4 others.

difficult to score on Xyl EMB

Arab. 5 plates.	+	−
	1	1
	1	11
	1	4
	1	4
	-	4
1-4	4.	24

S.O. plus on EMB Arab.

2+ mostly −. 6, 7, 8

Gal
4 plates.

Mal 1+
6 plates.

Lac
25 plates.
v. low yield all apparently scoring −! (Of course W466 is Lac, − !!)

1-4 Arabinose
5-9 Xylose
10, 11 Galactose
21-34 Maltose.

} All +!.

Sept. 21, 1948 + prec.

Streak out 5 single van. colonies from 262-0. and isolate from each 1 pure - and 1 pure + for nutritional tests.

1-5 are - ; 11-15 correlated +'s.

1	MT	11	M(T)
2	MTL	12	MT
3	M B?	13	MT
4	TLB$_1$	14	MT
5	MTL	15	MT

Save 3 as B?M -

4 as TLB$_1$ -

11 as B?M -

Sept. 22

① W478 x W583 ② W478 x W584. on Lac EMS.

Many Lac - colonies appeared in 20 hours on ②. Probably contaminants.

④.

① 34 tests. All Lac +. Novarey.

multi factor heterozygotes

Sept 25, 1948

1. W477 x Y470.
2. W478 x W483 25 plates only 15 lac+ colonies + 2 Lac-.
3. W126 x W466 Mostly + colonies.
4. W126 x Y87 ca. ½% +; Pick 2

(1) 100+ colonies tested. None Lac heterozygotes (Recheck 13, 41).

(2). 15 tested. (9 maybe variegated. — H51.

(3). See 323.

(4). 2 tested. Both ++.

→ streak out on EMB media. Gal ++
Mal ++
Sucr ++
Xyl +/-
Lac +/-

→ Both are Lac heterozygotes. H68, 69.

Sept 30, 1948.

W583 × W478

92 Lac+ colonies streaked out on Lac EMB.

from 17., 53, 54, 48 Streaks thereon EMS Lac. as 1-4. and test on Lac, Xyl, Mal, Arab.

(4) not heterog. 1-3 OK. ~~H~~70 – ~~H~~72.

1 Mal – colony noted on 3. S.O. Lac EMS and Mal EMB.

Sept 25, 1948 ff.

See 313.

40 lac+ prototrophs tested from W126 (Lac$_4$-) x W466 (Lac$_1$-)
Most of these are clearly heterozygotic for lac as seen on lac EMB plates.
Pure ++ (?): 34. 19, 20 (unavailable). At least 36/40 = 90% are heterozygotic.

#3, 8, 25, 28, 40 seem predominantly + & only small - sectors.

11 has colonies & moderately dark centers, mostly light margins with v. dark sectors (lac$_1$ + lac$_4$ + ? combinants ?).

36 has - colonies & dark centers. } Pick single colonies from the
26 shows fairly typical sectoring. } EMB and streak out on EMB.

Save 1-10, 11, 25, 26, 28, 36, 40 for H-series. Streak out suspensions on EMB and EMB-Mal.

H- 52-67.

P2. 4+ colonies from each tested.
36: mostly -, frequent ⊛ types.
26. the same. No pure + noted. Probably all Lac$_1$- Lac$_4$+ / Lac$_1$+ Lac$_4$-

Multiple heterozygotes

Oct 2, 1948

1. W477 x W67 (Lac$_1$- x Lac$_4$-). 15 plates.
2. W125 x W478 466 10
3. W133 x W478 466 10
4. W125 x Y87 5
5. W133 x Y87. 5

1. + and - colonies, variegation? } Restreak on EMS Lac.
2. Variegated, & small - sectors. } W73, 74

1. 10 colonies! Later 2 + noted? [redacted].
2. Numerous +. 64 picked to EMB. 11/64.
3. 1+ in 10 x 200 tests. = 327-3-1 Pure ++
4. Ca 20% +.
5. 0 or 1? + in ca 5 x 200 = 1000 tests.

59, 51, 47, 48 heterozygous 6, 1, 61, 20 Streak out on EMS Lac.

60, 43? ? 48h. (EMB.

H				
75	1	1	small cols.	Many nearly + colonies. occ ⊕
76	2	6	" "	Numerous ⊕
77	3	20	good growth; +; var	Nearly +
78	4	47	" "	" "
~~79~~	5	48	not heterozygous.	All +; sectored)
79	6	51		+ and ⊕ colonies. Also [redacted]
80	7	59	small cols	⊕ and ●
81	8	61	small cols	do.
82	? 9	60	mostly v. small +, - colonies.	Var. [redacted]
83.	? 10	43.	Good var., +, -	good growth [redacted]

H. Crosses.

Oct. 6, 1948.

all on Lac S.

1. W478 × WS 83 — Plates marked 1 in black (Xyl; Lac) are repeated 10/7/48.
2. " WS 84.
3. W477 × W45
4. ~~W477 × W186.~~

2. 40 tested; 11 selected for further test. S.O. EMS Lac

3. No yield

1: xylose: 72 tested 1: Lac: 44 tested.

5, 6, 7, 8, 12, 35, 43, 48, 49, 60 16, 19, 23 are Var. on Lac 1 plate

65, 66, 67, 71

Monologous EMS — Heterologous EMB — 5/31/1 → 48h.

H.

1	5	—	
2	6	— ?	
3	7	—	
4	8	—	
5	12	—	⊙
6	35	V	
7	43	V ?	V.
8.	48		○
9	49	—	
10	60	—	●
11	65	—	⊙
12	66	—	⊙
13	67	—	⊙
14	71	—	
L 15	16	V	ζ on EMB Lac, ●
L 16	19	V	
L 17	23	+	

— no reading, — colonies show rather pleiotic appearance. ⊙ darkish centers, but no well-defined sectors.

W478 × U58Y continued. 330 b.

583

October 13, 1948.

Test 330-1 isolates on Lac, Xyl. H-

	Lac	Xyl.	EMS Lac.	EMS Xyl.	H-	
1		Var	−			
2		Var	−			
3		Var	−	−		
4		Var.	−	−		
5		Var.	+	++		Reduels carefully.
6		++ ?	+	++		
7		Var.	−	+±		
8		Var.	−	−		
9		++	−	−		Not heterozygotes?
10		Var	−	−		
11		Var	−	−		
12		Var	−	−		
13	~~Var~~	Var	−	−		
14	~~Var~~	Var	−	−		
15	Var		++	++		
16	Var		++	++		
17	++.		++	++		Not heterozygotes

Cultures variable on EMS Xyl.

Pick to T(0) slants from Xyl EMS. Incubate Lac EMS further.

Test 330-2 isolates on all media available.

	Lac	Mal	Sal	Xyl	Arab.	H	
1	+, - V?	—	++	V	++		56 —
2	++	—	++	++	++		
3	V	—	++	++	++		57
4	++ ; —	—	++	++	++		58
5	++	++	++	V	++		59
6	+ ; —	—	++	++	++		60
7	V	—	++	V	++		61
8	V	—	++	++	++		62
9	V	—	++ -?	V	++		63
10	++ ; —	—	++ (-?)	++	++		64
11				++			65

Study additional isolates from 2 and 11 for study

-2

-11

1 ++
2 ++
3 +,
4 +, V, — H65.

Oct. 16, 1975.

Streak out H85-88, 91-97 on Lac EMB's. Pick papillae & purify on Lac EMB.

		Lac 1	2	3	4
1	H85	+	+	+	+
2	86	+	+	+	+
3	87	−	−	−	−
4	88	−	−	−	−
5	91	+	+	+	+
6	92	+	+	+	+
7	93	=1 definitely segregating for Lac+/−			
8	95	+	+	+	
9	96	+	+	+	+
10	97	−	−	−	−

+ 2 (+) 3 (+) 4 (+) *

* same papilla. Hold 3, 4, 10 for papillation.

EMB:	1	2	3	4	Notes.
1 v. wh+, opp. sect.	* → do	+	++ → do.	++, flat cols.	H110
2 wh. full +	−	+	−	−	H111
5 sectored cols.	(V)		(V)	(V)	H112
6 sectoring, weak +	+ →		→	→	H113
7 (V)	(V)		(V)	(V)	H114 dark, mucoid
8 bullseye cols some sectoring					H115
9. ++	++		++	++	H116.

choose * for purification as T/O)

Compare H93 (→ V) and H96 (+ → V) in detail. Streak both out on Lac EMB's for further papillae. See 3-15-11 [illegible]

Oct 5+, 1948.

A). Streak out single variegated colonies and pure colonies to heterologous + homologous media.
On original test plates, V colonies only were seen on xylose, but 3 Lac − colonies were in H72. Pick these as 333A: 1–3 and streak on xylose EMB.

R7 B) Streak 5 var. colonies each from Xyl plates H70, H72 to Xyl + Lac

		Xyl.	Lac
H			
70	A	++, −	Pure +
	B	+, −, & var.	almost pure +.
	C	−, +, var.	miscreable.
	D	"	almost pure +.
	E	+, −, var	" "
72	A	+, −, var.	Mostly −, +.
	B	+, −, var	+, −, var.
	C	+, var, −	−, +, var.
	D	+	~~±~~ "
	E.	" "	"

Series 70, especially, seems to show loss of Lac variability within Xyl segregants.
Pick var. colonies from Xyl plates to Lac + Xyl EMB.

70B-(1-3). [illegible]

R7. A). 2, 3 are pure xylose −. (1) Contains preponderantly − but some + or variable. Pick three to Xyl EMB and to Lac EMS. [Not isolated colonies.

1 +, −; var on xylose.
2 +, −
3 Pure +
4. Pure +
(0) to EMS. See 333a.

No partial segregation here

Pick to Lac EMS and Lac EMB.

333A: 1–6.

	Xyl.	Lac
1	±	±
2	±	±
3	±	±
4	±	±
5	±	±
6	±	±

evidently, not pure – when picked.

333A0 is a plate of EMS Lac, streaked ultimately from a ~~Lac~~ " Lac − " colony of H72. About 50% are Lac−. Test thereon Xyl EMB. Kup on Lac EMS.

c). Streak out H72 on EMS Xyl + Lac to look for − colonies.

P10

1−3 Lac − ?

4−8 Xyl − ?

	Lac	Xyl.	
1	−	±	(1)
2	−	−	(2)
3	−	−	(3)
4	−	−	(4)
5	−	−	(5)
6	−	−	(6)
7	±	±	
8	−	−	(7)

Take to Lac + Xyl EMS for papillae, except (7).
No papillae on Xyl. Also H72: no papillae.

P18. Papillae tested on EMB Lac. ∴ These Lac − prototrophs are monogenic for Lac −

	1	2	3
1	++	++	++
2	++	++	++
3	++	++	++
4	++	++	++
5	++	++	++
6	++	++.	

p10. B)

		Lac	Xyl.
70 B'	1	++; −	−+ V
	2	++; −	−; +; V
	3	+++	−; +
c'	1	++ −	−+ V
	2	++ V	−+ V
	3	++ −	−+ V
	4	++(−)	−+ V
E'	1.	++ mix	Var.
72. Ax	1.	−; V	−
	2.	+, −, V	−
BL	1		+−V
	2		+−V
	3		+−V
	4		+−V
Bx	1	+−V	
	2	+−V	
	3	+−V	
	4	+−V	
CL	1		V−+
	2		V−+
Cx	1	−V	
	2	−V	
	3	−V	
	4	−V	
DL	1		++
	2		++
Dx	1	−+	
	2	−+	
EL	1		++
	2		++
Ex	1	−V	
	2	−V	

Except for H72 D_L, and doubtfully for series 70) the segregation of Lac and Xyl is strictly correlated. Pick colonies and make 1/2 D_L to recheck on segregations.

		Lac	~~Xyl.~~
D_1	1	−	
	2	−	
	3	+	
	4	−	
	5	+	
	6	−	
	7	−	
	8.	−	
	(0)	±	
D_2	1	−	
	2	±	
	3	−	
	4	−	
	5	+	
	6	±	
	7	−	
	8.	−	
	(0)	±	

should all be papose +.

Note alteration from typical behavior of H72.

→ 333B1 +2.

Pick var. colonies from D_{23} and D_{26} and
a) test nutrition:
b) streak on EMB Lac:
c) S.O. on EMB Lac + Xyl to reverify of H72.

(1): Variegated, +, − both on Lac and Xyl
(2): " " "

The error was based on the use of Gal EMB as Xyl EMB.
No partial segregations here!

H Crosses.

October 12, 1948.

(1). W108 × W466	Mostly − !	From Lac EMS to Lac	
(2) W327 × W466	Mostly + !	From ~~Hal~~ EMS to Hal	
(3) W252 × W466	Mostly −	100+ pricked.	Lac EMS to Lac
(4) W108 × W478	Mostly −.	~~Hal~~ Lac EMS. to Lac	

(1) 24 tested. 10, 12, 18, 73, 3, 4. = 1 − 6

(2). 48 tests. No heterozygotes noted.

(3) 79, 64, 82, 49, 52 (97) (94) (96), 2 7 -15 Head lighter appearance on EMS Lac

(4). 20; others?) 16.

Retests:	Lac	Glu		
1	Var	++	H-100.	
2	?	V?		W108
3	V	++	H-101	×
4	V	++	H-102	W466
5	++	++		
6	V	V?	H103	
7	V	++	104	
8	V	++	105	
9	++	++		W252
10	++	++		×
11	V	++	106	W466
12	V	++	107	
13	++	++		
14	V	V?	108	
15	V	V?	109	
16	?	++?		

Retest selected from 16. None segregating.

Lac, Xyl segregations from H72-

10/16+/1948.

A) Grow H72 in Y2 broth overnight to allow segregation, and plate on Lac; Xyl EMB. Counted by N.2. + Calculated!

Xylose

	+	−	Var.	Σ
a.	20	274	6	
b.	25	345	8	
c.	16	196	5	
	61	815	19	895.

$-:+ = \frac{815}{61} = 13.3:1 = \alpha$

$\chi^2_4 = 0.15$

$p = .99!$

Lactose

29	228	9	266
15	178	4	197
32	248	10	290
76	654	23	753

$-:+ = \frac{654}{76} = 7.5:1 = \beta.$

$\chi^2_4 = 3.17 \quad p = .53.$

This gives linkages as Xyl-Af = ~~25~~ 7.0

Lac-Af = [redacted] 11.8

B). Take − and + colonies and test on heterologous medium.

	Lac−	Lac+	Σ
Xyl−	109	16	125
Xyl+	64 38	0	

Lac-Af = 16/128 = ~~12.5~~ 16.6 Interference?

	Xyl−	Xyl+.
Lac−	104	7
Lac+	102	1

Xyl-Af = 7/111 = [redacted] 6.3

+ colonies from 336a retested on both media.

1-16 " Xyl − Lac+"

17-23 Lac − Xyl +

24 Lac+ X-yl+.

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

not segregated for either Lac or Xyl (S.O.) test isolates.

(24 -)

	Xyl	Lac
1		+
2	−	+
3		
4	−	−
5	+	−
6	−	+
7	+	−
8	+	−

	X	L
9	−	+
10	+	−
11	+	−
12	−	+
13	+	−
14	+	−
15	−	+
16		+

and a mixture of Xyl+ Lac − and Xyl − Lac+.

Smi − − (4) was also found, the others say [illegible] a mixture.

+	Df	+
Xyl		Lac
—	x	y	—

Xyl− Lac−	(1−x)(1−y)
Xyl+ Lac−	x (1−y)
Xyl− Lac+	y (1−x)
Xyl+ Lac+	xy.

⑥. Interference: In A, $\frac{X-L+}{X-L-}$ should $= \frac{X+L+}{X+L-}$. $\chi_1^2 =$

Expectations in some columns are <5.

②. Linkage. Use only single crossover data.

$$\text{Lac}-\ \frac{\text{Xyl}-}{\text{Xyl}+} = \frac{1-x}{x} = \frac{1}{x}-1. \qquad x = \frac{1}{r_b+1}$$

(r_b).

336. $r_b = 17$ $\quad x = .055$

336a. $r_b = \frac{104}{7} =$ $\quad x = .077.$

mean: $r_b = \frac{111}{36}$ $\quad x = .061$

χ_1^2:

34	2	36
104 (104)	7	111
138	9	147

$Xyl - \frac{Lac-}{Lac+} = r_a.$

336: $r_a = 33/6$ $\quad y = 15.4$ $\quad \bar{y} = 13.4$

$r_b = 109/16$ $\quad y = 12.8.$

108. 109	17	16	125
33 34	5	6	39
142		22	164

$\chi^2_1 = \frac{1}{5} + \frac{1}{17} + \frac{1}{34} + \frac{1}{108} =$ .01 .20 .06 .03 .30

$p = .\cancel{06}\,60$

Summed data 336...

	Xyl-	Xyl+
Lac-	34	2
	104	7
	138.	9

Lac- Lac+ Xyl-

	33	6	
Xyl-	109	16	
	142	22	174

336a. Random plating. Assumed term absence of X+L+ class.

X-L-	1328
X-L+	167
X+L-	112
	1606.

gives $x = 7.1$ $\quad y = 11.2$

Oct. 15, 1918.

W583 × 58-161.

Low yields: Abandon expt.

1) EMS Lac

October 19, 1918. Repeat. ca 30:1 −:+

EMS Xyl B_1 :	Σ	+	−
ca 3% +	32	2	
	136	1	
	41	1	
	41	1	
	31	3	
	28	1	
	309	9	300

EMS Xyl:	Σ	+	Σ	+
	54	4	201	2
	339	1	120	2
ca 1% +	141	3	162	1
	277	3	178	1
	96	0		
	194	1	2218	23
	170	3		
	92	1		
	183	1		

2) EMS Lac B_1. Colonies picked indiscriminately to homozygous medium.

Classified by presumptive 1st original score + B_1 in plates:

1. Xyl + B_1
2. − B_1
3. Xyl + 0
4. Xyl − 0
5. Lac − 0
6. + 0
7. − B_1
8. + B_1.

This experiment unsuccessful on two counts

(1) Tests were not decisive, most suspensions tested being apparently mixtures.

(2). Confusion of classes.

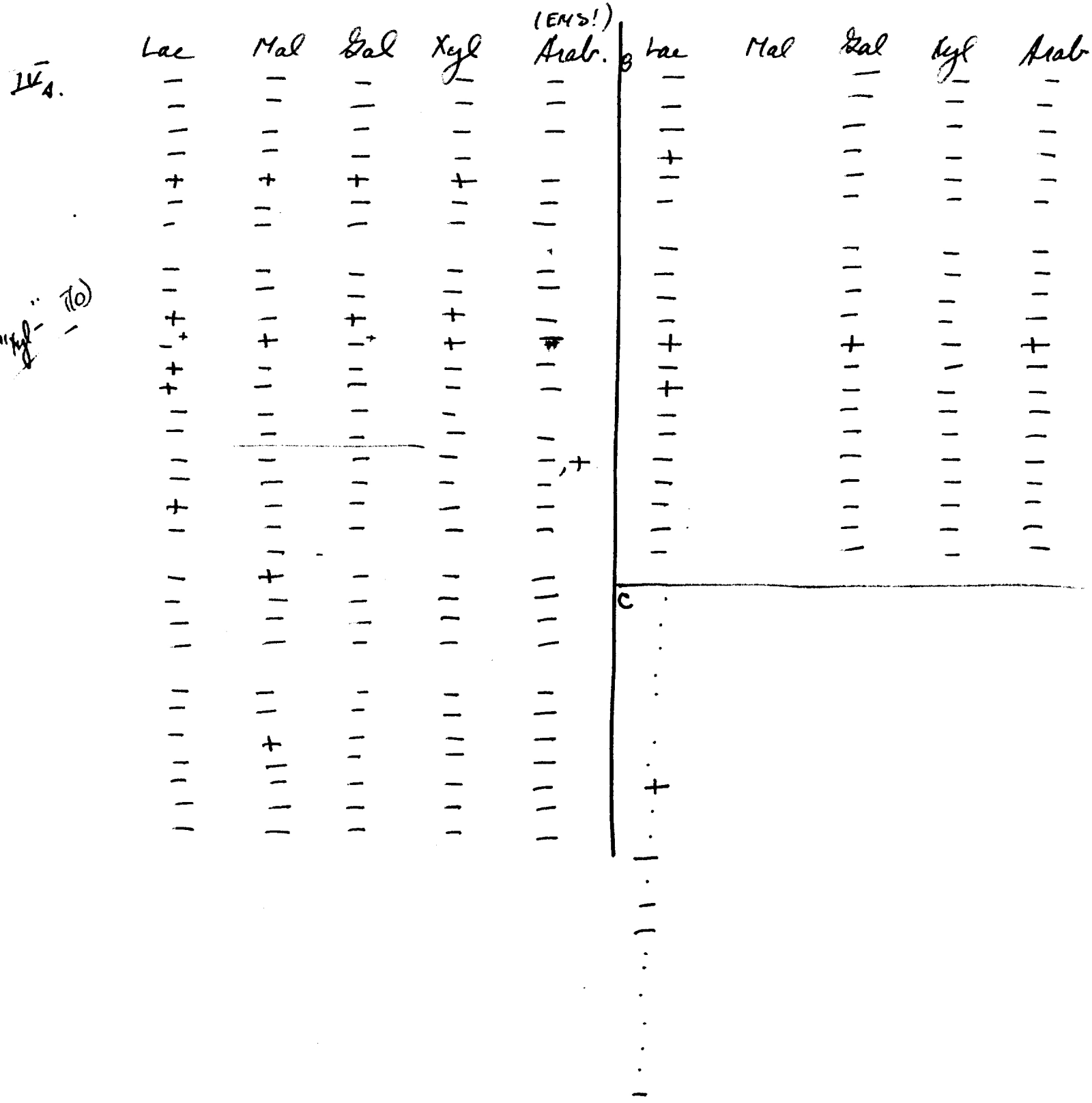
4a.

Lac	Mal.	Gal	Xyl	Arab	Lac	Mal	Gal	Xyl	Arab
−	−	−	−	−					
·	·	·	·	·					
−	−	−	−	−					
·	·	·	·	−					
−	−	−	+	·					
−	−	·	·	·					
−	−	−	−	−					
+	−	−	−	−					
·	−	−	−	−					
−	−	−	−	−					
•	·	−	•	·					
−	−	−	−	−					
−	−	+	−	+					
·	−	·	·	·					
−	−	·	−	−					
−	−	·	−	·					
·	−	−	·	−					
+	−	−	·	·					
−	−	−	−	−					
·	−	·	−	·					
−	−	−	−	−					
−	−	−	−	·					
−	−	−	−	−					
·	−	−	−	−					
−	−	−	−	−					
−	−	−	−	−					
−	−	−	−	−					

Group IB:	Lac	Mal	Gal	Xyl	Arab	
1	+	−	+	+	+	−
2	+	+	+	+	+	+
3	−	+	+	+	+	+
4	−	−	−	+	+	−
	−	−	−	+	+	−
	−	+	−	+	+	−
"Xyl+"	−	−	−	+	+	−
T(o).	−	+	+	+	+	−
	+	+	+	+	+	−
	+	+	+	+	+	−
	+	+	+	+	+	+
	−	−	−	+	+	−

IV	"+"	−	−	−	−
" T(o) ·	−	−	+	−	+
" Lac⁻	"+"	−	+	−	+

VI	+	−	+	−	+
T(o)	+	−	+	−	+
Lac+ · ·	+	−	+	−	+
	+	−	+	−	+
	+	−	+	−	+
	+	+	+	+	+


(EMS!)
Lac
Mal
Gal
Xyl
Arab.
IV A.
Xyl
B
Lac
Mal
Gal
Xyl
Arab
C

good reading! —

7

86.

Lac	Mal	Gal	Xyl	Arab
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	+	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	−	−	−
+	−	+	−	+
+	−	+	−	+
+	+−	+	+	+
+	. −	.	[illegible]	. +
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	+	+
+	+	+	−	+
+	−	+	−	+
+	+	+	−	+
+	−	−	−	−
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	+	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+

Lac	Mal	Gal	Xyl	Arab
−	−	+	−	+
+	+	+	+	+
+	+	+	+	+
+	−+	+	−+	+
−	−	+	[illegible]	+
−+	−	−+	−	−+
+	−+	+	+	+
−+	−	.	−	−+
+	+	−+	+	−+
.	−	+	. −+	+
−	−	+	−	+
+	−+	+	−	+
+	−+	+	−+	+
.	−	.	.	.
+	+−	+	−+	+
+	+	+	−+	+
+	+	+	−+	+
+	+	+	−+	+
+	+	+	−+	+
+	−+	+	−+	+
+	−+	+	. −+	+

8.

8a

	Lac	Mal	Gal	Xyl	Arab	Lac	Mal	Gal	Xyl	Arab
1	+	+	+	+	+	+	−	+	+	+
2	−	−	−	−	−	+	+	+	+	+
3	+	−	−	−	−	·	·	·	·	+
4	·	·	·	·	·	·	·	·	·	+
5	−	−	+	+	+	·	·	·	+	+
6	−	−	−	−	−	+	+	+	·	+
7	·	·	·	·	·	+	−	−	−	+
8	−	−	−	−	−	·	·	−	·	+
9	·	·	·	·	·	+	−	−	−	−
10	−	+	+	+	+	+	−	+	−	+
12	·	+	+	·	·	−	−	−	−	+
13	·	·	·	·	·	·	−	·	+	+
14	+	−	+	+	+	+	−	−	−	+
15	−	−	+	+	+	+	+	+	+	·
						+	+	+	·	
	−	−	−	−	+	−	−	+	—	+
	−	−	−	−	−	+	−	+	−	+
	·	·	−	●	·	+	−	+	−	+
	−	−	−	−	·	+	−	+	−	+
	+	−	+	−	+	−	−	+	−	+
	·	·	·	·	·	+	−	+	−	+
	+	−	+	−	+	+	−	+	+	+
	·	+	+	+	+	+	+	+	−	+
	−	−	·	−	+	+	−	−	−	—
					+	→	−	−	−	−
	+	−	+	−	+	+	−	+	−	+
	−	·	−	−	−	−	−	−	−	+
	−	·	−	+	·	+	−	−	−	+
	−	−	+	−	−	+	−	+	−	+

8.

(2) - Hadleyworth scoring.

(1)

Lac	Mal	Gal	Xyl	Arab
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
—	—	—	•	—
+	—	—	+	—
—	+	—	+	—
—	+	—	+	—

2a.

Lac	Mal	Gal	Xyl	Arab
—	—	•	•	•
	—	—	—	•
	—	—	—	•
	—	•	•	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—
	+	—	—	—
	—	—	—	—
	+	—	—	—
	—	—	—	—
	—	•	—	•
	+	•	—	•
		+	—	—

October 18, 1948.

Inoculate H72 fairly heavily into T(0) + T(B,). Shake.

P19. No growth A20. Heavy growth in T(B,); none in T(0).

Is H72 B, – " inviable?

① Streak out H72 on Lac EMB, EMS, EMS'.

② Plate out T(B,) tube on Lac EMS'; Xyl EMS'.

P21. ① On Lac EMB: almost all lac – ② Do. on xylose EMB.
(i.e. most of the stock culture is segregated.).
A few + noted on EMS.

② 2 plates on Lac EMB. 140 colonies. All Lac –
EMS' too small to read

A22. – only noted on all plates, ~~EMS~~ EMS' Lac + Xyl'

A22. Pick single + colonies of H72 from ~~E~~ EMS Lac to T(0) tubes to –
a) resuscitate H72 and b) continue expt. Streak out on Lac EMB from T(0) suspension. Use #6.

	Lac EMB. (OK).
1	V
2	V
3	V
4	V
5	V
6	V

See 348.

Oct 21, 1948.

~~W576~~ W478 x W583 on Mal, Gal, Ar EMS.
Low yields!!

101-120 Gal+
121-123 Arabinose. } test on EMB Galactose + Arabinose

1-100 ~~Mal+~~ Maltose. test on EMB Maltose.

D. 6.

100 colonies picked from Mal, not readily scored. Only 39 Mal+
Rechecks: 16, 25, 31, 50, 59, 87, 95, 99.

20 Gal+ colonies: All Gal+ Arab+. No heterozygotes.

3 Ar+. 2 Ar+ Gal+. 1 Gal− Ar +, − ? Rechecks 121.
1-8 on Mal EMS 9 on Ar EMS.

		EMS.	2
1	16	++	++
2	25	++	++
3	31		lost.
4	50	++	++
5	59	no +'s.	
6	87		
7	95	++	++
8	99	++	++
9	121.	++	++

2 colo from EMS killed.

Oct. 23, 1948

Spread H72 grown on T(0) (see 348) on EMS Lac + Xyl and expose for 5-15 secs. Of 348 for control.

Exp. n.g. Controls inviable NF

October 23, 1948.

Grow H72 on T(0) — see 344. — dilute 10^{-7} and plate on EMB; EMS Lac; Xyl for - colonies.

n.g. Culture inviable.

October 23, 1948.

See 345.

streak out storage media indicated

	EMS Xyl.	EMF Lac
H93	small +	v. small + cols.
H96.	n.g.	
H58	n.g.	
60	n.g.	
62	+- cols.	
	EMS $Ar_2(B_1)$	
H85	+ cols ?	
86	n.g.	
88	1 - col.	
93	v. small + cols on EMF	
94	numerous + and - cols.	
	EMBM. EMS Xyl.	
95	a few + and - cols.	Mal -
96.	n.g.	

---

93 → XylEMB, LacEMB, LacEMS. ~~Lac V~~ Xyl V; Ar - "Lac -"

85 XylEMB, LacEMB, ArEMB.

94 " Xyl V

95 " Lac - (slow ??)

62 LacEMB; ArEMB Lac V Ar +

H52 Stock. Lac V OK

H1S. OK

Reassociation in preparation of H1 [illegible]
Rechecked.

	EMFbac	EMSmac	EMBxyl	ZM84ab	
H723	V				
H72n	mostly -				
H93			V	—	
H62	V; +, -			++	
H85	(V) slow +, -			hot +; - ?	+ character here?
~~H22~~					
H22	++, - (probably V)				
H94	—			~~+~~ weak? +	
H95	+, - V			—	
H88			—	—	Segregating! Female lineage, in EMBxyf.
H70	++, - +				
H1	V, +, - ~~++~~	++, -			
H52	(+), -, (V)				
H71	(V), ++, - -				

Oct 24, 1948.

W478 × W583 Ara, Mal + Gal EMS.

Arabinose : 24 + colonies. All ++

Galactose : 28+ colonies All ++.

Maltose : 50+ " All ++ Checks 4, 5, 18, 19, 43 (N2)

0.005% T 2 / N.A.

test on Lac + Mal (3 sec)

58-161 Glu (-)

Oct 29. Inoculated to Perm. assay.

Oct 30 Irradiated 7 secs on Q.M.B. glu. plate

Oct 31 16 colonies picked strongly glu (-)
streaked out on Q.M.B glu.

Nov 1 four (4) apparently glu (-) streaked again on Q.M.B. glu

Nov 2 2 glu (-). streaked on T_1. both T_1 4 Glu.

	"N"	"2"		"N"	"2"
Nov 3. Tested on: Glu	Neg	Neg		pos	pos
Lac	Neg	Neg		~~pos~~ Neg	Neg
Mal	Neg	Neg		Neg	Neg
Fru	Neg	Neg		pos	pos
Mannose	±	±		pos ±	pos ±
RhaM	Neg	Neg	15 hrs.		
Arab	Neg	Neg		pos	pos ±
Gal	Neg	Neg		pos	
Agl	±	±		pos	pos
Tal	Neg	Neg		Neg	Neg

Nov. 8 Tested on K gluconate

"N"	"2"	15 hrs
± slow	slow ±	

W351

Nov. 16, 1948.

58-161 x W583. m EMS Lac B,

(1).

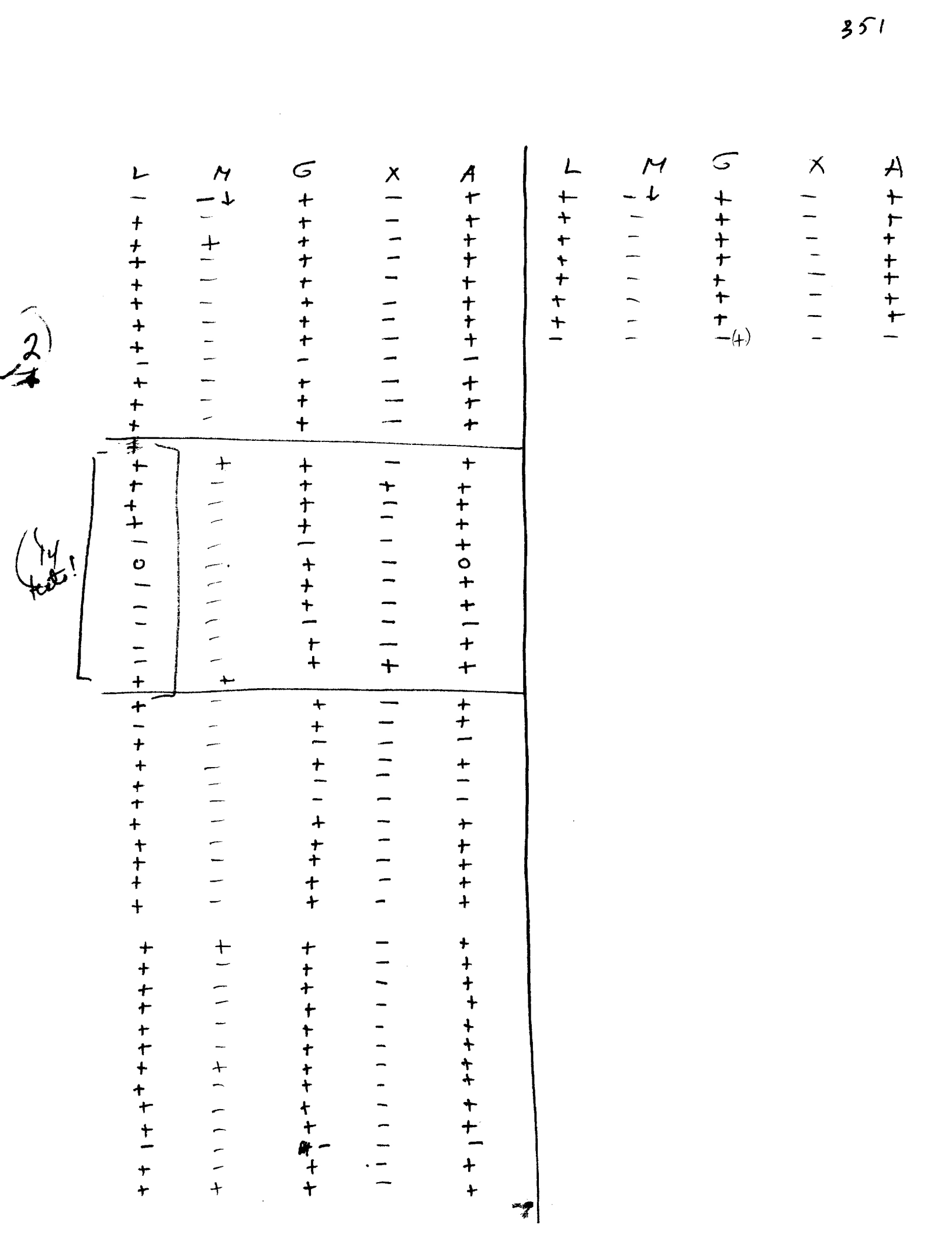
L	M	G	X	A
+	–	+⊥?	–	+
+	–		–	+
–	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
–	–		–	–
–	–		–	–
–	–		–	–

L	M	G	X	A
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
–	–		–	+
–	–		–	–
+	–		–	+

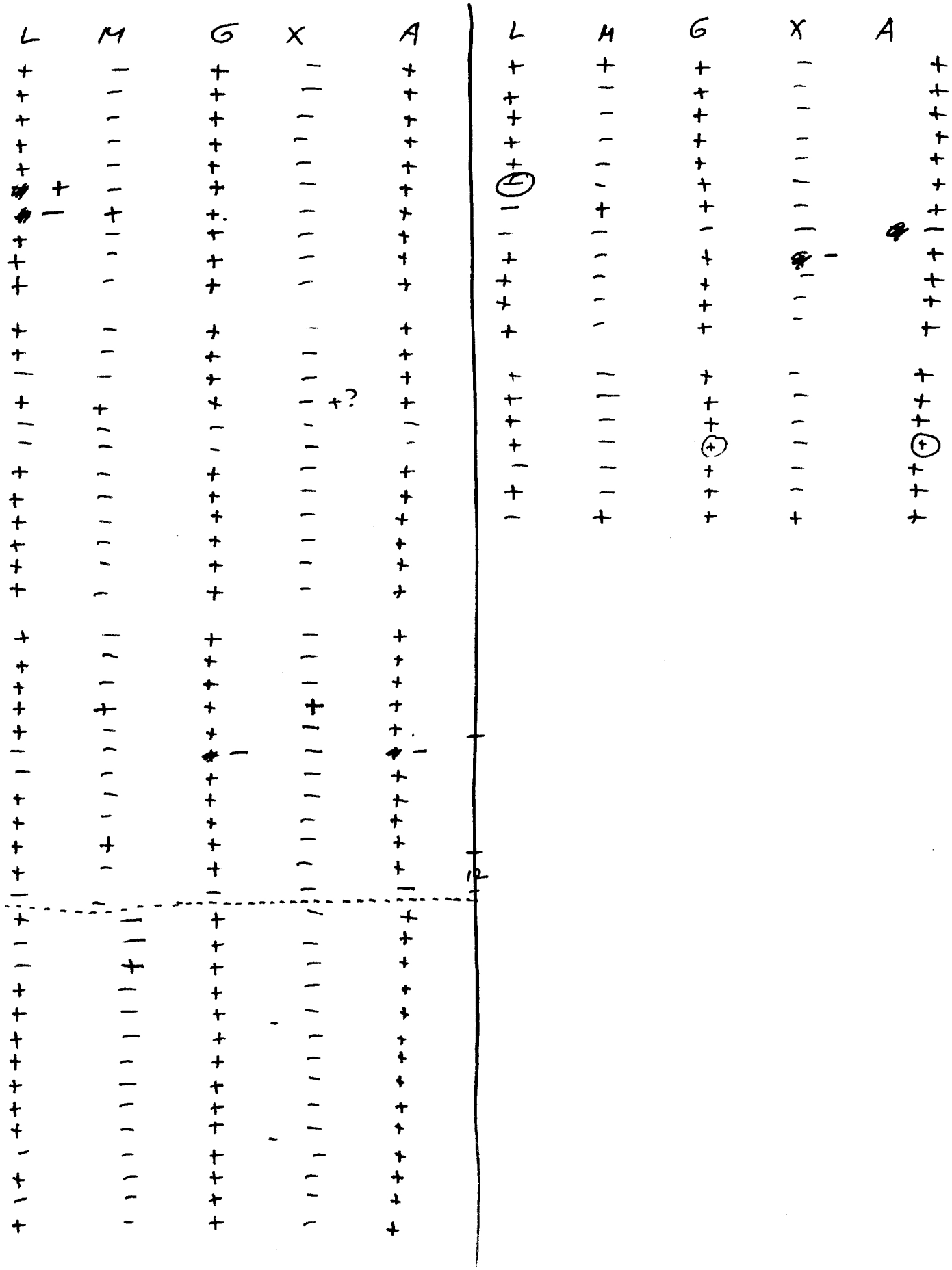
L	M	G	X	A
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	–
+	–		–	+
+	–		–	– ?
–	–		–	–
–	–		–	–

L	M	G	X	A
–	–		–	–
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	–		–	+
+	10 –		–	+
–	–		–	+

Cal plate n.g.


L M G X A
L M G X A
(2)
(14 pts!)

(3)



L	M	G	X	A
+	−	+	−	+
−	+	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
−	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	−	+	−	+
+	+	+	−	+
+	−	+	−	+

4

check TI sensitivity on Gal EMS.

20 Ar+ : All Gal+
19 S
1 R

23 Ar − : 1 ? Gal+
22 Gal −
all S ; no R.

58-161 : S

W583 : R

Summaries. 164 total.

Lac + 137

Lac − 27

Note excess of Lac+ !

Among 27 Lac −

Mal+	Xyl+	Gal−	Ar−

total :

Ar + Gal closely linked.

12 Gal- Ar −
1 Gal- Ar+
0 Gal+ Ar −
151 Gal+ Ar+.

test Ar with Lac.

Lac-	Ar −	15
Lac-	Ar+	19
Lac+	Ar −	5 (triples?)
Lac+	Ar+	126

apparent interaction of Ar ē Lac.

However, the distorted recovery of lac − makes the conclusion dubious. Suggests that Gal and Ar are very near to [redacted] V_1. Check directly.

W477 Lac R. W352-

Nov. 10, 1948.
Steelscript W477 on EMBlac

11/12/48. Picks ~~top~~ 2 papillae to (1). EMBlac +, - color
P14 to (2) .... to 5 purifications = W588.1

sub-suppressors

353

Nov. 11, 1948.

Streak out, on glucose, for papillation:

from W252,

1 W431
2 436
3 437
4 438.

from W327, Mal−

5 W441
6 443
7 452 ~~446~~
8 448

Mal 2

9 ~~447~~
10 453
11 439
12 440

252 stock apparently Glu+. Retest
Present stock apparently contam. or emutted.
12/6/48. W-252 received from Doudoroff. Checks OK as Lac+ Glu−

① 4 Glu+ colonies examined: all +. Store as 353+1. Probably ~~Lac~~ Lac3+.

③ 1 W+. Not Lac+!

	Glu	Mal	Lac
353-1.	+	+	+
2			
3	+	+	−
4			
5			
6			
7			
8			
9			
10			
11			
12			

11/16/48. Restreak from EMB/Glu plates above.

N17.

1. many +.	Pick to EMB Lac individually for possible Glu+ Lac− types.	
2. papillae in wood streak.	Restreak.	After +. As 1.
3. "	"	pap. hold.
4. all −	"	~~pap~~ − hold
5. "	"	− hold.
6. Several +.	As 1.	All (14) were Lac−. S.O. ① as 353-6.
7. papillae	Restreak	+, − As 1.
8. all −	"	Some slow + hold.
9. pap.	"	+, − As 1
10. pap.	"	do.
11. pap.	"	Some slow +. ~~hold~~. As 1
12. Same + cols, but	" .	As 1.

① → 11 tested. 2−; 2+ and −; 7 +. Pick 1− and 1+ for purification

11/25. Take these cultures up again which had been held for a week

2: 7 all Lac − (should be tested on Mal).

11: 8 all Lac − (" ")

1b Lac+ Glu+ ~~slow~~
c Lac − Glu slow.

9. Glu++ and Glu slow. Test on Mal
10. all Glu s

6+7 all − (7 slow + ?)

11/30/48.

-9. 3 colonies Glu++ → Mal++ } Purify 1 each. Kup as 353-9
2 colo. Glu± → Mal− → Glu ÷. T.O.

-2 3 Lac++ } (1 each. Glu++ Kup as 353-2
2 Lac− } Glu± T.O.

-3 5. all Lac− (1) Glu ÷. T.O.

-4. 11 all Lac− (1). Glu ÷. T.O.

-5 5 Mal+
1 Mal++ } 1 each. Glu ÷. T.O.
2 Mal−

-8 6 Mal− (1) Glu ÷. T.O.

-10 4 Mal− (1) Glu ÷ TO

-12 8 Lac− (1) Kup as 353-12

-11. Lac− Glu ÷ T.O.

11/11/48.

S-161 Hanovia UV lamp 7 secs.

83 plates T2 } glucose. Ca. 100/plate ≐ 16,800 tests.
85 EMB }

1 each from T2 and EMB.
W593 W594

Check T1, Lac, Mal, Xyl.

H_2S indicator media. 355

11/12/48.

To a base of Peptone 10
Fe ammon. citrate .5
K_2HPO_4 1.0
Agar 15
/ liter

Prepare plates with following supplements (/liter).

K-12 SW13.

1. Na thiosulfate .8 g
2. —
3. Cystine 100 mg
4. " + Nats
5. N-Case 20g
6. " + Nats

In 18 hours, all grew quite well, but none were discolored. 2a. 72 hours

Kligler's Pb acetate agar ~~also tried~~. neither gave sharp reaction ~~c~~ K-12 or SW13.

11/9/48.

S.O. stock suspensions on EMS Glu.

P11 Pick 4 cols. each to water. S.O. Lac (or Xyl EMB.)

	1	2	3	4	
H1	−	−	++	−	
H22	−	−	−	−	
H52	±V	±V	±V	−	OK 1-3
H62	−	−	−	−	
H72	±V	−	−	−	
H85	±V	±V	±V	±V	
(Xyl) H93	V	V	V	V	

These critical strains should be cured by repeated single-colony transfer.

So H52/1; H72/1; H85/1 and H93/1) on EMS Lac. and old stocks of the other strains here. ← Not recovered from suspensions.

Retest single Lac+ colonies, and s.o. concentrates on EMS.

Recover → from EMB to EMS Lac

11/16/48

H1. 8 tests. 1-4, 5, 8 OK.

H22 8 tests 6 best V; Ftlm OK.

H52. 4 tests 1, 4 OK.

H62. 8 tests 1-4, 5, 8 very good 6, 7 OK.

H72. from Glu EMS. 2 test both Lac −

H85. on Xylose EMB 2 tests both V.g. (on Lac EMS. Med Xyl ≠

H93 2 tests both OK. on Lac EMS near −.

H-72 needs be recovered! OK ✓ . 11/18.

11/12/48

.2ml serum / 10ml NaP 7.5 4/50. .001ml 319A.

	Serum	Di	Df	$D_i^{CO_2}$	Δ
1.	—	007	190	~~190~~	190
2.	11/11	580	630	522	108 ?
3.	11/6	437	546	397	149
4.	11/4.	350	481	315	166

See L.J. Case for definition of these sera.

Streak out individual mosaic colonies from each heterozygote to clarify with respect to Lac_1; Lac_2. Also test individual colonies, as seen, on Bugal in .5 ml tubes.

	Bugal.	S.O. on Lac EMB.
1	+	
2	−	
3	not H.	
4	−	−, V.
5		−, (V)
6	−	−, V, +
7	−	−, V, (+)
8	−	−, V
9	−	−, +
10	+	−, +
11	−	−, V
12		−, V
13		−, +, (V)
14	−	−, +, (V)
15	−	−, +, V
16		−, V
17		−, +
18	+	−, V
19	+	−, V, +
20	−	−, V, +
21	−	−, (V)

W477	+
W45	−
W583	+

Study, in detail, 1-4. Pick 8. colonies and test on Bugal.

(1). 1-3, 5-8 are ~~Lac~~/Bugal + #4 is Bugal −.

(2). 1-3, 5,6,8 are Bugal−; 4,7 Bugal +

(4). 1-4, 7 are Bugal −; 5,6,8 are +. streak each of these out again Isolate and restreak to cross tests on Lac EMB.

Segregation from $\frac{Lac_1 \pm}{Lac_2 \pm}$

December 2, 1948.

H-135. 8 colonies nutritional test:

	Bugal.	Nutr.
1	−	TB,
2	−	M
3	−	M
4	−	M
5	+	++
6	+	++
7	−	M
8	+	M

12/6. Originals, in EMS Lac. of these cultures cannot be found.

11/5/48

W583 × 58-161 as T(0) [= ~~Gentian Violet~~)

Lac	Mal	~~Sal~~ Ar	Xyl	~~Ad~~ D	L	M	A	X	~~B~~
−	−	−	−	−	−	−	−	−	−
+	−	+	−	+	+	−	+	−	+
−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	+ −K	−	−	−	− −
−	−	−	−	−	⊖	+	−	−	−
−	−	−	−	−	−	−	−	−	−
+	−	+	−	+	−	−	−	−	−
+	−	+	−	+	−	−	−	−	−
−	−	−	−	−	−	+	−	−	−
					+	+	+	+	+
−	−	+	−	−					
−	+	−	−	−					
−	+	+	−	−					
−	−	+	−	−					
−	−	−	−	−					
−	−	+	−	−					
−	−	+	−	−					
−	−	−	−	−					
− S +	+	−	+	− −					
− R +	+	+	−	+ +					
−	−	−	−	−					
+	−	+	−	+					
− S +	−	−	−	− −					
−	−	−	−	−					
+	−	+	−	+					
÷	−	+	+	−					
−	−	−	−	−					
−	−	−	−	−					
−	−	⊖	−	−					
−	−	−	−	−					
−	−	−	−	−					
−	−	÷	−	−					
−	−	−	−	−					
−	−	−	−	−					
⊖	−	−	−	−					
⊖	−	−	−	−					
−	−	−	−	−					
+	−	+	−	+					
+	−	+	−	+					
−	−	~~⊖~~ ÷	−	−					
+	−	+	−	+					
+	−	+	−	+					
−	−	−	−	−					

(1) from T(0).

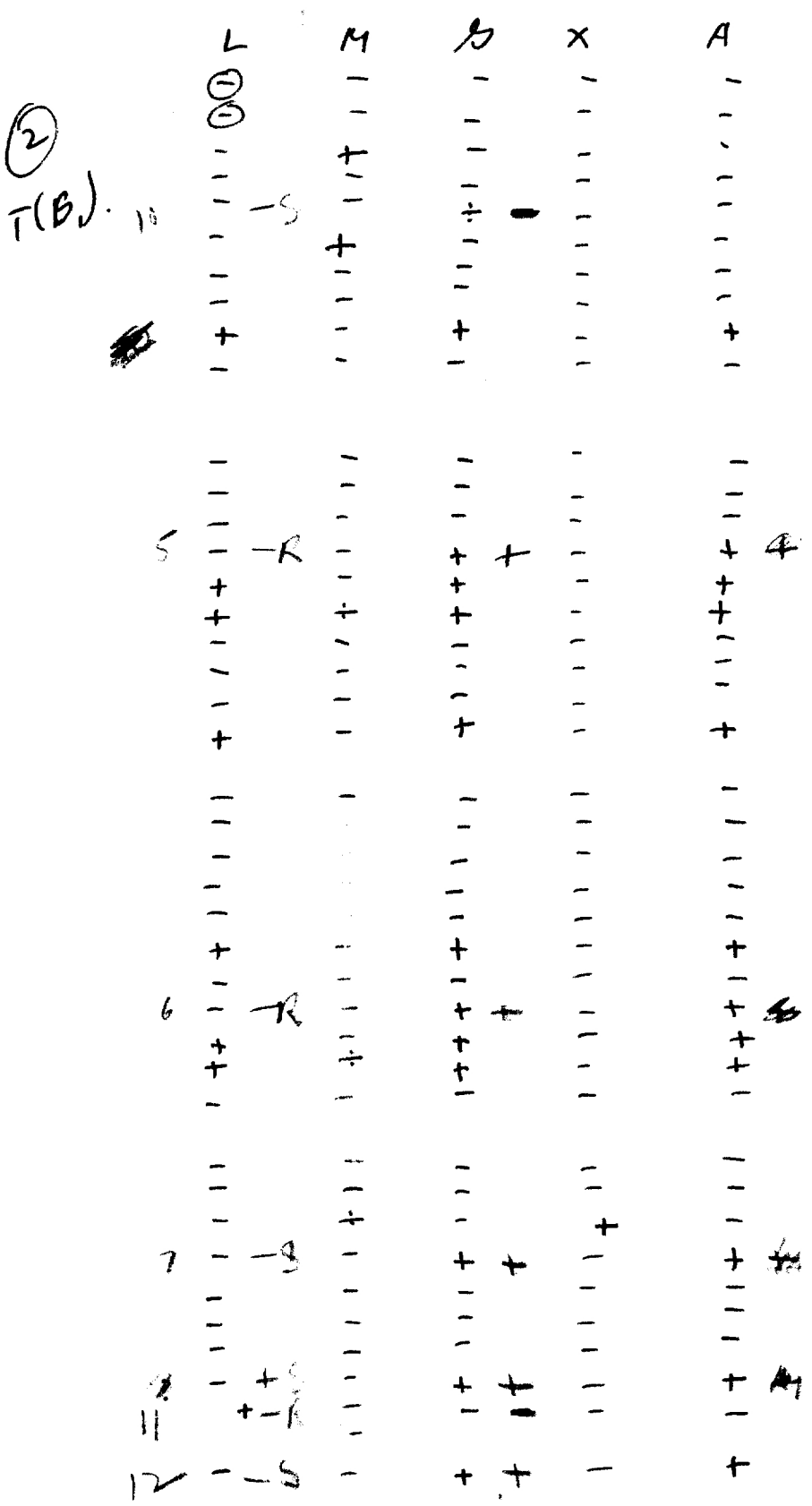
Lac − Gal+ 6 ∴ This is the right one.

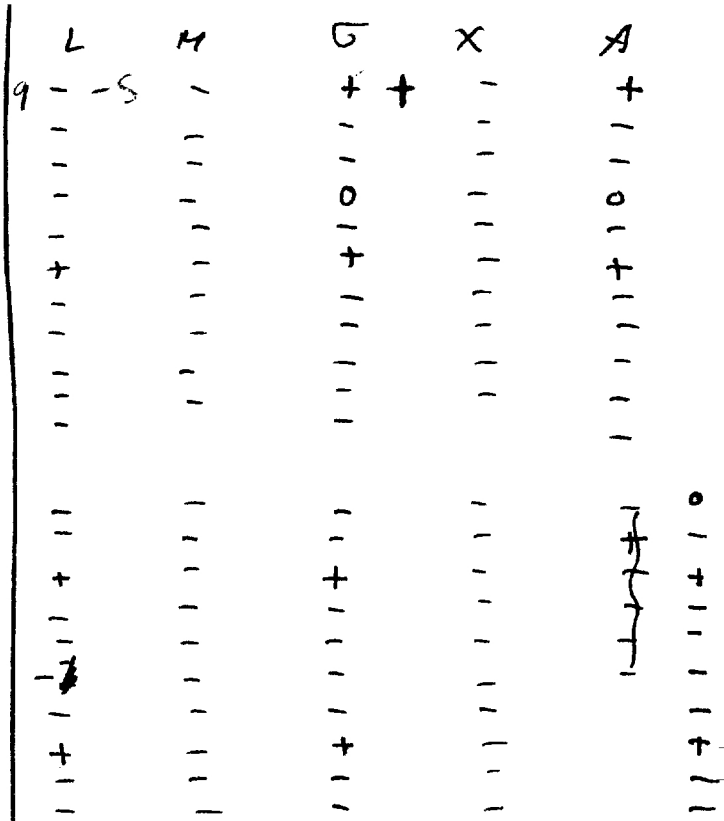
Lac+ Gal− 0.

BM Lac ca 4u. Gal V,

1/14/49

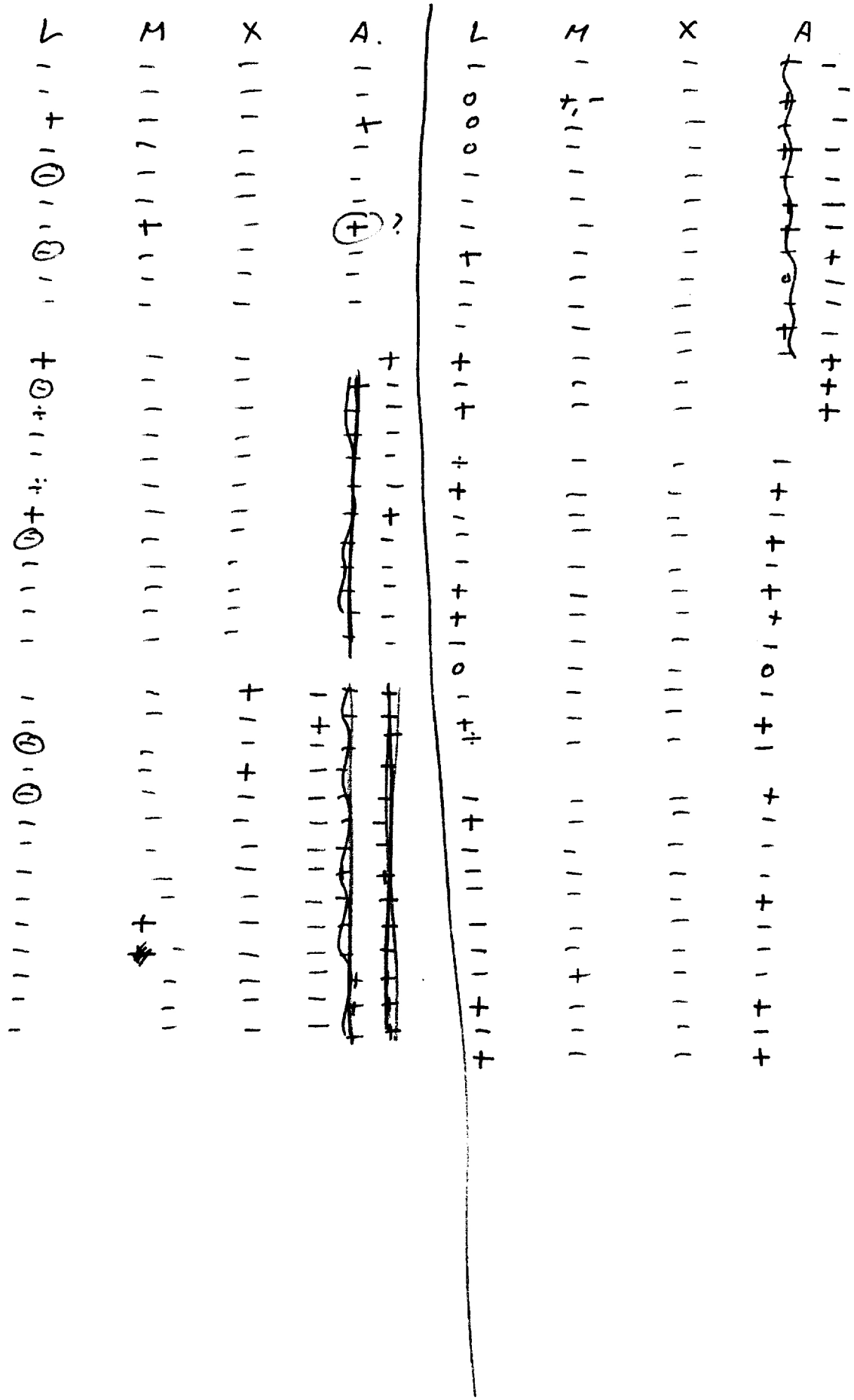
This class is missing because Gal- is epistatic to Lac+.





Rel and trab are clearly linked to Lac., but ~~dark~~ relative positions are not clearly established. The critical recombinants, i.e. B ± L ∓ should be rechecked for classification

additional tests (sugars limited).



(3)

T(B,)

(4)

T(p.)

L	M	A.
+	+	+
+	−	+
−	−	−
−	+	−
−	−	−
−	−	+
−	−	−
−	−	−
−	+	+
+	−	+
+	−	±
−	−	+
+	−	+
+	−	+
−	−	−
−	−	−
−	−	−
−	0	0
−	0	0
−	−	−
−	−	−
−	−	−
+	−	+
−	−	−
−	−	−
−	+	−
+	−	−
−	−	−
−	−	−
−	±	+
−	−	−
−	−	-
−	−	+
+	+	+
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
−	−	+
−	−	−

L	M	A.
−	−	+
−	−	−
+	−	−
−	−	−
+	−	−
−	−	+
−	−	−
−	−	−
−	−	−
+	+	−
−	−	+
−	−	−
−	+	+
+	−	+
−	−	−
−	−	−
−	−	−
+	−	+
+	−	+

A v. difficult to score here, mostly +.

(5).

$T(B_1)$

L	M	A
−	−	−
−	−	−
+	−	+
−	−	−
−	−	−
+	−	+
+	−	+
−	−	−
−	−	+?
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
−	−	−
÷	−	−
−	+	−
÷	−	−
o	−	+
+	−	−
−	−	−
±	−	−
−	−	+
−	o	−
−	−	−
+	o	+
−	−	−
−	+	−
−	−	−
−	−	−
−	−	−

L	M	A.
−	−	−
+	−	+
−	−	+?
−	−	−
−	−	−
+	−	+
−	−	−
−	−	−
÷	−	−
−	−	−
−	−	−
−	−	−
+	−	+
+	−	+
+	−	+
−	−	−
o	o	o
−	−	−
+	−	+
−	−	−
+	−	+

A v. difficult locus, [illegible] close linkage of A + G to L is shown. Order of A + G not established.

Pick colonies to Xyl CHS(B_1) for scoring of this character above.

	Xyl+	Xyl−	Σ
− B_1	4	95	99
T(0)	30	73	103
	7	82	89
+ B_1	41	250	291

= 14%

omitted:

Y	95	99
7	82	89
11	177	188

5.8%

T(B_1)

3	81	84
2	75	77
3	111	114
2	118	120
1	101	102
11	486.	497

= 3.3%.

There are definitely a higher number of Xyl + among the B_1+ than among the B_1−.

B_1− Xyl− BM+ TL−

B_1+ Xyl+ BM− TL+

There should be a greater discrepancy between B_1− and B_1+, but this seems to place Xyl in the indicated position, between BM and B_1.

11 "Xyl +" tested on Mal. 10 were Mal+

1 Mal−

T(B_1) This establishes a linkage between Mal and Xyl.

Heterozygotes Lac, Lac$_2$

351
359

11/15/48

357 W45 x W477 on EMS Lac

359.. W145 x W466 " 11/16.

359. - 27 + colonies from 10 plates. S.O. on Lac EMB.

357 38/65 + colonies. 17 are Lac Var. (ca 40%)

			to EMS lac for retest	Xyl EMB	Lac EMB →
357:	1	1	H-133	+	V ⊙
	2	5	H-134	+	V ⊗
	3	6		+	— ●
	4	10	H-135	+	V ⊗
A	5	12		+	V ⊙ and ⊗
	6	13		+	V ⊗ + pud.
	7	15		+	V ⊗
	8	16		+	V ⊗
	9	17		+	V ⊗
	10	19		+	V ⊗
	11	20		+	V ⊙
	12	21		+	V V type ?
	13	22		+	? ⊗ + pud.
	14	25		+	V ⊗
	15	28		+	V ⊗
	16	30		+	V ⊗
	17	31		+	V ⊙
	18	33		+	V
	19	37	also 20 & 21 20 is ⊗ 21 is both.		

			Xyl	Lac	Sucr	Mal
359.	21	2	++	++	++	++
	22	3	++	++	++	++
	23	5	++	++	++	++; 2-colo'
	24	8	++	++	++	++ prob contam.
B	25	9	++	++	++	++
	26	12	++	⊗	++	++
	27	13	++	+, —	++	++
	28	16	++	++	++	++
	29	17	++	⊗	++	++
	30	18	++	⊗	++	++
	31	22	++	++	++	++
	32	26	++	+, —	++	++

∴ all tho possibly 5 Lac+/Lac- may prove of these.

⊗ = sectorial variation

⊙ = pericinal variegation.

Chloroacetate papillation as a test for ploidy

Streptomycin resistance

11/16/48.

Take single colonies from 356a H. stocks to water and streak on T(0) + Na Chloroacetate 1mg/ml and streak out on EMB Lac. cf. K-12.

	Stock	Quadrant (v. 356a)	T(Cla)	EMB lac	T(0)
1.	H-1	1		V	+++
2	"	2		V	+++
3	"	3		V	+++
4	"	4		V	+++
5	"	5		V	+....
6	"	6		++	+++
7	"	7		++	+++
8	"	8		V	+++
9	H52	4		V	++
10	"	2		—	+++
	K-12.				+++

H17: No growth or papillae on T(Cla)...

Plate W478 heavily on USA ē 100u/ml Streptomycin.
11/16/48.

PIP - no colonies.

Repeat Chloroacetate at various conc. /ml: on T(0).

	100 u/ml	200 u	500 u	1 mg.	T(0).	EMB lac.
K-12	- pap	—	—	—	++	++
H-72-1	- "	—	—	—	++	V
-2	- "	—	—	—	++	V
Aerogenes.	++	++	-, smear pap	gl. residual removed.	++	

Mannitol mutations here.

11/17/48.

73 plates × 300/plate 21,000 tests.

W583, 7 secs UV, Mannitol EMB.

Quite a few slow, like 1.

			Mannitol	Sorbitol	Glucose		T1
	1.	W583 slow.	−	−	+		S
	2.	~~W596~~ mal- purification	+	−	+		S
W597	3.	− or slow?	−	−	+		S
	4.	− throw out. not certain	−	−	−		?
W598	5.	−	−	±	+		S
W599	6.	−	+	−	+		S
	K		+	+		K.	R

Repeat tests.

	Sorb	Man	
1	slow +	slow +	
2	slow +	slow or ++	
3	+	v. slow +	
(5)	slow +	− −	W545.
6		+	

V-retests.

~~364~~
363

Streak out/stories, heavily on EMS Xyl.

H: 87. no cols.

88. 2-? colonies.

85, 86 4. col.

91 mostly −; occ. + cols.

92 ca 5+ cols.

93 no cols; 2 cols smoother.

94

95 } no cols.

97. }

11/22/48. ×H88. both Xyl− Gal− no longer heterozygous for Xyl.

H91. Xyl +, − cols. [Restricted on Xyl EMS.] + and − cols. noted.
Gal − but 2 kinds of colonies noted: "R" and "S"

×H92 Pure Xyl+ on EMB.
Gal −

H93. Gal− Lac(s)− #3 is Xyl (V).

Streak out H93 for papillations on Lac; Gal EMS.

→ Retest on Xyl EMB., 8 cultures. All ++. No heterozygote.

Gal-Reversions. 3636.

11/22/48.

Streaked out M93 on EMS Gal, Arab + Lac. and on EMB Xyl.
To look for reversions.

11/27. Papillae from Gal + Lac to same EMS. Arab turns + slowly, indicating selection

11/29. Numerous Lac+ colonies from papillae 1 & 2.
1 2 3 4.
— 11/x.

↳ EMSlac + EMBLac.

All 4 are Lac variegated! Confirmation of Lac- homozygous.

Gal papillae on EMS Gal are not clear cut.

↳ They have the form, however, (*), being + only in the center.

The lipbids on Lac EMS, enlarge more slowly, but have a comparable appearance.

---

4. Gal papillae taken to Gal EMB EMS.

	Gal EMS	Gal EMS-
A	Mostly intact, strong ++	All — Probably segregated
B	"	++
C	Numerous colonies which have dark centers and light margins. Not obviously variegated.	+ like EMB.
D.	like A.	++.

12/2. Streaked out A B C + D from EMS to EMB Gal and Xyl.
Also streaked out C from EMB.

Xyl: A Var B Var C ++ D —. A + B are variegated.

Lac$_2$ + / − heterozygote

11/19/48.

W45 x W588

20 Lac+ colonies picked

#17. for retest. This does segregate for Lac and is presumably $\frac{Lac_2+}{Lac_2-}$.

H118. Predominantly +. Streaks out on Lac EMB. Maintain on EMB

From mosaic A + B obtain – cols. and test nutrition.

& centime.

A1	mg.		B1	MTL	C1	MTL	W ~~600~~ 607
A2	+++	TO	B2	MTL	C2	M−	
A3	+++.	TO	B3	MTL.	C3	M−	

Control on Bugal fermentation and selection of Lac −.

P28. Inoculate lightly, 58-161 and Y10 each into 2 tubes of Bugal..

P29. Streak out on Lac EMB. Bugal tube:

58-161 −	1	about 20% −	A	+±	Some Lac slow? = D.
	2	all +		++	E
Y10	1	about 50% −	B	+±	
	2	about 1% −	C	++	W 602 – 5.

P30. Purify one – from each culture.

Restreak all 4 cultures. P30.

A1.

58-161	A	1	as above.	
"161	B	2		
Y10	A	1	1 : 1	+/−
Y10	B	2	100 : 1	

Retest D and E on Bugal.

D: Bugal ++ Streak out on Lac

E: Bugal − rf.

additional Lac - recovered

Zygotics from H118 for Lac$_2$ - ~~T~~ T - L — 364

Streak out mosaic colonies and test (1-3) Lac - from each.

12/2. 1. ++

12/3.

A. MTL

B. MT

C1 MT
C2 ++
C3 MTL

D1 M? TL
D2 MT
D3 M

12/4.

A1 M	B1 M	C1 MTL	D1 MTL
A2 MT(B_1)	B2 M	C2 MTL	D2 MTL
A3 M	B3 ++		

12/5.

A1 M	B1 MTL	C1 MTL?	D1 M
A2 M	B2 M	C2 MTL?	D2 ++
A3 MT	B3 ++(B_1)	&	

12/12 Culture in T(TLB,) ligrid. streak out on EMS Lac + TLB$_1$ and test single Lac - colonies. Hfr 10 was B_1 -.

Utilization of further substrates.

11/19/48.

1% x ____ EMB.

A. K Na tartrate

B. Propylene Glycol

C. Dextrin

D. Gum Arabic

E Sucrose

	A	B	C	D	E
K-12	– pop.	–	–	–	–
Aerogenes	±	–	–	–	++

S. typhimurium
Mal+

" Mal–

11/21/48. Streak out papillae of K-12 on EMB tartrate. Also S.O., 58-161 W583.

11/29/48. No evident ~~gas~~ acid production. Streak out to EMB Ammonium H Tartrate, which may be more oxygenic.

11/21. Y8 7 on EMB Sorbose. No obvious fermentation. No marked inhibition

11/29 neg. papillae noted. Restreak to EMB Sorbose.

11/24/48....

P23. Duplicate 10 ml washed suspensions of Y87 and W1G in H_2O,
A) 10 secs. in open Petri dish. Inoculate 1 ml/10 Y2 broth for crossing.
B) for control, Y87 × W1G. (see 367).

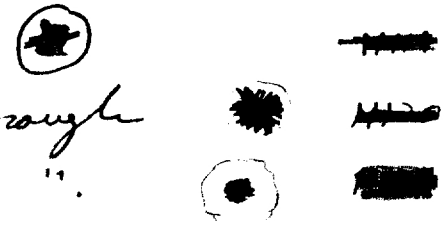
N24. Cross

10 plates × ca 200 prototrophs/plate. N26., 4 Lac + segs.
1 from B. 25.

Streak out on Lac EMB and ~~Malac~~ EMS. N27 A: #6.

N24.1 to EMS.

	EMB	
1.	Lac ++	Lac ++
2.	missing, 1st test.	
3.	variegated or incomplete +	
4.	" " , maybe mucoid very rough	
5.	" " " " .	



See 371

11/24/48.

P24 irradiate, as 365, 5 secs. in open dish. Inoculate 1ml 1/10 Y2 for cross.

Cross 11/25.

P27: ~~Very~~ heavy yield, ca 100/plate. V. few + 10 plates
9 Lac+. S.O. Lac EMB + ~~suspicious~~ Lac EMS

Lac EMB.

① Mostly −; occ. + probably Var.
2 Lac ++
③ ⊛
4 mostly −
⑤ ⊛
6 Lac++
7 Lac++
8 Lac++
9. Mostly Lac− ; + may be many.

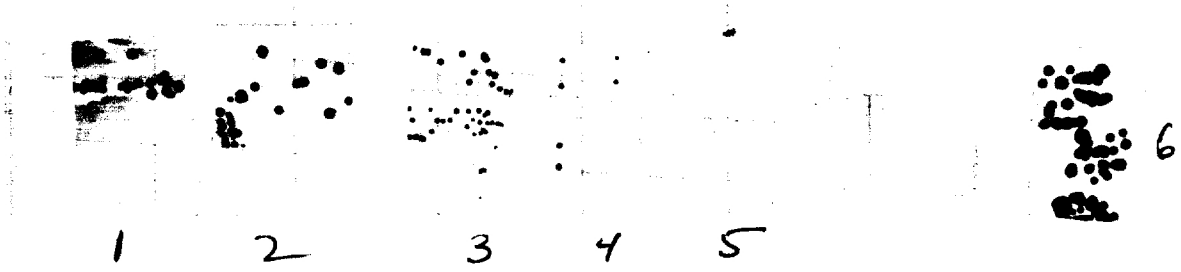
Restreak from Lac EMS.

See 371

11/24/48.

Cross Y87 x W126 on a variety of EMS media + variable supply of N as Amm sulfate.

A.S. g/liter.	K-12 control.	Rh.	Acid.		
5.0	1. = A	++	++	8 plates, ca 300+ each.	4+ 11/26.
1.0	2. B	++	++	5 plates ca 40 ea.	1? +
0.1	3. C	+±	+	5 plates ca 10 ea.	No +
0.05	4. D	+	±	ditto	
0.01	5. E	±	−		
5+ 5 ml glycerol	6. F	+++	+	Glycerol addition seems to inhibit acid production	



Yields are very much lower on "2" than on "1" suggesting a dependence on ammonium concentration.

367A: 4+. 1 ++
2. +/− ? and −
3. +
4. +

S.O. on EMS Lac and retest

B. 1. +.

See 371

-6.

P27. All colonies mad + (Glycerol +).

See 371.

-2]. 1+ picked for test /5 plates.

-3. Very low yield. Colonies appear very rough + dry.
1+ formed + picked for test.

-4. Ditto No +.

-5. Very tiny pentagons, few in number. Not scoreable

11/25/48.

W-595 (Lac, Mal, Xyl, Gal, Ar, Man,) × 58-161 n
EMS ± B_1 (Xyl v Mal).

Mal B_1 plates have too heavy a background to enumerate Mal+

Xyl (0) yield very low - only a few + colonies.
Mal (0) somewhat heavy background.
Xyl (B_1) colonies v. small but more numerous; occ +.

Incubate Xyl (0) further.

	+	−	Σ
Mal (0).	4		32
	1		43
	6		85
	4		57
	4		88
	10		130
	2		37
	31		552
Xyl (B_1)	2		30
	3		42
	2		48
	3		42
	3		89
	0		28
	4		94
	4		68
	21		441

11/24/48.

50-161, str. Fructose EMB. 67 plates × ca 300 = 20,000 tests
(plates are not properly gelled, but can be streaked.

		Lac	Max	Dul	Glu
#2. very slow on fructose.	W596	++	++	++	++
5 —, sm. cols.	597	–	–	–	–
7 —, sm. cols.	598.	–	–	–	–

Check on lactose, glucose

~~W596 (may show segmentation) + mannitol + sorbitol ±).~~

W596 is also slightly slower than type on mannose.

test on mannitol + sorbitol:

	M	S.
W596		

4/30 Streaked W108 on Mannose, fructose EMB.

11/29/48.

Test [illegible] EMS Mal- on EMS Xyl

Xyl+ " Xyl B$_1$ " Mal B$_1$.

a). Mal+ : 16 Xyl+
(o) 15 ~~Xyl~~ Xyl−

b) Xyl+ : 4 Mal+
(B$_1$) 14 Mal−.

streaks out Mal+ on Mal EMS; Xyl+ on Xyl EMS for mixtures of heterozygosis

1-16 a Xyl+ } Mal
17-29 a Xyl− } Mal

30-33 b Mal+ } Xyl.
34-47 b Mal− } Xyl

1-3, 5-8, 9-12, 17-20, 21-24, 25-28
29-32 Intact Mal+.

29, 31-32, 33, 41, 42, 46, (2), ~~Mal+~~ Xyl+.
~~30~~, Xyl− : 30, 34, 35, 36., 37-40, 43, 44

(#4)

Many Xyl+ Mal− were mixed and should be Xyl−

which alters ratios!

↓ #4 was ped. Mal− with some peculiar Mal (+slow). streaks out on Mal EMS. Mal+ and Mal− each pure. No sign of segregation. What are slows? not clear. May have been Mal+

365 - 366 - 367.
Nine heterozygotes.

11/28/48

Summary of apparent heterozygotes from cross of Y87 × W126.

	H-			Check from EMS.
~~365~~				
1.	119	365-2	~~not complete~~.	Varieg.
2.	120	365-3	Lac +/-	Varieg.
3.	121	365-4	+/-	Varieg.
4.	122	365-5	+/-	Varieg.
5.	123	366-1	+/-?	Pred. -; Reqmify.
6.	124	366-3	+/-	Varieg. (rel. stable).
7.	125	366-5	+/-	Varieg.
8.	126	366-9	mostly -. Some + may be Var.	Mostly - on EMS. pmify 11/29:
9.	127	365-6	on EMS only	Varieg. (rel. stable)
10.	128	367-2	"	Varieg.
11	129	"A1"	+/-	Varieg.
12	130	"A2"	mostly -;	+/-? Varieg.
13	131	- B	Lac +/-	Varieg.
14.	132	- C.	+/-	Varieg.

Obtain & characterize segregants from various of these.

1.	~~H121~~ H120B: Lac- ✓	M-	}	W	599
2.	H120A: Lac+ ✓	T1-		W	600
3.	H119A: Lac -	TLB1-	}	W	601

Mapping + numbers of ...

November 30, 1948.

A. W595 × W65.
B. W595 × W48 } Lac EMS
C. W595 × W182
(D) W595 × 58-161 } Mal Xyl Man EMS ± B_1.

No prototrophs P2.
A3. A - no prototrophs
B+C Very few, unreconcilable + or —.
Pick 12 from B and 8 from C for further test - 12/3 on EMS Lac.
all Lac -

D:

Mal EMS +	Mal EMS −	Mal B_1 +	Mal B_1 −	Xyl +	Xyl −	Xyl B_1 +	Xyl B_1 −	Man +	Man −	Man B_1 +	Man B_1 −
2	16	1	84	0	0	12	109	0	5	0	81
0	7	3	169	0	0	3?	18	0	33	1	46
7	31	6	210	0	0	5?	28	0	3	3	54
2	34			0	0	0?	26	0	1	1	5
0	1			0	0	3	54	0	1	0	23
0	7			0	0	3	36	0	5	0	35
1	9			0	0	2	14	0	3	0	48
0	13			0	4			0	3	+1	
12.	118	10	463	0	4	28	285	0	57	6	292
9.2% +		2.2% +		0		9% (Counted to ca 1%)		0		1.7%	

Pick +'s to homologous medium.

1 - 6 are Man B_1,
7 - 10 are Man (0)
see 372a.

Mal B_1 plates turbid; Xyl plates empty!
work difficult to score

Retests: all Mal correctly scored
All Man " "
Hostapp. "Xyl +" are Xyl -

Recount certain plates:

(M&L) Mannitol EMS.

+	−
0	7
2	14
0	4
1	5
1	1
0	4
4	35

Mal EMS.

+	−
3	15
4	6
1	0
8	21

X-yl EMS B_1

+	−
2	129
0	62
2	191

ca. 1%

This late appearance of mannitol+ recalls interaction of glycerol+ and B_1− noted in 1946.

Pick to homologous EMS and S.O. on EMB.

Mal (o)+ 16 tested on EMB. #1 pred.−, occasional+ others are +++.

M&L (o) 10+ tested on M&L EMB All +

December 1, 1948.
Streak out Y87 and W126 for single colonies to repeat 371.
Use mi crosses and buffer, record on EMB lac plate.

A. Y87A x W126A. } 8 plates each.
etc.; : B, C, D. }

E. W599 x W588 i.e. M'? x H. Wrong strain used. had in mind that 588 was a Lac+ reversion of 583.
F. W601 x W352 (Lac+ Xyl-).
~~G. W600~~
O. W600 x Y87.

12/3: Yields variable; Lac - very small. Ca 100-200/plate.

A. 7+.	11 Var. (#1)	6 ++	
B. 1+ (-yields low).		1 ++	Should be repeated.
C. 6+	4 Var	2 ++ (#3, #5)	
D. 8+.	6 Var	2 ++. (#1, #7)	
E. Numerous ++.	11 Var.	11 ++.	Equal numbers of Var & ++.
F. No yield.	High yield + increase. Good plate; sharp definition + no background.		
G. Small Lac+ colonies.			

E: 28 streaked out on EMB lac 6 are Lac variable: #5, 13, 14, 8? + others

G. 60 " " " " . #34, 37, 38 restreak on Lac EMS.
All others ++.

34+37 all -. 38: ++

December 3, 1948.

A. W65 × W595 on Lac EMS.
Lac_x × Lac, –

No yield. 12/6

12/2/48.

70 plates W596 (58-111, Fruc ÷) mediated 7 secs on EMB Glu.
ca 300/plate ⟶ 20,000 tests.

Numerous mucoid and slow colonies intufund with sampling:
Following finally screened.

	Glu	Lac	W
1	—	—	610
2	—	—	611
3	—	— pap	W612
4	slow ++	+	
5	"	+	
6	"	++	
7	"	++	
8	"	+	
9	"	++	
10	"	++	614
11	— s.r.	— thin	
12	++ and —	++	- 613
13	slow +	±	

Save 1, 2, 3 from glucose and respecify 12.
Do not keep slow mutants except 10

New heterozygotes

December 4, 1948.

A. W65 × W595

B. W48 × W595 No yield

C. W182 × W595

12/5/48.

~~By~~ W45 × W595 on Lac EMS.

12/8. No yield! (3+ colonies in 15 plates!)
2 not cli 3'd +4.

Note. Hfr. A12 Streaks out W-1 to W595 series to establish mutability.

		on lac
Y53	Lac −	M (irregularly; many colo. stable).
W1	" Gal −	M consistently.
W566	" Gal −	S
W582	" Xyl −	S
W583	" Mtr −	S
W595	" Mtl −	S

The mutation to Gal- seems to have been accompanied by stability of Lac, −, possibly fortuitous.

12/28/48. Test other Gal- mutants of this series on Lac EMB for mutability:

W 565	Stable, thin colonies	575 mostly small stable colonies; some large mutable.
W 566	" heaped-up centres.	
567	" (very occasional papillae).	576 small colonies uniformly.
568	Stable.	
569	v. sm colonies; some revertants	
570.	typical mutable.	
571	like 565	
572	like 567	
573	stable, large colonies	
574	typical mutable	

12/6/68

A. W126B x Y87B see 373.

B. W495 x W45

C. " W48

D. " W65

E. " W182

Yields low:

A. 5 plates 100/plate. 3+ colonies. S.O. on Lee EMB + EMS. ++

B. 10 plates ± 2/plate 2+ colonies. ++

C. " ca 1/plate No +

D. " ca 1/plate No +

E. 9 " " No +

12/5/58.

Pick 1 – colony from each of four mosaics of H119 - H122 & test as indicated.

		Lac	V_1	Bu Gal	Nutr.
119	A	– S	S	–	TM
	B	– M	R	+	TM
	C	– S	S	–	TL
	D	– M?	S	± M	T * ✓ –
120	A	– M	R	+	M
	B	– M	R	+	MT
	C	– M	R	+	M
	D	– M	R	+	T
121	A	– M	R	+	MT * ✓ –
	B	– S	S	–	(MTL ✓
	C	– M	R	+	~~(MTL)~~ M * ✓ –
	D	– S	S	–	MTL
122	A	– S	R	–	MT ✓
	B	– S	S	–	TL ✓
	C	– M	R	+	MT ✓
	D	– S	R.	+	MT ✓
temp new data	Y87	– M	R	+	BM
	W126	– S	S	–	TLB,

6S:10R

M = mutable
S = stable

Summary:

Bug	V
8	+ R
1	+ S
2	– R
5	– S

suggesting linkage of Lac, and R.

Note preponderance of T- and M- S streaks out indefinite Bu Gal tests. *

There is a general correlation between mutability and Bu Gal – but it is not perfect here

Maintenance of heterozygotes.

12/14/48.	H1	v?	vv	
Lac	22	All+	All+	Returns to previous EMS plates.
Lac	52	v	vv	
Lac	62	v	v	
Lac	72	vv	v?	
X-gl	85	v	v	
X-gl	93	vv	vv	
Lac	118	v	v	

+ colonies from previous EMS plates restreaked on EMS. These restreaked, 2/type, on EMB and streaked on EMS; also on Nutrient agar slant (subculture 1).

A6. Streak out NA slant from H1 and H118 to determine feasibility of recovery at this stage.

4 tests each.

H1. 1- 3 Var. 4++ or Var? H118. All 4 are Var.

This may be a suitable method

Dec. 13-14, 1918.

A. W45 x W595
B. W45 x W583
C. 58-161 x W595.

} EMS Lac

(1). 15 plates each P13. A16: all but blank.

A: 7 colonies altogether. 5 possibly +. All --
B: 17 " " . 10? " +. All++ or --
C. - + 4 tested: 3++ 1-

} No Var.

Pick all possibly + colonies and streak out on EMBlac, ~~sucrose~~ + EMS.

(2). 15 plates A + B A14. Low yields, but quite up apparent +'s. (58)
Mostly — mostly scored as +. Not variegated.
Some weak(?) + noted. Type by on Lac EMB

#19, 2, 6, 14, 16 Keep as W-460
#16 is Gal++ Lac++ others are Gal- Lac very weak

Papillae seen on lac + gal plates. Streak out on both media.

Gal + pop:	Lac	Gal
6	++	++
14	++	++
Lac+ from 6		
0	++	++
1	++	++

Note: on lactose, residual Gal- colonies show near + reaction when they are situated in vicinity of + colonies.

Conclusion: The Gal- in these stocks is also an inhibitor of lactose fermentation, in distinction to W-255. H93, therefore, may now be Lac, ± and Gal-. ∴ It is not proven that Lac- can be homozygous!

12/23/48.

Cf. W460 on 1% and 3% Lac EMB.

At 48 hrs. W460 is nearly +++ on 3% lactose
still slow on 1% ".

Streak out W695 on EMB galactose for reversion.

Test revrts on lactose for mutability.
(W660.) #4. Allac Lac mutable like Y53.

Dec. 18, 1948.

Cross W45 × W595 on lac synthetic media.
A) "EMA" .5% asparagine as C source.
(B) EMS, fresh batch. Na succ " .
(C) EMA+B. Asparagine + Succinate .5% each.
(D) Lilac B. Bact standard.

} Very heavy (4× conc.) inocula.

A) 8+ /11 plates. A few lac-. Pick+ test +
1-8.

B) 7+? /13 plates. Surval -.
9-15

C) 12 plates. Poorly scored, but yields much higher.
16-48 25+.

D) 4 plates. 1? +.
42

Very few scored + on EMB. Some were lac mutable. (W45?)
6++ altogether. Numerous slow + à la 389.

Test media for crosses.

December 22, 1948.

Cross W478 × W595 on various media using constant inoculum. (1 drop ½ del. points)

Also conc. inoculum on Lac EMS.

+B$_1$: ——→ 1-6.

5 plates each.

	+	Nutrient (Difco)	.59	.59	rusty,
		Nutrient	.59	.19	blue
4		Mg SO$_4$	0.01g	0.01g	mottled
		agar	1.5g	1.5g	
		K$_2$HPO$_4$	.2	.2	
		Eosin Y	0.04	0.04	
		Methylene blue	0.0065	0.0065	
		Lactose	1g	1g	
		B$_1$			

12/24:

T(B$_1$).	51,	43	49	46	43	m = 46.4
T(0)	4	3	5	10	1	m = 6.6
(1) EMB.	1	0	0	0	0	.2
(2) MB	0	0	0	0	0	0
(3) E	0	0	4	1	0	1.0
(4) No dye.	10	8	14	4	10	9.2

The dyes are certainly inhibitory, but the minimal medium base is certainly not very satisfactory, possibly due to use of lactose as main carbon source.

From 20 plates mainly on Lac EMS, about 2% pink (+) spots + streaks, 4 of 4 from typ. mucoid and/or grossly Lac +.

D478 x D57.

3934.

12/29 - /48.

1. 26 ✓
2. 46
3. 171 ✓
4. 188 ✓

not heterozygous.

: H139, 140, 141.

	Lac	Xyl	Mannitol	Gal	Malt
139:	±	±	−	++	
140	±	±	±	++	
141	±	+	−	++	

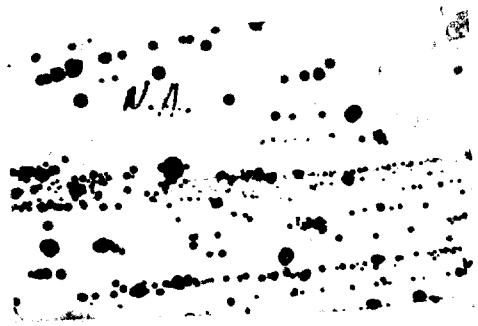
12/23/48.

Recover H93 from nutrient agar slant and from Xyl. S plates from NA to Xyl EMB. Pred Xyl-. Ca 2% mosaic colonies.

nutrient agar probably remains a preferred means of maintaining heterozygotes.

similarly on EMS Xyl. Pick a few to Xyl EMB to test recovery of H-93.

from EMS plate 7½ are still mosaic. Recover heterozygotes from EMB; EMS Xyl.



Where a heterozygous colony is streaked on EMB:

Gal negative

Xyl almost all mosaic colonies

Lac slow + (1 or two colonies finally ++).

Lac 3% full +; no sign of variegation.

H93 is therefore probably Xyl +/- Lac +/+ Gal -/-

December 24, 1948.

S(lac) 7 carboys ca 30 hours

1/20 mL .251 .340.

i. Each ml of culture will provide ca equivalent of [illegible] of 319A.

104 g. collected from two carboys (70 Liters). i.e. equivalent to 20 ml 319A.

58 g. suspended in a very heavy cream in 1/80 for grinding but ~~no~~ pump did not draw properly. Return cream & remaining paste.

12/25/48. Re carbeties mill & grind remainder of cells. Use entire basis. (ca 40-50 g. paste probable).

Ca. 10 ml of extract. Assays 3970 u/ml.

Galactose mutations
Xylose.

12/24/48.

Y87 7 secs. etc. 80 plates ca 200/plate
Galactose 16,000

W870 7 sec. etc 33 plates. ca 300/plate
10,000

→ W641.
642 :
643 very thin.

Xylose.

Galactose :

W			Lac
644	1	—	M
45	2	– thins	S
46	3	slow +	M
47	4	slow ++	M
48	5	– small col.	S
49	6	slow ++	M
50	7	slow +	M
51	8	—	S
52	9	slow +	M
53	10	slow +	M
54	11	slow ++	S
55	12	slow ++	M
56	13	slow ±	M
57	14	– thins	pap. to thin colony, prob. sat +
58	15	– thins	S
59	16.	– thins	S.

use 644 for 2 pathways.

Inhibition by various batches of MB; Eosin. – Crosses.

12/9/5

Weighed out 40 mg Eosin Y and 6.5 mg MB of batches indicated. (Certification numbers ...)

	Eosin	MB	Colonies −	Colonies +	Average.
1.	23	27	58	18	
2.	23	28 ✓	113	38	
3.	23.	29	93	25	
4	24/24	11	56	8	
5	14/24	28 ✓	103	23	
6.	24/24	29.	49	8.	
7.	22±	28	236	23	
8.					
~~12~~					
8.	7(0).		146		

all batches gave results comparable to 7(0).

"Spontaneous heterozygotes" II

Jan 4 ++. 1949.

1. W644 x W126	14 plates.	ca 100/plate.	16 picked.	2H
2. W660 x W45		26 picked.		2H.
3. W595 x W45		2 picked.		
4. W660 x W69		1 " . Wood yield.		
5. W595 x W17		No yield whatever.		

1: A1, 3 are heterozygous	12 others probably lac−	1, 2
2: ≠ #7, #12	2 prob. lac−.	3, 4
3. #1 H.		5.
4. — −		

Additional:

2): 8 tested All ++

3): Two tested Both slow +. (Gal- Lac +?)

4). 4 tests. 3 − 1 ++.

Test + purify as lac EMB, EMS.

1. Clearly lac heterozygous.

2. " "

3. May be lac heterozygous; colonies fade quickly.

4. ++.

5. Mixture of +, − colonies. Probably not heterozygous, but test sample of + colonies from EMS lac. ++

January 18, 1971.

streak out as indicated:

402, -1, 2, from mosaic colonies, on Lac, Gal EMB. (note W6YY may be suppressive for Gal-). H136, 137 Gal+. (maybe heterozygous for Lac, Lac+ Gal?)

3. from a "mosaic +", on all sugars: Lac, Mal, Xyl, Ar, Xyl, Man.

5. from Lac+ on EMB on Lac ++.

? H137 may have some Gal+ I

3. (4 cols, identical). H138 Lac, Lac 2.

Lac variable Gal+ (as reputed)
Xyl −
Mal −
Ar +
Man variable.

Note: on lactose, colonies are pinky peripherally −, show sectoring in centers ◔ etc. These colonies tend to fade: Almost full + on EMB.

On mannitol, almost all colonies are as under with well defined central region ◉; occasionally colonies show sectoring.

~~H136 + 137. have been streaked out as Lac EMB to provide segregants for fertility study.~~

irradiations. -

January 9, 1949.

6. W644 on maltose. This culture was supposed to be galactose negative. When irradiated, it showed many Mal slow. Reinvestigation shows that there are two components in W644

(1) Gal slow Mal − mucoid on galactose.
(2) Gal + Mal +

(2) W660 on galactose. 50 × 100 = 5000.
= W595 Gal + irradiated.

(3) W656. on arabinose 20 × 70 = 1400 3 mutants:
Ar. Xyl. Glu. Lac

W-667 1.
W-668 2.
W-669 3.

W670 1
671 2
672 3
673 4
674 5
675 6
676 7
677 8 +
678 9
~~679~~ Mucoid.

Sp. heterozygotes

Jan. 12, 1948.

(1) W45 x W660

(2) W182 x W660. not.

contaminated ⊂ Aerobacter.

26 "+" tested: None heterozygous. ca 1/3 —.

Segregation of H136, H137.

Jan. 13, 1949.

Streaking out of mosaic colonies of these cultures gives about 50% mosaics; 50% −. Full + is quite rare.

e.g. 25− : 21+. This is rather lower rate of segregation ~~that~~ than shown by previous H stocks.

Picked well isolated − colonies for characterization with T1 and nutritionally. Also picked possible Lac+ ~~one~~

H136 A.

	T1
1	R
	R
	R
11	S
	R
	R
	R
	R
	—
	R

B

	T1
2	R
	R
12	S
	R
13	S
	R
	R
	R
	R
	R

C

	T1
3	R
	R
	R
	R
14	S
	R
	R
	—
	R
	R

D.

	T1
4	R
	R
	R
15	S
	R
	R
16	S
	R
	R
	R

H137. A.

17	S
	—
5	R
	R
18	S

B

6	R
	R
	R
	R
	R

C

7	R
19	S
	R
	R
	R

D

8	R
	—
9	R
	R
	R

Total: 47R : 9S. Highly abnormal. (non-random).

Test ~~mut~~ nutrition of 9S and 9R cultures.

January 14, 1949.

	ALL $Lac_2 - V_1^R$
1	+
2	+
3	+
4	+
5	+
6	+
7	+
8	+
9	+
	$Lac_2 - V_1^S$
11	L
12	TL
13	MTL
14	TL
15	MTLB,
16	TL
17	TLB,
18	TLB,
19	TLB,

Kupa W - 721.

M+ > M-

T, L ca equal. (linkage to R.S).

Segregation of 11138

Streakout from segregating plate, grossly, to EMBLac.
Rather large proportion of Lac+ segregants, also Lac I.

Mal mutations.

Jan. 12, 1946

50 × ca 300 = 15,000 colonies. 487 / Mal EMB.

	W	Mal	Glu
1	679	slow	+
2	680	−	+ faded
3		S+	+
4	681	− s.c.	+
5		++	+
6	682	−	+
7	683	−	+
8	684	− ±	+
9	685	—	+
10		S	+
11	687	−	+
12	688	− s.c.	+
13	689	± −	+
14	690	±	+
15		+	+
16		++	+
17		+	+
18	690	—	+
19	691	−	+
20	692	−	+
21	693	−	+
22	694	−	+
23	695	−	+
24		+	+
25	696	—	+
26	697	±	+
27	698	−	−

segregations of sp. [illegible]

Jan. 16, 1919.

H138: (Lac; Man. variable). sp. W45 × W660

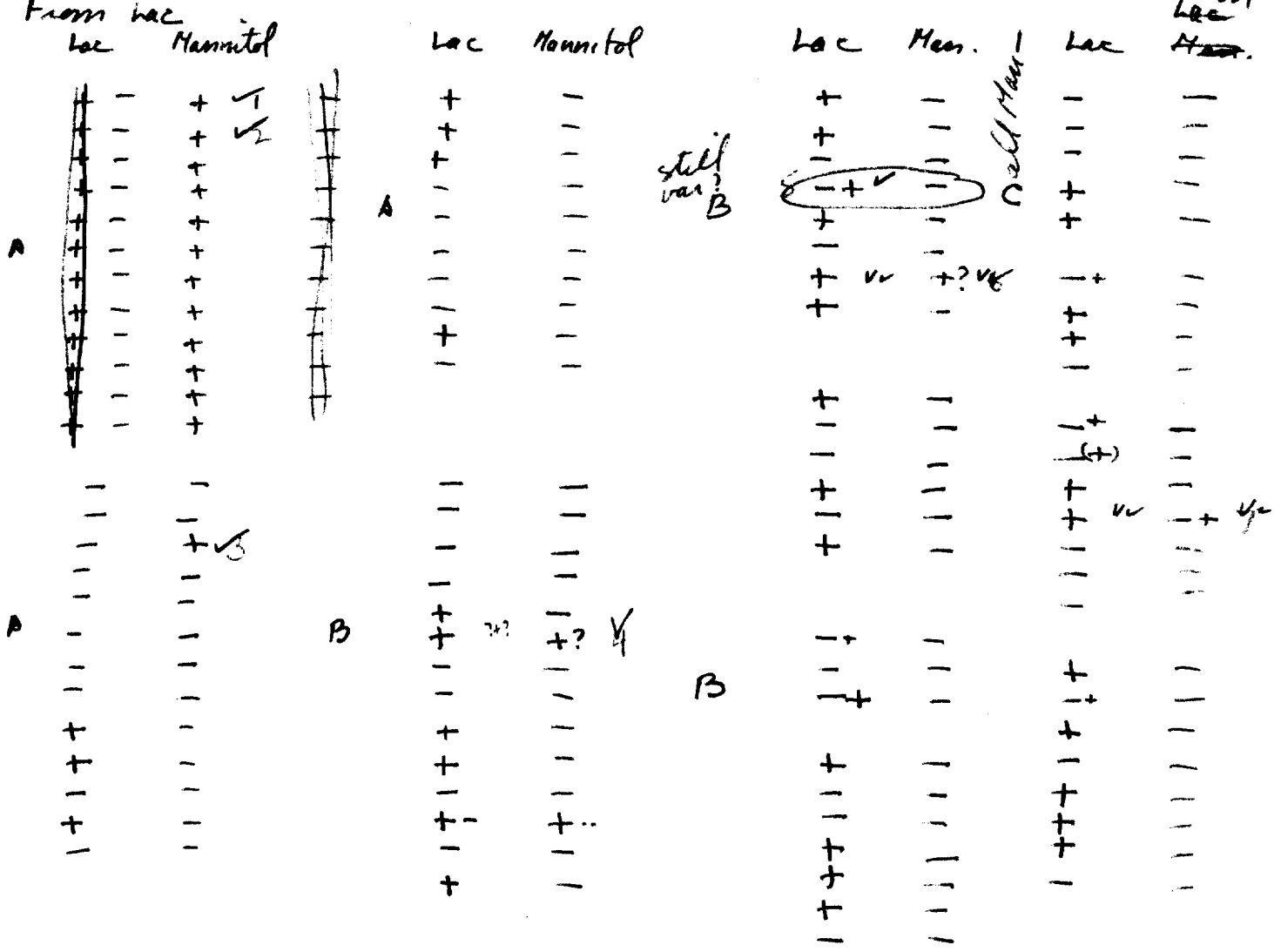
~~H134~~ H140: ~~(W45 × W660)~~. W478 × W595.

[H139 on Mann EMS.]

Streak out H138 + H140 on Lac EMB for starter inoculum.

Pick all isolated ~~colonies~~ colonies + or − from individual mosaics and score on other media.

H138. From Lac

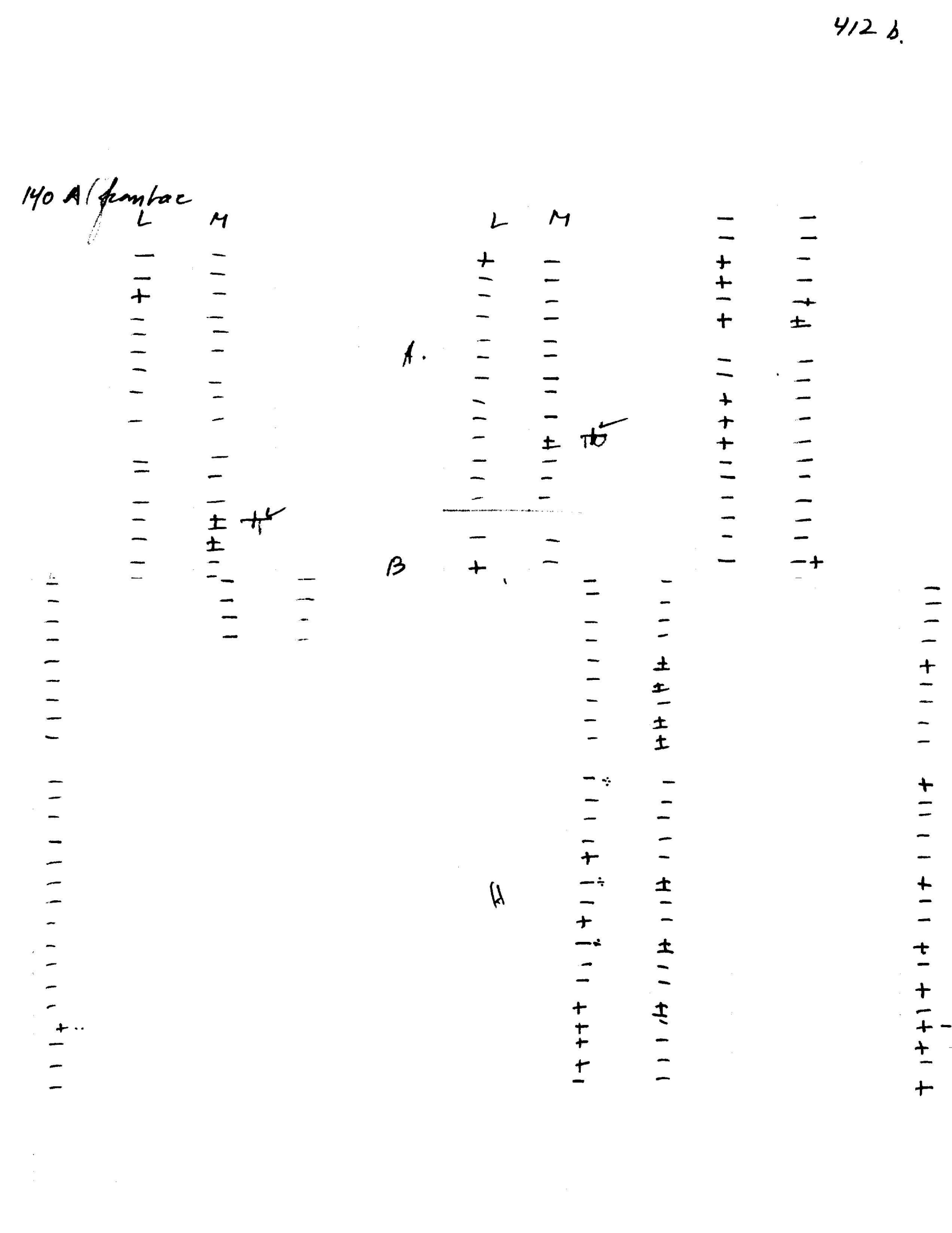


Segregation of nitrogen fixers

H138 (from Man)

	Lactose	Mannitol	L	M	L	M	L	M
D	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	+	−	−	−		
	−	−	−	−	−	−		
			−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	+ (−+?)	−		
	−	−	−	−	−	−		
	−	−			−	−		
	−	−			−	−		
from M.								
E	+	−	+	−	−	−	+	−
	−	−	−	−	−	−	+ (−+?)	+ −
	−	−	−	−	+	−	−	−
	+	−	−	−	−	−	+	−
	+	−			−	−		
	−	−	−	−	+	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	+	−	−	−		
	−	−	−	−				
	−	−	−	−	−	−		
			−	−	+	−		
			−	−	−	−		
			−	−	−	−		
			−	−	−	−		
			−	−	−	−		
					−	−		

140 A (femtac



1/21. Tests on reisolated segregants.

	Lac	Mtl.
1	−	++
2	−	++
3	~~+~~ −	++
4	+?	V
5	V or +, −?	−
6	V	V
7	V	V
8	~~+~~ +, − V?	−
9	−	+
10	−	+

Picks from Mtl^{var} columns.
Rechecks on Lac.
" " "
" "

1/22 Rechecks

1
2
3
4 Lac variegated (not pure +)
5 Apparently Lac+ and Lac−. No definite Lac^{v}. Mtl for recheck.
6
7
8. 1, 2, 4. Lac −. 3 Lac$^{v?}$ and Lac −. Restreak Lac$^{v?}$ on EMB Lac + Mtl Lac+.

No partial segregations. High correlation in a colony. Suggests that sectoring may result from very few segregations per colony. Should try to find evidence for reversal of trend in +/− segregations!

Maltose deadication runs.

1/19/49.

W668. 40 plates EMB/Mal × ca 400 cols.
16 mutants

W700-715.

6/28/53 ~~Adaptation~~ W583

Cavalli's data 58-161 x W583

Gal \ Lac	+	−	
+	43	21	64
−	91	286	377
	134	307	441

[Need V_6 rather than Lac to map Gal.]

Assumed M Gal Lac

but too many doubles.

W478 x W583: Xyl, Lacv's isolated

330] Noted that Xylv were mostly "peculiar Lac−"

Gal doubtless provided by Lac ~~segregation~~ episteses.

330-2 (on EMS Lac) mostly Gal+ Mal− Lacv.

340: 58-161 x W583. close Lac, Gal correlation

351: Lac+ excess. ~~Ex~~

273
13

58-161 x W583

	Lac +	−	
Gal +	43	21	64
−	91	286	317
	134	307	441

Gal + Lac + if independent would be $\frac{64}{441} \times 134$ = about 20

Interaction in scoring? Use V_6.

	Mal +	−	
Gal +	8	53	61 Unlinked.
−	18	355	373
			434

	Xyl +	−
Sal +	6	
−		

Crosses for heterozygotes

1/18/49.

1. W677 × W478 ca 100/plate: No + probable.
2. W182 × W677 14 plates 44+ : 150-
3. W45 × W677 15 plates 58+ : 155- Probable not 1 mosaic noted

(1) In 20h., quite a few V noted. However, in 40h., someone not easily scored. (Medium rather dilute). 7 picked for recheck. 1-7

(2) 1? for recheck. Lac+ 8 ✓ Picked. "+" to Lac EMS + EMB.

(3) 1? " " Lac+ and Lac-. Var? — 9. No var.

H (1) 1/81 Mostly +. 1 V? Restreak on Lac EMB. Lac Mal Gal Mal Xyl Not heterozygotes.

(2) 1/82 Mostly +. 1-. Wait for EMS-plated. not heterozygotes.

1/83	3	+, −, V	} Restreak.	✓	+	−?	+	+	+
1/84	4	+, −, V.		✓	−	−?	+	−	−
1/85	5	"		V	+	→	+	+	+
1/86	6	"		V	V?	→	+	−	+
1/87	7	+, − and V.		V	−	→	+	−	V

Additional 100 Lac+ colonies streaked from (1). 9 probable V. picked and restreaked.

H 142, 148 are vacant

Heterozygotes from W677 x W478. 4179.

		Lac	Xyl	Mtl	Gal	Mal	Ar
~~H142~~		~~V~~					
3	143	V	+	+	−s	+	+
4	144	V	−	−	−vs	−	+
5	145	V	+	+	−s	+	+
6	146	V	+	V?	−s	−	+
7	147	V	V	—	−s	−	+
~~+~~	~~148~~	~~V~~					

These are no more satisfactory than ~~H142~~ H-140 which has already been analysed to some extent. H144 and 147 are useful for getting Mtl+ recombinants of H139 (Lac, Xyl^{v} Mtl −)

See 4176 for additional heterozygotes in this series.

Heterozygotes from W478 × W677

	Lac	Xyl	Mtl	Mal	Arab	Gal.
1	V	V	V	+	+ V?	V
2	V	V	V?	+	"	V
3	V	—	—	—	±?	V
4	V	V	V	—	V?	V
5	V	—	—	—	±?	V-
6	V	V	V	—	V?	V
7	V	V	V	—	V	V
8	V	V?	—	—	V	V

Gal may be regularly variegated in these strains. May be associated with the Lac, -

Second observations:

		Lac	Xyl	Mtl	Mal	Arab	Gal	H	
H 167	1	V	V+	V	+	+V?)	V?	165	
168	2	V	V+(B)	V	+	(+V?	V?	166	
169	3 A	V -pur.	—	—	—	A V??	V	167	
170	4 A	V+pur.	AV-	V-	—	B V??	V -pur.	168	
171	5	V+	—	—	—	?	V?B	169	See 3/10/49. Pur+
172	6	V-	V+	V	—	?	V?B	170	
173	7	V+-	V?+pur.	V	—		VB	171	
174	8	V+	AV -pur.	—	—		V	172	

almost all gummy on galactose

(1) Restreak 1V colony from all arab + galac segregations.

(2) Retest all (4) segregations.

Jan 28., 1949

H168 is confirmed heterozygous for Lac, Xyl and Mtl.
The following ectets were made with Galactose and Arabinose.

H 165	(1).	Probably mixed	Gal / Gal^s	Arab. slow++
166	(2)	segregating for	Gal+ / Gal s	slow++
167	(3)	very clearly segregating		"
168	(4)	segregating	+/s	"
169	5)	Segregating	+/s	slow++
170	(6)	segregating		slow++, but some radiating colonies
171	(7)	Segregating	?	slow++
172	8)	Segregating	Gal+/s	slow++

check by streaking out a slow and + colony separately.
H168: heterozygous for four factors; use for crossover studies.

W589 Crosses.

1/20/49.

W589 (Luria's tryptophane-adenineless) is not fermentatively normal:

Slow on mannitol, galactose, Maltose – Lactose – Glucose ± → +.
+ after 2 days.

1/21

1. Cross with W477 (ILV, Lac–). × W589
2. Y-161 × W477. (Lysine-histidine).

Yield of ① very high in 48 hours. Sharp segr. +/–
② Less marked yield. Tests:

② : 40 + tested. 5 for retest. 1-5
Selection from "strong" + prototrophs.

Y : 28 + tested. 16 probable Lac⁻ 6-21; 26-29
Selection from "weak" prototrophs.

Overall: 21 Y /68+ or ca 34%.
an additional 40 are formed from the first group. On reexamination;

① 100 tested. 4 possible mosaics noted. 22-25
None of these is very sharp. Restreak on EMS, EMBLac etc.

Store 20 m EMS-Lac- T1 test plates
Work up 5 additional

In addition to routine restreaks, take 8 colonies and 1 gross streak of H149 (419-2-1) and streak out on EMB Lac.

H148 = 419-2-2 Lac EMB: Many +; occ. – and V.
H150 = 3 Mostly +; occ V and –
" 151 4 Mostly – occ V and +
" 152 5 " " "
153 6 ca = +, –

W589 x W477

1/25/49.

W589 x W477
Series (1). 22-25. EMB Lac:

22: Most colonies either large, rough spreading lac+ or small, smooth lac−, with some sweice combinations. 1? still variegated colony. Not sharp.
48+: 10− 1 or 2 V. H 154

23: Majority −. 44−: 17+. 1 or 2 V. H 155

24: 20+: 11+ H 156

25: 54+: 27− H 157

None of these seems to be strictly mendelian. The colonies which are probably variegated are not very sharply defined, and some of them may represent the slow fermentation type of W589. At any rate these do seem to be heterozygotes. Wait for EMB plates to develop before proceeding.

A 25. Add'n'l 108 colonies picked and tested on Lac EMB.

W1977 x Y-161. 4196.

(H149 segregation, etc.).

Jan 25, 1949

Bind. colonies of "H149" had been streaked out on EMBlac.

1. Only + and V? or lighter colonies noted.
2. +, vague + or V, and a few −
3. + only. Probably miscored as mosaic.
4. Mostly "mosaic"; few, = + and −.
5. Mostly +, spreading. A few −
6. All +
7. All +
8. + = −. A few mosaic.

0 (gross streak). + sl. > −. Recover H149 to EMS from 8 to avoid possibility of losing this strain.

Corrigenda !

January 25, 1949.

A considerable part of the work done this day used contaminated tubes for suspending colonies, etc.

Following can be recovered from original plates:

(1) Resolution of H148-153 (Lac+/- from Y-161 x W477)
T.O. H149 [too much trouble]

(2) 417-7 (H147) from EMS Xyl plate.

(3) H138 M+ from EMS Mal.

Repeat: H144 on Mtl

January 23. 1949.

1. W126 × W701 Lac_4^- × Lac_1^- Mal- Gal- Ar-
2. W589 × W677 Tr^- Rd- × F_6^-.

Yields poor on ①. V. few + as expected. high on ②.

②. 100 picked P25. A26. No clearly segregating colonies.
streaked on Lac EMB (N2)

1 is picked. Show peculiar mottled appearance on Lac EMB (Is this another Gal-epistasis?)

After 36-40 hours, on Lac EMB, These colonies (7 of the 8) show definite sectoring, especially #6. Assign H159-164 to these cultures. Streak out Mosaics of H163 (#6).

②. Additional 100 picked P26. A27: 1 very questionable ±.
Streak out on EMB; EMS as 420-2-1 Not variegated.

∴ "2" has given no reasonable heterozygotes.

Jan 26, 1948.

S.O. H139 and H141 on EMS MtL, Mal and EMBLac to select reversions.

On EMS MtL, H141 shows speed +, a few − colonies. At this time is MtL ±.

To confirm, streak out on EMSLac, EMBLac + EMBMtL

H139 OK.

P30. 16 papillae from H139 picked to MtL EMS (or EMB). Later 4 more
P1 Restreaked on EMS MtL; EMBLac and EMBMtL.

	Lac	EMB
1	V	
2	V	
3	V	
4	V	
5	V	
6	V	
7	V	
8	V	
9	V	
10	V	
11	V	
12	V	
13	V	
14	V	
15	V	
16	V	
17	V	
18	V(?)	
19	V	
20	V	

All the cultures are obviously still lac V.

On mannitol, however, they show an indefinite reaction never fully +, rather gummy, and sometimes against a vaguely sectored background. Pick the most clearly sectored colony in each set and restreak on MtL EMB.

On EMS MtL, similarly, the colonies show an intermediate response. (This may be due to vigorous reduction of M.B.)

Segregation of M163

January 28, 1949

After 48 h.

1.) Inoculate from EMBlac to Purassay. Dilute and spread on various sugar media (Lac, Mal, Gal, Ar EMB. Two sets, A + B.

2.) Streak out single variegated colonies from EMB Lac to same

(1) P31 A. Lac EMB: 1) 58− 1+ 9± /68.
2) 76− 1+ 9± /86
3) 76− 2+ 14± /92
210− 4+ 32± /246 Σ

Lac − 1/two kinds, one pinkish; one blueish.

Mal	105+	4−			Corrected for heterozygotes.
	109+	6−			
	214+	10−	/ 224.	195	
Gal	151+	17−			
	96+	5−			
	247+	22−	/ 269	234	
Arab	122+	6−			
	73+	2−			
	195+	8−	/ 203	177	

Lac EMB	75−	1+	11±	Mold. tests for M−(Rb) on all media.
	90−	0+	8±	
~~Vacuoles~~	145	1+	19± /165	
Gal	107+	5−		
	104+	6−		

In series A, Lac plates show 87.0% segregation. Of the segregants there was: 1.87% Lac+; 5.13% Mal−; 9.4% Gal−; 4.5% Ar−.
In series B, there was 88.5% segregation.

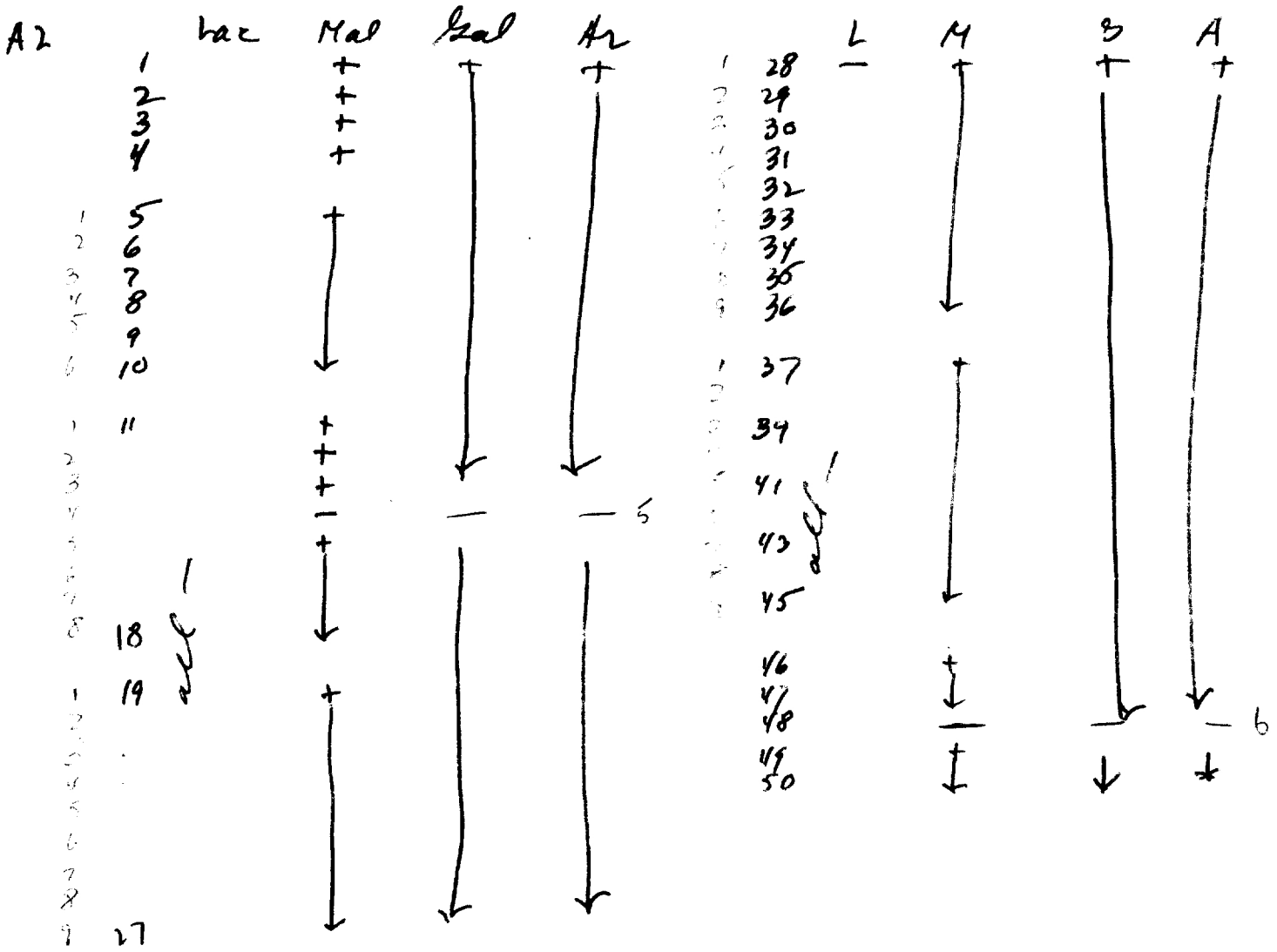
Pick Lac- at random and test:

A1.

	Lac	Mal	Gal	Ar	
1	−	+	+	+	
2	−	+	+	+	
3	−	+	+	+	
4	−	+	+	+	
5	−	−	−	−	1
6		+	+	+	
7			+		
8			+		
9			+		
10			+		
11			+		
12			+		
13			+		
14					
15					
16					
18					
17					
20					
21					
22		−	−	−	2
23		+			
24					
25					
26					
27					
28					
29					
30					
31		−	−	−	3
32		+	+	+	
33		+	+	+	
34		+	+	+	
35					
36					
37					
38					
39					
40					
41					

	Lac	Mal	Gal	Ar	
42		+	+	+	
43					
44					
45					
46					
47					
48		−?	+	+	4
49					
50					

42YAA


A2
Lac
Mal
Gal
Ar
L
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B
A
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8
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11
18
19
27
28
29
30
31
32
33
34
36
37
39
41
43
45
46
47
48
49
50
all 1
all 1
5
6

[illegible] throughout

All – hue tested are glucose-negative.

∴ W701 carries such a factor, probably introduced as Hal–.

Albert alone to invert + test inversions in Xyl and Ar.

	Lac	Mal	Gal	Ar.	
Bl.	−	+	+	+	46 tests.
except.					
Row B-6	−	−	−	−	
E 1	−	−	−	−	
G 2	−	−	−	−	
A4	−	(−?)	+	+	1, 1, 2
B2	−	+	+	+	49 tests.
except.					
A2	−	−	−	−	
A3	−	−	−	−	
B4	−	−	−	−	
B8	−	−	−	−	
C2	−	−	−	−	
E4	−	−	−	−	
E8	−	−	−	−	
B4	+ input	+	+	+	

Total: 195 tests of Lac−. 15 were Mal-Gal-Ar−
180 Mal+ Gal+ Ar+
2 were possibly Mal slow.
Retest these as 424-1 and 2
Recheck some of the ---- on glucose. There may be an epistatic−

Feb. 1, 1949.

H163B. Ar-: 11 tested. 10 are Lac- Gal- Ar- Mal- V_1^R

[#8 is Lac ± Gal + Ar + Mal +. Streaks out on Lac ± ' S
omit. Still Lac vary.

Nutritional tests on 1-7, 9-11.

February 1, 1911.

H 163A lac+ : 1-4
B 5
A Mal- 6-14
B 15-19 No 20
A Gal- 21-36
A Ar- 37-44
B " 45-46.

	Lac	Mal	Gal	Ar
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	—	—	—	—
7				
8				
9		—	—	
10				
11				
12				
13	all others —			
14				
15		—	—	
16				
17				
18				
19	—	—	—	—
—				

	Lac	Mal	Gal	Ar
21				
22				
23			—	—
24				
25				
26				
27				
28				
29				
30				
31				
32				
33			—	—
34				
35				
36				
37				
38				
39				
40				

W589 crosses.

425

January 28, 1949

(1) Y10 x W589
(2) W477 x "
(3) W677 x "

P30-31. (1): + colonies only. 20 picked for retest, all ++ Lac.
(2). 100 picked + streaked on EMB Lac Hold 2 for retest.
(3) " " All ++

425-1-2 on EMB Lac for retest.

January 28, 1949.

Streak out single colonies from Lac EMS of H 154 - 157.
8 colonies from each on Lac EMB

P30: Each shows Lac+ only! Reason from initial plates: Test 8 colonies from Lac EMS:

None of these show any signs of segregation as Lac EMB

Conclude: H154-7 are not heterozygous.

January 30, 1949.

Y-161 7 recs. on lac EMB UV. 30 plates ca 6 × 10⁸ = 18,000

12 recombinant lac- mutants (1 slow)

Test segregant from "sp." heterozygote for "H"

1/31/49.

W721×440 100 tested all Lac++. Retest #7+ : Lac+

~~Test for "4" in a spontaneous diploid zygote~~ 4/29.

W589 ×

Feb. 1, 1949.

~~W-721 x 440. nLac EMS~~
W477 x W589. nEMSlac

20% Lac+ colonies streaked out. No clearly Lac v.
Restreak 1-4 on Lac EMS for verification.

1A, B ++

2C, D are Lac v-; A+B are Lac ++. H173

3. ++

4. ++.

Streak out 4 cols. H173 nLac EMB; Take 1+ and 1- and test nutrition:

	BMTLB,	BMT, Ad	TLB, T, Ad	Σ.	V,	Lac
1A	+	—	+	+	S	+
1B	+	+	+	+	S	—
2A	+	+	+	+	S	—
2B	+	—	+	+	S	+
3A	+	+	+	+	S	—
3B	+	—	+	+	S	+
4A	+	+	+	+	S	—
4B.	+	—	+	+	S	+

Growth in Σ rather sparse in 1A, 2B, 3B, 4B; Very heavy in others.

Check from Σ tube on T1.

February 2, 1949

W ~~991~~ 251 (Lac_3-S+) x W478 (Lac+ "H") on GluEMS.

Majority are Glu+. High yield. Streaks out on EMB glucose.

100 tested on GluEMB. All Glu+

Chicks on $BaLac_3$ - ?) 431

2/2/49.

Rectuals stock cultures of Glu on EMB Glu. → Lac

1 W249 h.g.
2 386 small cols. v. slow +
3 387 compact
4 388 erythem.
5 389 slow +
6 423 minute cols
7 432 good −
8 433 " −
9 434 "
10 435 many +: − good size.
11 467 good size −.

chick plates of W434 and W435 on EMB Lac show papillae in their stools with lytic clearings around them!! See 437

chicks crossed: 2 plates each. x W108

a. W433: 0+/400.
b. 435: 0+/150
c. See p. 434. W467
d W432 0/300
e. 4434 No prototrophs. a few hundred microcolonies

Stocks for suppressor cross x. 432

Feb. 2, 1949.

~~Spread~~ S.O. W251 ($Lac_3 - Sl_3^{L+}$) on EMB/Glu to select for Lac_3+ revertants. Picks 8 papillae and restreaks to purify + and − colonies noted generally. One colony was noted which looked (*) as if it might be segregating. (Heterozygous?)

Picks from dark center and restreaks as 432-1.:

Picks pure + from ~~str~~ remainder and restreaks, for confirmation; on EMB-Mal.

432-1: mostly +. +few −. None could be identified as segregating and this will be true except for the most stable heterozygotes.

Feb 1 ff. 1949.

Inoculate Y10 into T(m)TLB, Lac and Glu. Thereafter loopful transfers in homologous medium.

B) A4.
C) ~~A5~~
D) P6
E) P8
F) A9
G) A10 → EMB Glu Lac
H) A11
I) A12 ←

etc.

W589 - Inhelia type

February 6, 1949.

A. W589 x W~~5~~466. 92 tested; Retest 6 for Lac v.

B. W589 x 471 100 tested. No Lac v!

A): 1-3 fairly certain Lac v; 4-5 ? 1-3 yield approx. proportions of Lac - prototrophs Cf. 429 where several Lac - tested were prototrophic

1, 3 are Lac v H-174 and 175 (A) (B)

Test nutrition of Lac + segregants:

A 1 + on BM TLB, i.e. Ad T-r +
2 "
3 "
B 1 "

8 additional A 8 Ad T-r + ~~+ T-r -~~?

8 " B. all Ad T-r +

These strains do not seem to be segregating nutr. req.

Suppressor tests

February 6, 1947.

W463

A. R21 w112 (+ Su.) x W461.

B. W108 " .

B. 5 plates, ca 200/plate. No +. ∴ Lac_3^-.

A. Picks + and streaks out on lac EM3

Feb. 5, 1944

Test Σ - segregants of H167 nutritionally to select for further "H" derivatives.

		W
1.	TL	[redacted]
2.	LB,	
3.	TLB,	735
4.	B,	
5	TL	736
6	TL	737
7	TL	738
8	TL	739
9	TL	740
10	TL	

Feb 6, 1949.

Inoculate Y10 on Y2 galactose + Y2 glucose Picks Ga and Gl colonies to

a) .1 ml M/1000 ONPG + .2 ml KP buffer 9/10 pH 7.5

b) .2 ml " ".

After 3 hours incubation, blue cells were — in both series; all but one was + from Gal series, I was rather weak. Streaked ↳ Justifying Method on Lac EMB as #436-1.

Mixture of Lac+ and Lac−!

2p phage.

Feb 7 ff. 1949.

See 435. Stock cultures of W435 and W435^{1} on Lac EMB, showed signs of plaque formation a central papilla.

Pick uncontaminated colonies, and spread as detector, pick papillae and streak out a) on W435^{5} (sensitive indicator) and b) Lac EMB for purification.

A 8.] Plaque formation clearly evident on 435^{5}.

Pick individual colonies from EMB and s.o., testing for phage also.

A9: All 8 cultures carry considerable phage (comparable to number of bacteria in collated streaks.

P9: Repeat

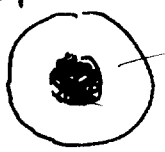
A10. Results in same sense. Conclude that these cultures are lysogenic on W435.

Stock, streaked, does not show this response.
W435

Filtered, heavy suspension of 437 "lysedans". Test on 410, W435:

Feb. 11, 1949.

1. N11: passage lysate in NSB from ~~single~~ plaques of 437-H on W435.
   437 mixture. | 5 further growth.
   No action of control plaque noted on Y10, although active on W435.
2. Continue single colony isolations from 1 and 2 (lac + and − resp.) testing lysogenicity concurrently: 8 single colonies from 1 and 2 and 1 each from 3-8 were all lysogenic!
3. Test nutrition of 437-(1-8).
   1-1 : TLB$_1$ 1-2 : TL(B,?) 2-1 : poor ++ ? 2-2 : do.
4. Typical appearance of a plaque is:



   In heavy regions of overgrowth, occasional clear spots are seen, possibly virus mutants? Picks and ~~cultivate~~ ~~437-1.~~ mass containing such clearings and spread: no clearings noted!

5. Tests of Phage sensitivity.

	T1	T2r	T3	T4	T5	T6	T7	C	
Y10	S	S	R	S	S	S	S	R	
438-1	S	S	R	S	S	S	S	R	
W435	S	S	R	S	S	S	S	R	O.K.

   stocks ng.

   ∴ the lysogenic derivative has same phage reactions as the sensitive and standard strains.

Feb. 12, 1948

look for Lac v among progeny of crosses of W167 segregants.

x 58-161

A. W-736

B. W-737

C. W-738

D. W-740.

A. ~~3~~ 56 tested. No Lac v.

B. 64 " No Lac v

C. 100 " No Lac v.

D. 16 " No Lac v

∴ Segregants of #167 do not carry Het.

Feb. 12, 1949.

W705 × W126. 15 plates. Ca. 50/plate = 750 colonies.

1, (mucoid), lac+ formed. 2.0. on EMB lac All mucoid.

Feb. 12, 1949.

W705 × W126. 15 plates. Ca. 50/plate = 750 colonies.

1, (mucoid), lac+ formed. 2.0. on EMB lac all mucoid.

seperation of λ from W153.

Feb. 12, 1949.

Harvest cells from EMB plate and suspend in 10 ml saline. Sediment and remove supernatant as λ. Filter most of the supernatant to remove bacteria. Keep a portion unfiltered, but substantially bacteria free. Wash sedimented cells with saline and resuspend.

A) Dilute cells 10^{-3} and plate out: a) on EMB Lac b) on W435 for λ determinations.

B). Dilute λ' ~~10^{-2}~~ 10^{-1}, 10^{-3} and 10^{-5} and plate as above 11 plaques

c) Titrate λ on W435.

D) Heat 1:10 dilution of Cells A at 56°C. 1 hour. Titrate cells and phage

A) Ca 50 colonies per plate on EMB Lac. 46, 38, 57

Plaques very difficult to count ~~3~~, 25, 17, 34

B) 10^{-5} 17 plaques; 7 bacterial colonies.

10^{-1} Almost continuous lysis + occasional colonies. Bacteria continuous

~~10^{-3}~~ 10^{-3}. Several hundred colonies; do. plaques.

c) No plaques at any dilutions

E) Test W753 as other coli strains:

34 single rods. 58-161

and 35 of K-12 tested by short stroke + .

pinpoint on plate spread ± W Y 35°. Each caused +

Most readily scored on strokes.

E. W435: Patchy lysis.

Y10	No plaques
Y40	"
ML	"
B/1	"
B/1,5	"
B/4	"

W435 seems to be unusually sensitive

Note: W753 is T-L- Lac ± Glu++.

W754 is (B)-M- Lac_3-. Where W753 could have come from is not clear; possibly the original source of λ.

For further study, use W754 as a marker strain.

Test R+S colonies from (B) above for lysogenicity on W435.

34 colonies tested, all carried λ.

Transfer of λ.

Feb. 13, 1949.

Inoculate W754 in Y2 galactose with a series of other λ-, 2 types to look for transfer of λ.

1. W754
2. 58-161
3. W-108
4. K-12

12 58-161 + W754
13 W108 "
14 K-12 "
15 W477 + "

On plates, test various cultures for sensitivity + content of λ.

Cannei → / Host ↓	W754	K-12	Y10
W435	L	L?	L?
K-12	O	O	O
W108	O	O	O
W467	O	O	O

Conceivably, K-12 cannis λ and W435 is a mutation sensitive to it! See 443.

An important problem now is to devise best methods for scoring λ and obtaining it free from bacteria. (recover)

1. Show that λ is transmissible. Mix K-12 and W435 and streak out. Test Lac- colonies for λ, testing for its transfer.

2. Rapid methods of testing susceptibility + infection:

   a. Try cross streaking

   b. Spray disturbed colonies with suspension of λ^s.

February 15, 1949.

Test K-12, W753 + W754 for λ by shielded W435 streak + W435 old culture susc, in N/34. Also plate ca 150 K12 on W754 + mmi with batting.

1) Plate: K-12 : no plaques; W754: 1 hazy plaque

2) Streakout: K-12: a few plaques noted in both sets of streaks. Rather more are found in 753 + 754. ∴ K-12 is lysogenic.

W435 is a susceptible mutant, and W753, etc. are mostly standards carrying λ.

Cross-streak tests for λ. 444.

Feb. 15, 1949

Lay down heavy streaks of W~~5~~754 (for λ) and 435 (for λ^5) Cross streak ē each other; K-12 etc. on EMB Lac

	NSA v. 754	NSA v. 435	EMB Lac v. 754	EMB Lac v. 435
1. ↻	λ +++	λ +++	++	+++
2. K-12	−	λ ++	−	++
3. SP-161	−	λ +	−	++
4. W478	? ±	±	−	++
5. W477	λ ++	++ ?	Patchy. −	+
6. W108	−	++	−	+
7. W595	−	++.	−	+.

Patchy appearance along entire streak

Cross-streaks are very difficult to read. However, lac + λ on lac-S, or EMB lac are not so bad.

(N7) Lysate from penicillin treatment of K-12, 10^7/ml initially, filtrate. 2/17/49. .1ml had no λ, was sterile.

Transfer of λ

2/15/49.

Grow K12 with W435 for 8 hours in Y2 broth. Plate out on EMBlac.

(179+ : 8−) = ca 5% − .

A) Pick − and test for λ on W435 EMBlac plates. Keep in order.

This test was inconclusive. Repick 16 and streak out on 435 film. 13 were apparently ~~sensitive~~ non λ. 3, all of which had a lac+ component, showed λ. Pick + and − from each of these for retest. Pick others to test sensitivity (445a). K-12 controls had λ; W435 did not.

#14.	All+ cols.,	λ+ and prototrophic.	No transfn.
#16.	2− cols.,	λ−; lac+ was λ+.	" .
#11.	2− "	λ−; 2+ " "	" .

14 other − cols which were λ − were tested and all found sensitive to λ

B). W756, λ+ streaked out on W435. λ developed. Pick from confluent area to find lac −. Streak out on EMBlac. Only 1 lac − colony found. Streak out ~~#~~ 2/5 W435 background. (not well isolated)

On lac EMB − Pure lac − ; on W435, lysogenic. Therefore transfer of λ can occur under these conditions. Induced λ strain is W767 Check institution. 4 colonies all H− like W435. See 445a.

c) Sensitivity tests in A were done with λ from K-12. Streak out zones of lysis to find λ+ lac − (for evidence of transfn. Do. ∈ 448).

Transfer of λ.
Retest original λ⁻ strains.

4/5/9.

2/20/49.

B). All 4 s.c.i. of W767 agree in Glu-, M-, and λ+. All are pure from sup⁰ as tests of induced lysogenicity.

C. Many plates were primarily Lac- with patchy lysis, and lysis at intersection of mutant colonies. Picks Lac- colonies to test for λ+.

	λ.	Autolysis
a.	1-3 λ+	1-3 −
	4 λ−	4 +
b.	1 +	1 −
	2 −	2 −
b'	1-2 +	1-2; −
c	2-3 +	1-4; −
	1, 4 −	
d.	1-4; +	−
e	1-2; +	1+; 2−
e'	1-2; +	1-2; −
f	1-2 −	1-2 −
f'	1-2 +	1+ 2−
g	+, +	−, −
g'	+, +	−, +

W	λ	Autol.
432	+	−
433	+	−
434	−	−
435.	−	−

W434+ 435 are, therefore, merely λ⁵−. Their sensitivity was detected presumably as a result of mixture or contam. with W753, possibly related to W108.

1 clear plaque noted. Picks as possible virus mutant and streaked on λ− and λ+

Autolysis probably indicates a sensitive strain which has phage mixed with it. ~~In most cases~~, the autolysis and lysogenicity are comparable, consistent with this picture.

UV - Lac Mutationsum.

446

2/16/49.

1) W760 43pl x ca 50 / 2500. colonies. Very high yield of mutants apparent.

2) W758. 40pl. ca 5% / 2000 cols.

1) 5 -- W768-772
   2 ± W773-774
   3 slow W775-777.

2). 5 - W778-782.

	Lac	Mal	Glu	Ina	Gal	
W 768	−	−	−		−	Hex -
9	−	+	+		+	Lac -
770	−	−	−		±	(108)
1	−	+	+		+	Lac
2	−	+	+		+	Lac
3	±	÷	−		±	(108')
4	±	÷	−		±	(108')
5	±	+	+		+	slow lac
6	±	+	+		+	slow lac
7	±	+	+		+	"
8	−	+	+		+	Lac
9	−	+	+		+	Lac
780	±	+	+		−	Gal - slow lac
1	−	±	±	+	+	(108'?)
2	−	+	+	+	+	Lac

Lac_3 suppressors.

2/18/49.

Streak out W467 on EMB lactose. Restreak, and pick lac+ colonies to EMB Mal + Glu.

27 tested on Glu + Mal. 3 Glu − Lac+. Alternate all ++. Later, 33 tested on Mal. All +.

Purify the Glu - Lac+ on EMB Lac. All were Mal - W764-766.

Inoculate W766 on Glu EMB to recover revertants.

2/21/49. Inoculate W768 on Glu, etc. media to find specific reversions. Maltose: slow +. Lactose full +'s. Nothing on gal., Mtl or glucose

2/23. Collect W677 lac+. Check on other sugars. W814

3/1. Test 1 Mal+, 8 lac+ purified from homozygous plates.

	Glu	Mal	Mtl	Gal	Lac	
1	− −	+ ++	− −	− +	++	W815
2	− −	+ ++	− −	− +	++	
3	− −	+ ++	− −	− +	++	
4	− −	− −	− −	− +	± +	W816
5	− −	+ ++	− −	− +	++	
6	− −	+ ++	− −	− +	++	
7	− −	+ ++	− −	± +	++	W817
8	− −	+ ++	− −	− ++	++	

not spec. lac response! Save 1, 4, 7. as W815-817

3/9/49.

A set of "Hal+" colonies was tested on 4 sugars. Many undoubtedly subs.

Types.	Mal	Lac	Gal	Glu	W
1	+	−	−		856
2	+	++	++		857
3	++				856

3/2/49.

5 Gal+ isolated and tested:

	Gal	Lac	Glu	Mal	Mtl	
1	+	—	—	+?	—	840
2	+	+	—	+	—	
3	+	+	—	+	—	
4	+	+	—	+	—	
5	+	+	—	+	—	841

Fermentation of Gal is sluggish; Mal and Lac slow.
Picks as W839 + 840

Routine tests for λ.

2/18/49.

Preliminary tests have shown λ in K-12 and a number of derivatives. Retest + check by streaking out on EMB 5 sugar, (for autolysis) and on W435.

		λ On W435.	Autolysis.
1.	K-12	+	—
2.	W754	+	—
3.	58-161	+	—
4	Y40	+	—
5	Y87	+	—
6	Y70	+	—
7	W677	+	—
8	W70~~81~~	+	—
9	W45	+	—
10	Y10	+	—
11	~~Y83~~ Y77	+	—
12	~~Y44~~ W	+	—
13	W~~58~~ 125	+	—
14	W680	+	—
15	~~W677~~	+	—
16	W478	+	—
17	W467	+	—
18	W108	+	—
19	W145	+	—
20	W126	+	—
21			

∴ Most standard strains still carry λ and are resistant to it.

λ- sensitivity in W518 "A" and "B" 448

2/18/49.

EML noted that W518 A + B were lysed by Y70. W518 itself, when streaked out was autolytic, suggesting a mixture of λ- and λ+.

1. Streak out W518 on EMB Lac
2. Test A + B for lysis of each other, of W435, and by K-12.

Cann. → / Mat ↓	A	B	435	K12
W435	λ-	λ-	λ-	λ+
A	-	-	-	+
B	-	-	-	+

∴ 518 A + B show same pattern of sensitivity as W435 and are λ^S, λ-

Test for transfer of λ from K-12 to 518 A + B. [Streak out plaques to find λ+ Lac-]. Cis λ/518.

Mostly + colonies. Some - had plaques. Pick clear Lac- colonies + streak out on EMB Lac; EMB W435.

A). ~~1 + 2 + 3 + 4~~ 1-4 λ+ (3 had 1 plaque), 1; 3 are autolytic. Use (2).

B. 1-4 all λ+ no autolysis. 3 has papillae, probably not pure -.
Use #1.

C.)(W518 λ+). /435 /auto.
1 - (1 pl.)
2 -
3 - no growth -
4 ++ - → → W518 λ+

λ− x λ+.

2/18/49.

Y10 x W435

8 colonies found in 15 plates. Very low yield! All lac+

Streak out on Lac EMB. Use 2 isolates colonies per isolate, to gave (A-D)(1-4).

		/435	/auto.
A	1	+	−
	2	+	−
	3	+	−
	4	+	−
B	1	+	−
	2	+	−
	3	+	−
	4	+	−
C			
	1	+	−
	2	+	−
	3	+	−
	4	+	−
D	1	−?	−
	2	+	−
	3	+	−
	4	+	−

Retest D1:

D1/518 No plaques.

Y10/D1 Some questionable plaques.

Y10/518 Numerous plaques ± central papilla

D1.

Retest: was sensitive to λ. Recheck 4/7/49.

Sensitive to λ. λ−

2/23/49. Test 19 segregants from 10 mosaics of Y1/176 (W518 x W538) for λ.

		/518	auto.
A	1	+	−
	2	+	−
	3	+	−
	4	+	−
B		+	−
		+	−
		+	−
		+	−
C		+	−
		+	−
		+	−
		+	−
D	1		
	2	+	−
	3	+	−
	4	+	−
E		+	−
		+	−
		+	−
		+	−

Attempts to obtain free λ

2/19/49.

A. Scrape area of lysis of W756/W435⁻ into H_2O. Sediment and filter supernatant (sintered glass).

B. Extract 100mg dried K-12 ~ 10ml H_2O. Sediment 1:10 dil. and filter supernatant. B' is test on sediment. 9 colonies K12/1ml (noted. Numerous others cont. (from water?))

C. Inoculate Y2 ~ W435 and K-12, young cultures. Shake + incubate ca 2-3 hrs. Sediment and filter.

C' Let grow overnight and filter.

A). .1ml: ~~80~~ plaques on ~~W758~~A.W435⁻ on EM13-s. ✓
A hopeful streaked out was similarly effective.

B. No plaques.

B' 2 plaques in hopeful, probably from K-12.

C. No plaques in .1ml. 1/plate; 2 plaques in another.

Free phage from A) only.

C': ca 500 plaques/.1ml i.e. titer of ca 5000.

Synergisms in Lac tests
Lac_1 .... Lac_7.

450

2/19/49.

1. [redacted] W112 — Cross streaks on EMB Lac.
2. W45
3. W108
4. W126
5. W145
6. W125
7. W133.

	1	2	3	4	5	6	7
1	−	−	−	−	−	+++	−
2	−	−	−	−	−	++	−
3	−	−	−	−	−	−	−
4	−	−	−	−	−	+	−
5	−	−	−	−	−	+	−
6	++	++	++	++	++	±	+++
7	−	−	−	−	−	+++	−

At 24 hours, Lac_6 – reacted regularly. Its isolated response was irregular, sometimes ++, sometimes −! Needs study in liquid medium!

Hold for 48 hr. Rdg! : No change Lac_6 shows most interesting interactions

Segregation of M179. 460

2/26/49.

M179 is W126 x W778. (TLB, Lac$_4$− x IV-M18t Lac$_3$−).

Streak out 4 original var. cols. on Lac EMB. Practically no pure +. Purify 1 - from each. Pick + centres for new segregations Test nutrition of 6 additional - segregants from diffrent Lac v Also streak out 16 additional v colonies.

d −−
D guarm - H only.
c ++ (weak on - T)
a −−
1 −−
2 ++
3 −−
4 −−
5 −−
6 TL

nutrition diff. to establish. Probably IV requirement interferes.

2/20/49.

Plate K-12 and W435 heavily together on EMB + NSA, to look for clear plaques of λ'.

All-defined nothing but no clear plaques seen in 8 plates.

2/21. 445g. Clear plaque. Streak out on W435 and on K-12, also seeing a turbid plaque and λ 450c'.

	W435	K12
1. Cl. pl.	+	-
2. Turb pl	+	-
3. λ	+	-

"Clear plaque" was probably purely ~~phage~~ free phage particle-initiated. Free λ gives essentially the same picture. Lysogenicity in this system is not evoked as readily as in Burnet's.

However on 2d day, lytic zone was clear, not hazy, and individual (resistant ?) colonies were noted. Pick to NS 3 + 518 to grow out this phage, and streak out the "resistants".

Purify 8 cultures and test for sensitivity. All but #5 are λ- and λ^r as determined with W435 and pure λ. #5 is λ^s.

Keep as ~~W8??~~ for #1 (Lac -) and ~~W8??~~ for #6 (Lac +, reversion)

W- W-

~~48~~

Y516

3/5/49.

E14B Lac —
E14B —
E14S Lac —
E14S —
T(0)
Y2 Smith
Pennsylvania
NSA

Steaks out single plaques from λ' on W518. 4 tested.

a). All gave clear plaques on W518

b) All gave no plaques on W811 (518 λ+)

c) When streaked out alone, all were ~~c~~ virulent, with a few resistant colonies.

3/5. Test c) resistants for lysogenicity on W518 16 tests.

		W518	Aut
A	1	−	−
	2	+++	+++
	3	+	+
	4	−	−
B	1	++	−
	2	−	−
	3	++	−
	4	++	+?
C	1	++	++
	2	−	− ±?
	3	−	−
	4	++	++
D	1	−	−
	2	−	−
	3	−	−
	4	+++	+++

Possible exceptions to "no lysogenicity with λ'". Should be rechecked.

Plaques of λ' are certainly clearer, and may bespeak a less frequent development of lysogenicity.

Check on B1 and B3:

B1 λ, rather small, clear plaques.

B3: larger plaques, some filled heavily or with granular overgrowth.

Keep ~~B3~~ B1 as W-855

New spontaneous intergrades

2/20/49.

1. W-126 x W705
2. " x W706
3. " x W707.

Lac⁻ heterozygote "Het"

2/20/49.

A } ~~W770 x W477~~

B } ~~W477 x W769~~

~~C. W770 x W769~~

W769 x W478.

C

Lac⁻ Lac⁺

W769 x W478 (BM).

W769 x 477 (TB, Lac, -). No Yield.

A+B } 100 tested on Lac EMB for Lac V. 2 Lac V, ~~[redacted]~~ H177-178

Purify on Lac EMS.

56 add'l tested: No Lac V. 1?

February 20, 1949.

W126 x

1. W 769
2. 771 — 2+ Not Lac ±
3. 772
4. 778 — 4+. 3++ 1 Lac ± (H 179)
5. ~~779~~
6. 782 — ca. 50% + 2+ Not Lac ±
7. W770 x W677 — 770 ♂ : ca 10% recurrent.

49 tested. No Lac

Stocks for Lac reversion in heterozygotes

Febr. 28, 1949

A W478 x W660 on Xyl EMS.

B. x 677

p23. Yields very few 1 + col. from B. Not Xyl v

10 from B. 1++ 9 mixture. Recisolate

3/1. Repeat W478 x W660. on EMS Lac + Xyl.

a) Picked + test 16 Xyl+ for Xyl v. 6 likely heterozygotes. (1-6).

Retest on Xyl EMS and Lac EMB.

	Xyl EMB	Lac EMB	Xyl EMS.
1	v	−	H189
2	+, − v?	+, −	
3	v	−	
4	v	v	
5	+, −	v	
6	++	−	

#1 3 6 are suitable for reversion studies of Lac.

B) 3/3. 6 y added'l tested on Xyl EMB Mass purified +/−; 6 Xyl v.

48 " " " Lac ". 8 likely Lac v

vm Lac EMB		
1	+, −	= X 11
2	−, +	X 12 } do not keep.
3	−, +	X 13 }
4	−, +	X 14
5	−	X 15 Keep. H190
6	−, +	X 16

Recorded as Gal v.

Later tests show Gal+!

Lac = Xyl v heterozygotes.

3/6/49.

W478 x W660 (Lac, Xyl, Mal, Ar, Mtl).

Remember in X and L series.

X 1-6. An Xyl EMS.

H-189 1. Growth ok; numerous - eswellas + colonies. Picks +'s to Lac EMS, Xyl EMS Xyl EMS.

2. No isolated colonies. Heavy growth in streak. 1 or 2 "papillae" in streak. Picks to EMS.

3. Good growth. 1 poorly isolated Xyl+ S.O.

4. Lilac 1 Lac v+ Xyl v-

5. Freq. + colo; - background

6. do.

H189 is Xyl v (except #4 of 6 isolates). ε - pseudom.

Do. H190 (except #2 of 4 isolates).

455L series. test on other sugars. (Mal, Arab, Xyl, Mtl).

	Lac	Mal	Arab	Xyl	Mtl	
1	v	—	++	—	—	
2	++	—	++	—	—	
3	v ✓	++	++	v+ *	+	? shows patterning
4	v ✓	++	v? s	+ —	+	
5	v	—	+	v	v+	
6	v *	—	+ s	v * - +	v -	* many pure +
7	v *	—	v? +	v	v	
8	v ✓	-	++	—	—	

Arab can scarcely be scored. Note correlation of Mtl with Mal.

~~Segregation of H180-181.~~ New heterozygotes

2/22/49.

W588 × W769. ~~Hayick~~ 5 tested. 2 were lac v!

~~H~~ H180-181.

2/25 Repeat W588 × W769 [should be het. for lac^{769}, V, ^{IP}, IV, Arg., TLB, ...]

3/1 100 tested. (4/plate 25 plates: 19 plates had 1; 3 had 2; 13 had no lac v. 18 altogether).

Many of these appear to be "bullseye" colonies

See 464 for segr. of W180 + 181.

→ 18 retested from Lac EMS. 2+ colonies from each.

All but #13 are clearly lac v. Preserved ☐ on Lac EMS Save momentarily.

At 48 hours:

1 Mostly sectorial; 1 bullseye (change of type? Streak out!)
2 Empty Sectorial; 1 ~~bullseye~~ annular. [both yield both]
3 Annular
4 " (almost pinpoint)
5 Sectorial; — pseudo.
6 ~~Sectorial~~, almost all bullseye (Annular)
7 Sectorial
8 " and bullseye nearly equal nos.
9 Sect. + pseud
10 Sectorial
11 Annular (large); occ. sectorial
12 Sectorial
— ++
14 Sectorial, very complex
15 Sectorial, some very simple
16 Sectorial
17 Sectorial, — pseudo
18 Sectorial

2/22/49.

Pick growth in center of plaques of λ (450 c') on W435 and streak out on EMB Lac.

A23. Mostly + colonies (occasional of W435 previously noted). Test for lysogenicity on W435 and auto. (16 colonies; 2 from one plaque)

		Lac	λ/435	auto.
A	1	—	—	—
	2	+	—	—
	3	+	±	±
	4	—	++	—
B	1	+	—	—
	2	—	—	—
	3	+	+	—
	4	+	—	—
C	1	+	++	—
	2	+	++	—
	3	+	—	—
	4	+, —	++	—
D	1	+	++	—
	2	+	++	—
	3	+	++	—
	4	+	++	—

∴ 16 trials, 9 lysogenic cultures isolated from plaques of λ/W435. Maintain A4 to test for persistence of λ.

New phages for 2 study

2/22/49.

Broz. NSA ē W518 and 1 ml sewage filtrate, overnight. Streak out unfiltered lysate on W518.

A23 Pick plaques to water. 1-7 large 8-28 small and very small. ~~Pick to~~ Streak these on W518 and on Y10 / EH13. to find any φ ē differential activity

Pick plaques of #2, 7, 8 13 and 20 to Penassay and add droplet of W518.

P213.

	W518	Y10
	~~++ M~~	
1	~~++ M,S~~	++ M,>
largest 2	++ M	++ M
3	++ M	±± M,S
4	++ M	++ M
5	++ ML	++ M
6	+ ML	+ M
7	++ ML	++ M
8	+ MS	+ S
9		
10		
11		
12		
13	+ M	± S
14	+ M	+ S
15	+ M	+ S
16	+ M	+ S
17	+ M	± S
18	+ M	+ S
19	++ M	+ S
20	+ M	± S
21	+ M	± S
22	+ M,S	++ S
23	± M	—
24	+ M	± S
25	+ M	S
26	+ M	++ S
27	+ M	S
28	± M	± M

maybe a difference.

Dif.

3/1/49. Cross test 12 and 120

φ	518/12	120
2	R	S
7	R	S
8	S?	R
13	S?	R
20	S?	R

Make 518/20 /2.

λ - specific phages. 458a

2/20/49.

A). Lysates of 458-2, 7, 8, 13 + 20. Last three were completely clear overnight; 2 + 7 were fully grown + had to be sedimented before filtration. Steile filter (sintered glass).

B) Picks 8 plaques each from 13 and 20 to Y10 and W518.

		Y10	518		Y10	518
13	1	28 M	8 M	20		
	2	11 M	23 M			
	3	20 M	16 M			
	4	0	0			
	5					
	6					
	7					
	8					

same plaques as in 518 but smaller on Y10. No absolute difference

4/5/49. Test strains against 458-2, 1 and 458-20.

	2	20
"518/2/20"	S	R
"518/20/2"	R	R
518	S	S
B/1,5	S±	R
Y10	S	S ±

20 resembles Bordet small in pattern.

518/20/2 is suitable for selection of additional 2. Plate with Raw filtered sewage. 5 plaques seen. Streak out on 518, 811.

Checks new phages

458c

3/5/49.

From Hershey.

	B/1,5	W811	W518
T16	++++	++++	+++
Bordet large	+—	—	—
" small	—	++++	+++
ϕ10-174	—	—	—
C36	++++	++++	+++
~~Luria's~~ (SP13)		++++	++++

Hershey sent 3/2/49.

Sp10 (not on B/6 or B/1) acc. Hershey. (same host range as T1).

Sp11 Not related to T. N. G. on B.

(acts on H, not B).

Sp12 all coli.

~~Sp13~~ (= C36)

These phages evidently do not differentiate between λ- and λ+.

Bordet large and ϕ 10-174 may be related to T1 and T5.

Bordet small does not attack B/1,5 although it is active on K/1,5.

Mold for lysogenicity tests.

1. Plaques very hazy, clear, regular centers; opaque margins
2. moderate plaques, "Resistents: a few papillae in background Some lact!
4. moderate-large; sharp borders. " a single pop. " ".
3. v. large plaques, spreading lysis.
5. large and small plaques. Restricts. small + large both → large

Streak out 518/— above for lysogenicity tests.

C36: no resistents! Test 2 colo./water suspension. Streak on sens. basis

Lysogenicity tests on new phages. 458d.

March 7, 1949.

Test newly received and isolated phages for the induction of lysogenicity in W518, by simple streaks over sensitive smear.

	1c36.	5 tests	None lytic
	1T16	5 tests	None lytic
1Sp17	~~1Sp17~~	3 tests	None lytic
	1Sp14	5 tests	Each lytic: lysogenicity? or carryover.

Streak out bacteria of 1Sp14 and retest lysogenicity. 458d1 and d2
Lysogenicity confirmed. Sp14 is, then, ~~Sp~~ lambda -2.

Sp15	4 tests	all λ-
Sp14	1 test	λ-

518/13 No stable resistants
518/18 6 colonies streaked out and isolates tested on W518:
None lytic

Test Hershey's Phages:

2/24/49.

	1 HP21	2 HP13	3 HP15	4 HP18	5 HP20	6 HP22	7 HP23	8) Sp 1	
SW36	L ++	++ S	++ ML	++ M	- hazy? small	M ++	S ++	—	bacteriogenic.
Y10	—	—	—	—	—	—	—	a few hazy plaques.	
W518	—	—	papillae! —	—	—	—	—	a " **	
SY20	—	—	—	—	—	—	—	+ *	
SY21	+	+	+	+	+	+	? +	?	hard to thin
SY23	—	—	—	—	—	—	—	—	
SY61	—	—	—	—	—	—	—	—	Lac+!!
SY83	very large ++	++	++	++	hazy ++ capture plaques	++ small.	++ sm. pl.	—	

very large plaques here!

* Hazy confluent lysis with a few clear plaques. (Reduced lysogenicity?)

** Several large plaques with hazy borders, and medium small sharp bordered. Y10 is similar, only scaled down.

SY23 all- maybe doubtful as it was spread very thin.

The most distinctive phages here seem to be #5 (probably inducing lysogenicity), #1, very large plaques, and #7, very small plaques.

Also Sp-1 which acts on K-12. clear plaques should be picked to purify.

λ and T1-T7.

2/28/49.

Plate W518 and T1-T7 on Lac EMB.

T1. Ca 10^2 plaques, noted, probably of λ, as W518 is V_1^R
T5 Ca 40 " " " " ".

∴ lysates of K12 contain λ as well as specific phage.

T2h. Confluent lysis and ca 300. resistant colonies. Some are smaller + smooth, others larger + rough.

T3. 6 very large plaques (ca 1 cm.) and 10^2 λ.

T4. Complete lysis ca 100 resistants, a few mucoid. Very small cols.

T6 Ca 400 "

T7 ca 500 ". Many nibbled or suicidal.

W435/T1. ca 100 resistants
T5. ca 10-15% mucoid.

518: 458-2 Nearly confluent lysis. ca 10^3 resistants (large plaques).
458-20. Complete. Most of 10^{2-3} survivors very rough.

Heat sensitivity of bacterial λ.

2/28/99.

A. Titrate out unheated W811 on EMB and C WS18.

B. Heat aliquot at 56° 1 hour and titrate for bacteria + λ.
Bacteria sterile. (No colonies at 10^{-1} ml).
No plaques seen at 10^{-3}

Ca 100 colonies. Only 7 plaques (1 confluent group included as 1)
The plates used were very wet + plaques may have smeared.

Segregation of H180-181 (W588 × W769)

March 1, 1949.

H181. 4/ Lac U streaked out. Lac− is very predominant

H180 12 Lac U so. − pred. also, not so markedly.

Test for V_1^R = .

Test 9 or 10 cols. from each of 10 mosaics.

	Lac−V_1^S	Lac+V_1^R	Lac−V_1^R	Lac+V_1^S
1	8	2	0	
2	2	7	1	
3	5	5	0	
4	5	4	1	
5	7	3	0	
6	4	5	1	
7	7	3	0	
8	5	3	1	
9	5	4	1	
10	4	1	5	
	52	37	10	0

The proportion of Lac+ is probably exaggerated due to bias in attempt to sample this fraction. It is clear that the −R crossover is more frequent than the +S, although it is difficult to say how representative a sample this is. Certainly, the crossovers are not randomly distributed. (4^0; 5^1; 1^5)!

T1- T7 lysogenicity.

March 2, 1948

Picks colonies streaked out from WY35/— or W518/— and test for lysogenicity on Y70., and control alone on EMB.

WY35/T1. A, B. 43 tested. None lysogenic.

W518/T2h. A, B 43 tested " ".

W518/T4. 20 tested. Most did not observe on control plates!
#20 showed ca 40 plaques on Y70; 2 colonies. Pick these colonies and recheck: not lysogenic

1458-2. 10 tested No lys. only 2 grew on control.

1458-20 7 tested No lys. 3 grew.

1T T. 6 tested No lys. 4 grew

W588/6. 54 tested. all grew No lys. 1 doubtful (#54; recheck). not lysogenic

435/5. 55 " " No lys.

Attempts to remove λ by ultra-violet

3/3/49.

29 W811 picked from UV irradiation ~~on~~ EMB plate. Tested on W518 for λ. all +.

12 addnl. All λ+

3/2/49. 60 tested. All λ+

101 tested λ+.

3/5/49. Test 100 each of u-v treated W826 and W828, from Lac EMB plates in a mutation run.

828, #6 ~~maybe λ—~~, 94,
826, #13, 16, 27, ~~59~~, 64,

6/200 = ~~3%~~ 3.5%

Reisolate and recheck.

I did not grow in 826 series. Check others by using as basis for W811 streak.
These cultures are not susceptible to W811 λ. Reisolate their lysogenicity — lost in course ??

March 6, 1949.

20 plates x 400 cols = 8,000 each. W826 and W828 4 UV 1 second on Lac EMB.

(See 466 for λ tests)

1–6	W826	→ W847-852
7-8	W828	W853-854.

W847 is lactose –, very like W768

W852 is very slow, not – on lactose.

Zelle's single cell isolates

March 5, 1949.
Slants, Lac- segregants.

	Lac EMB	Xyl EMB	TS
47	—	++	S
48	—	++	S
99	—	++	S
100	—	++	S
101	—	++	S
102	—	++	S

A51 +, −, ± ++ (some −?) A51 seems to be pure Xyl+ but Lac ± !

on Lac EMB, A51 gave + : − ca 2-3:1. 1 mosaic noted.
streak this out on EMS, EMB Lac and EMB Xyl.

A51, A53, A77, A78, A219-222 are all Xyl++, Lac+ and − or ±.
Are these from H-72 ??

H72, from slant is Lac ± Xyl +. ∴ these isolates are from a different heterozygote sent Zelle in error. (Air Mail Letter)
Rechecks from older Lac EMS plates.

3 plates marked H72 were found in refrigerator
"A" is verified as H72 (Xyl ± Lac ±)
B did not grow out
C is like slant. (probably H62)

Absorption of λ.

5/7/49.

Heat both cultures of W518 W811 (→ K.) and λ 1:5 at 56°, 90mins.

A. Titrate λ at 10^{-2}. ($\frac{1}{11} \times \frac{1}{10}$).
B. Test W518K and W811 for sterility
C. Test W811K for free λ (multiply by 5 to compare with A.)
D. Test heated λ for inactivation

E. Add .1ml λ to 1ml W518K. ~~At 10 mins., dilute to 10ml and~~ At 16 mins., assay .1 ml on W518. Do in triplicate.

F do. using W811 K.

3/8. A. 114, 112, 119

B. Both sterile (.1ml.) ✓ at 48h.

C 34 41 plaques! Some λ survives within W~~8~~811 and can be cleared!

D. No plaques at 10^{-1}, 10^{-2}

E Numerous plaques. 81, 126, 144, 152. m̄ = 126

F. Numerous plaques. 146, 127, 159, 158 m̄ = 147

No evidence of absorption.

Note that some plaques are mottled, with clearer patches

Repeat C+D.

C. 26 plaques. (i.e. ca 300 λ/ml ~~resist~~ survive heating of W811)

D. No plaques.

(Repeat C+D)

3/9/49.

Sediment suspension of heated W811 used in W470 to locate λ as free or in cells.

Cells	25
Supernatant	11.

Reversible absorption is indicated, even from heat killed cells!

Segregation of H-72

3/10/49

1. Test with 8 and some H72' for T5, T1 sens.

	Lac	T1	T5	V_1^c	V_1
W478	+	P	S^P	R	S
1	−	P	S^P	R	S
2	−	R	R	—	R
3	−	R^P	P	R	S
4	−	R	P	R	S
5	+	R	R	—	R
6	+	R	R	−	R

(V_{1c}^R reaction)

The coupling is probably Lac− V_1^R ; Lac+ V_1^R , so that X had occurred prior to the establishment of the heterozygote.

Reduction segregation of H168 (4176)

3/10/49.

		Lac	Mtl
165	1	V	V+
166	2	V	V+
167	3	V	V−
H168	4	V	V−
169	5	+	−
170	6	V	V+
171	7	V+	−
172	8	V+	V−

→ Choice for crossover studies.

Pick four Lac− and 4 Lac+ ~~and~~ from H168 and test nutrition and φ.

	Lac	T1	T5	V_1	V_{1c}	Nutr.	Xyl	Mtl	Gal	
1	−	P	S	S	R	TB_1	−	−	S	
2	−	R	R	R	−	B_1	−	−	S	
3	−	P	S	S	R	B_1	−	−	S	
4	−	R	R	R	−	TLB_1	−	−	S	Parental!
5	+	P	S	S	R	TB_1	−	−	+	
6	+	P	S	S	R	+	−	−	+	(test for Het).
7	+	P	S	S	R	TB_1	−	−	+	
8	+	P	S	S	R	TB_1	−	−	+	
W677	−	R	R	R	S	TLB_1	−	−	S	
W478	+	P	S	S	R	BM	+	+	+	

seems to be predominantly B_1− L+ M+ V_1^S Lac−. Bacterial samples tan as Lac+.

Parental configurations were T−L−B_1−M+V_1^R Lac− ....

3/11/49.

See 4584.

When the cultures of 518/14 were first grossly tested for lysogenicity, they lysed W518. However, when streaked out, no lysis ensued from single colonies thus purified.

Repeat isolations of 518/14 and 811/14.

A from lysed area.
B from "halo"

			λ	Autol.
811 ~~518~~.	A. Gross streak		+	−
	Single colonies.	1	+	+
		2	+(3 plaques)	−
		3	−	−
		4	−	−
	B.	1	−	−
		2	−	−
		3	−	−
		4	±	±
518.	A Gross		+	+ −
		1	−	−
		2	−	−
		3	−	−
		4	−	−
	B	1	−	−
		2	−	−
		3	+++	−
		4	−	−

Picks 518B3 and rechecks for lysogenicity. When streaked out → 518, a considerable content of λ was indicated. Test single colonies and gross streaks.

3/13/49

None of the 12 single colonies tested showed lysis, but gross streak lysed W518.

Restreak and test on W518. Also, inoculate broth & gross streak. assay now + after growth for λ_2.

Test 3 single colonies and W518 as ✓ for sensitivity to sp 14.

Recheck lysogenicity:

1, 2 + 3 were not lysogenic on W518; sp 14 control lysed.

When tested for sensitivity to sp 14, there was no lysis or plaque formation, but the area spread showed the same increased opacity as seen in the margins of halos from sp 14 plaques.

Initial. At a 10^{-6} dilution: 127 bacterial colonies. 23 plaques.

∴ probably each bacterium does not carry the phage.

At 10^{-2} dilution there was confluent lysis of the background and granular overgrowth.

At 10^{-4} there were about 10^3 confounded plaques.

Final: supernatant inadvertently discarded. Plate out washed bacteria.

λ_2 can grow on W811, but many of the bacteria are readily disinfected. Test a series of sci from the 10^6 dilution plate for λ^+ and λ^s. Also maintain #1 above for further study as W874

3/25/49

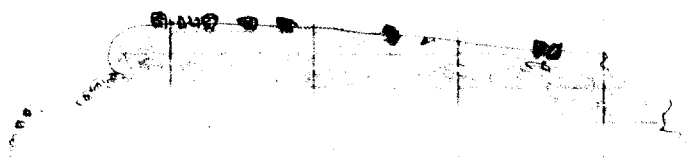
Test 70 s.c. from 473 a plating on W518 for λ_L.
None were lytic. 70 tested were resistant to λ_2.

This fluid gross streak showed λ, but not as markedly as the previous.

Restreak the streaks $\bar{c}$ and $\bar{3}$ W518 made layers.

Plating of culture from 473a gave ca 300-500 bacteria; just 1 plaque. This does not correspond to any growths.

Note 473(2) which had an appreciable amount of λ showed a light background growth with heavy growths. The background is probably responsible for only a part of the lysis for λ.



Type A: opaque outgrowths; B: more manoli and background

Some B did not grow, or grew sparsely, showing some regions of autolysis. Amount of λ from A streaks was variable, and much less in proportion to the bacterial growth than from B.
Pick 1 single colony from a sparse B brush, and that grew somewhat more densely, and streak out for purity + λ.
473(4): Heavy streak shows λ. S.c. do not. Streaks from heavy portion.

Sp 14 (λ_2?) 473c

3/17/49 ff.

473(5). None of 10 single colonies is λ+. Brush is more active than ever. Turning lac+ on continual selection. ~~Test brush + single cols~~ Hold for outcome of B2:

On B, 1 is λ−; 2 is λ+. Brush + streak.

8 single colonies from B2 were not lysogenic. ~~Str~~ Brush was.

3/18. Streak out bacteria from brush of B2 (473(6)).
Brush heavily λ+. No single colonies were.
Pick brush (1) and 8 single colonies (2-9). Test these for λ. Also (10) mix 8 colony suspensions and streak out for λ. 1 only lysogenic.

Compare cells from this brush for sensitivity to vaccinia C $\bar{C}$ W518, its parent.

↳ Take to slant as W877.

Segregation of H168

3/13/49.

Inoculate from EMS streak to two tubes Pennassay. Aerate overnight (5 humidifier; volume ca halved!)

Dilute 10^{-8} and spread on EMB media:

		Lac	Lac	Mtl	Mtl	Gal	Gal	Xyl	Xyl
A	Σ	528	365	651		510		651	
	+	500	341			486	too crowded to count well.	1	
	−	26	18	647		24		647	
	V	2	6	3		0 ?		3	
Rel %		4.9	4.9	0.5		4.7		0.5	

B.	Σ	204	229	201	163		236	187
	+	~~200~~ 199	225	201	1		0	0
	−	4	3	0	161		230	186
	V	1	1	0	1		1	1
Rel %		2.0	1.3	0	0.6	~~0~~		

ca 200 all −

By error all Gal was A
Xyl was B

Ca half the plates of B were cont E. aerogenoides type.
Pick rare type to all sugars.

A. Lac −
~~B~~. Gal −
B. Xyl + 1st 1
Mtl + 2nd 1

Segregation of H168.

3/15/49.

A. Lac− : 33 picked.
All are Gals. 32 are Xyl− Mtl−
1 Xyl+ Mtl+

B. 1. Xyl+ : Lac− Mtl+ Gals
2. Mtl+ : Xyl+ Lac− Gals
54 Gals : All Lac− All but 1 Xyl−Mtl−
1 Xyl+ Mtl+.

∴ Xyl, Mtl are emphatically linked (3 ++ segregants; all others −− ++)
Gal, Lac ~~fairly~~ very closely linked. (87 −− segregants; all others ++).

The Xyl Mtl + segregants are crossovers.

This segregation may not be entirely valid because of the very high population density which was reached.

Test some Lac− from A for V_1^R.

4 Mtl+ Xyl+ Lac− Gals from above: all R.
of the − − − −; 19 were V_1^S 6 were V_1^R. From parent background at lytic bunch. all V_1^S judged to be V_{1c}^R
Additional Lac− tested: − probably nucleoreable

Corol. Lac+ can be taken to be exclusively (or nearly so)

Gal+
Xyl −
Mtl −

that is, the dominant type.

Lac− is usually Gal− and v.v., but may be either Xyl Mtl + or −.
is often V_1^R.

3/17/49.

W847 x W769 on Lac EMS.

40 Lac+ prototrophs streaked on Lac EMB.

None Lac-. Later 847 retested: mostly Lac+!

3/20: W842 x W859. on MH EMS.

40 MH+ tested: all ++.
48 additional "

Note! In this cross, MH+ appeared to exceed MH- by at least 10:1.
(on EMS Lac; no B,)

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!

Lysogenicity, and [illegible]

3/15/49.

Nos.	Pick		
1-6	1	} all λ−	
7-10	2		
11-14	3		
15-17	4		
18-21	5		
22-27	6		
28-31	7		
32-35	8		
36-45	9		
46-49	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:	2 Autolytic; titers not?
98-101	23	99-100:	Autolytic
102-104	24	103-105	"
105-106	25	106	Not autolytic

on W318	on W311
±	±
++ 1 plaque	++
±	−
++	++
++	++
++	±
−	−
−	−
++	++
++	++
++	++
++	++
++	++
++	−
++	++
++	++
++	++
++	++
++	++
++	++
++	++
++	++
++	++
++	++
++	++
++	++

plate. Infl sewage with 518. 25 plaques found on 3 plates. Pick and a) streak, streak on 518 to obtain isolates, test 2-3 of each for λ on 518. b) centrifuge from 2 plates and compare action on 518 and 311. ± 14 is the only 1 plaque 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 W311. Continue 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

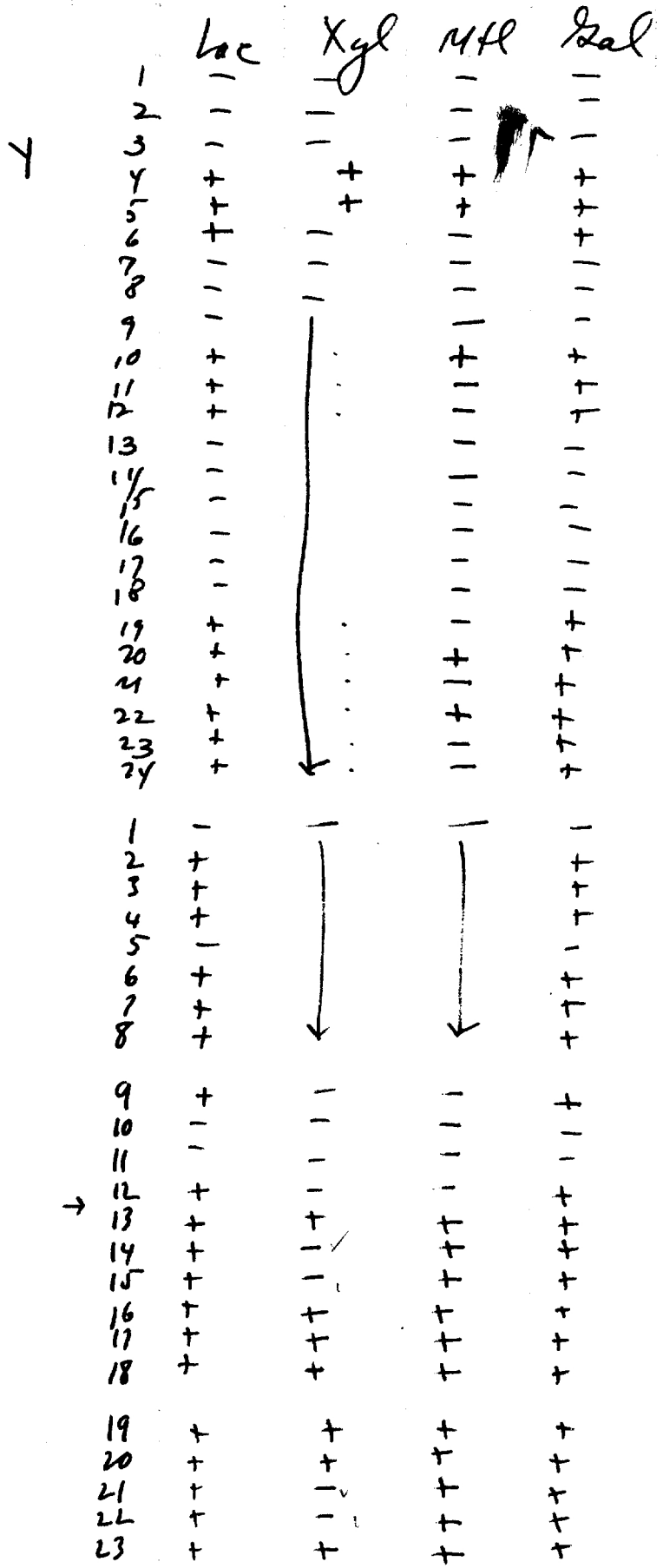
	L+X-	L+X+	L-X-	L-X+
	7	10	0	0
	0	0	1	13
	[illegible]	5	0	0
	0	4	2	14
	7	19	3	27

Segregation ratios:

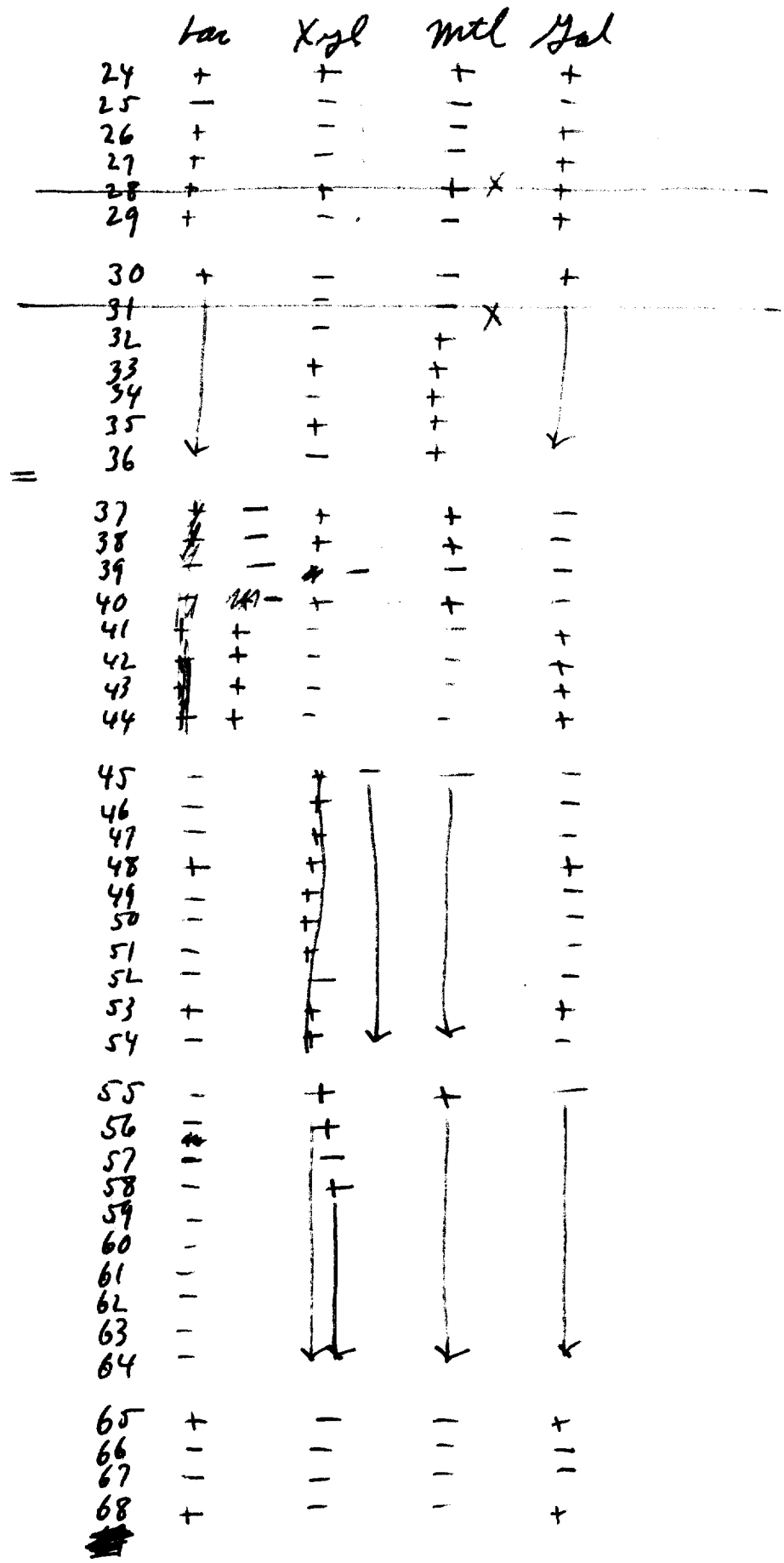
168-	Lac +	Lac -	Lac % +	Mtl +	Mtl -	Mtl % +
6	231	18	93	61	95	39
1	21	159	13	73	98	43
5	1300	147	90%	20	698	3
7	50	390	11%	74	212	26

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 + + + +
7 + + + +
8 + + + +
9 + − − +
10 − − − −
11 − − − −
12 + − − +
13 + + + +
14 + − + +
15 + − + +
16 + + + +
17 + + + +
18 + + + +
19 + + + +
20 + + + +
21 + − + +
22 + − + +
23 + + + +

H168'


Lac
Xyl
Mtl
Gal
24
25
26
27
28
29
30
31
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34
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38
39
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41
42
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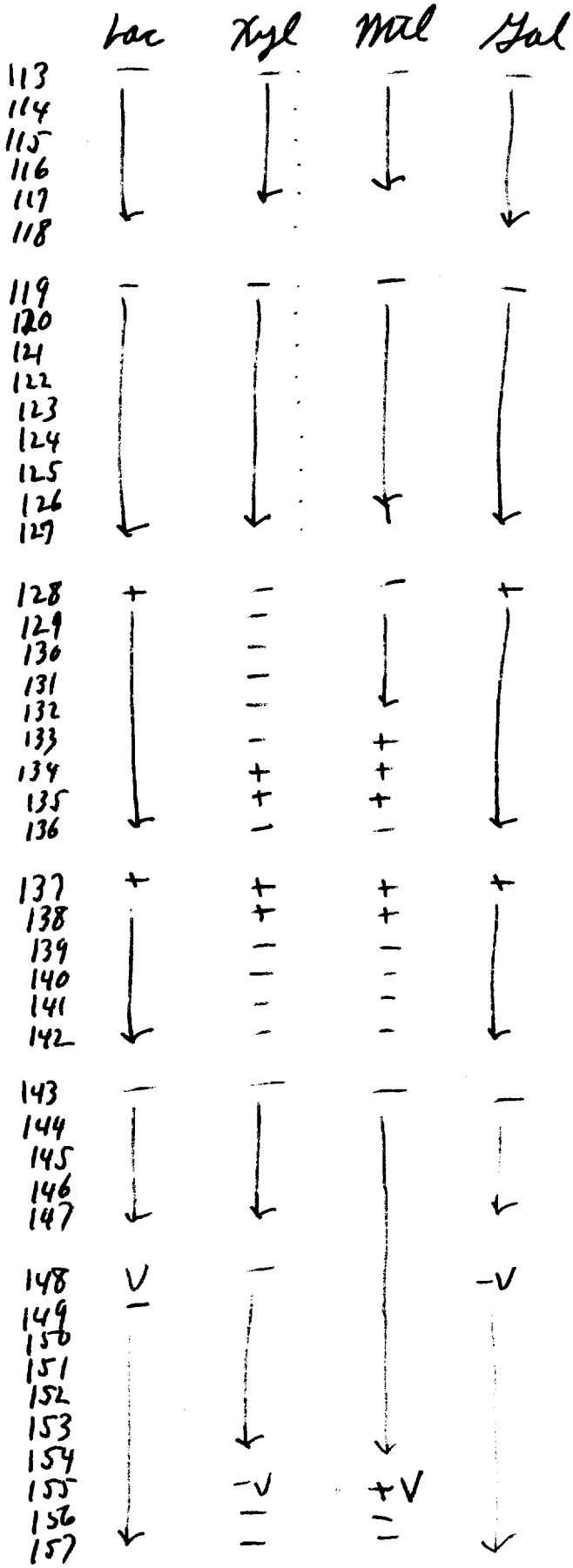
64
65
66
67
68

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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127
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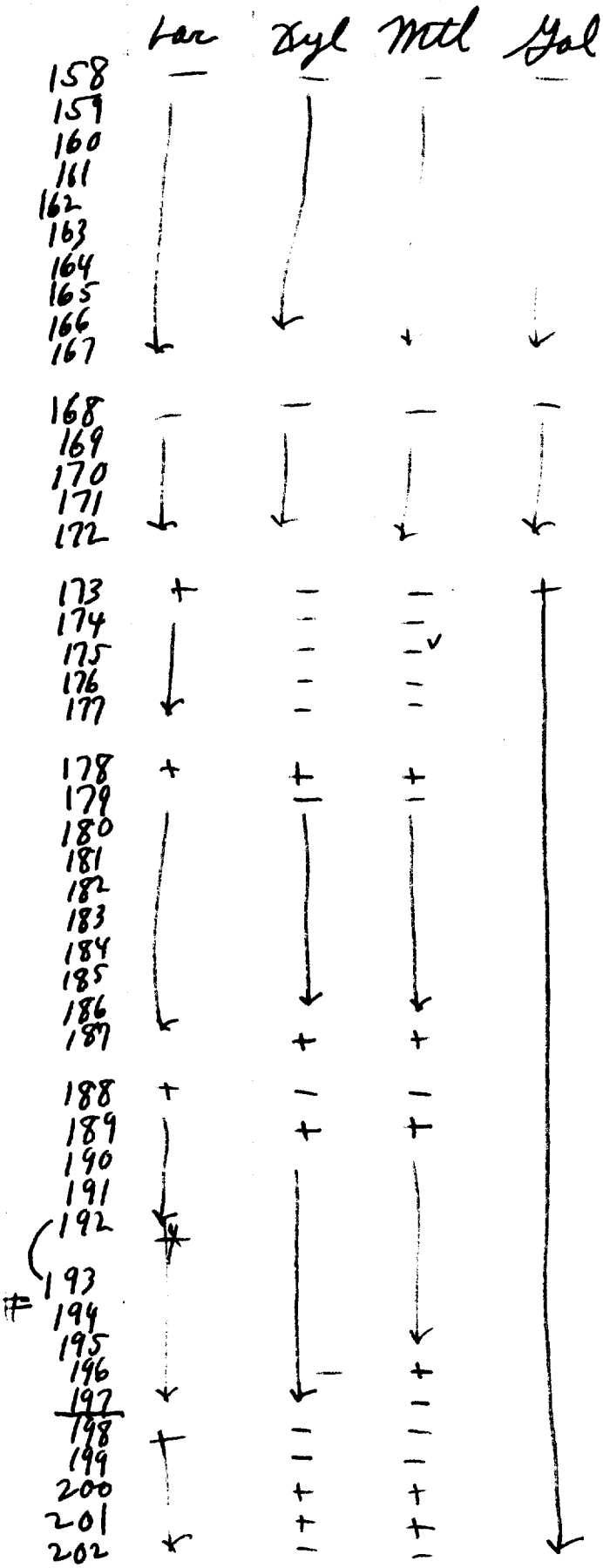
H168


Lac
Xyl
Mtl
Gal
158
159
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172
173
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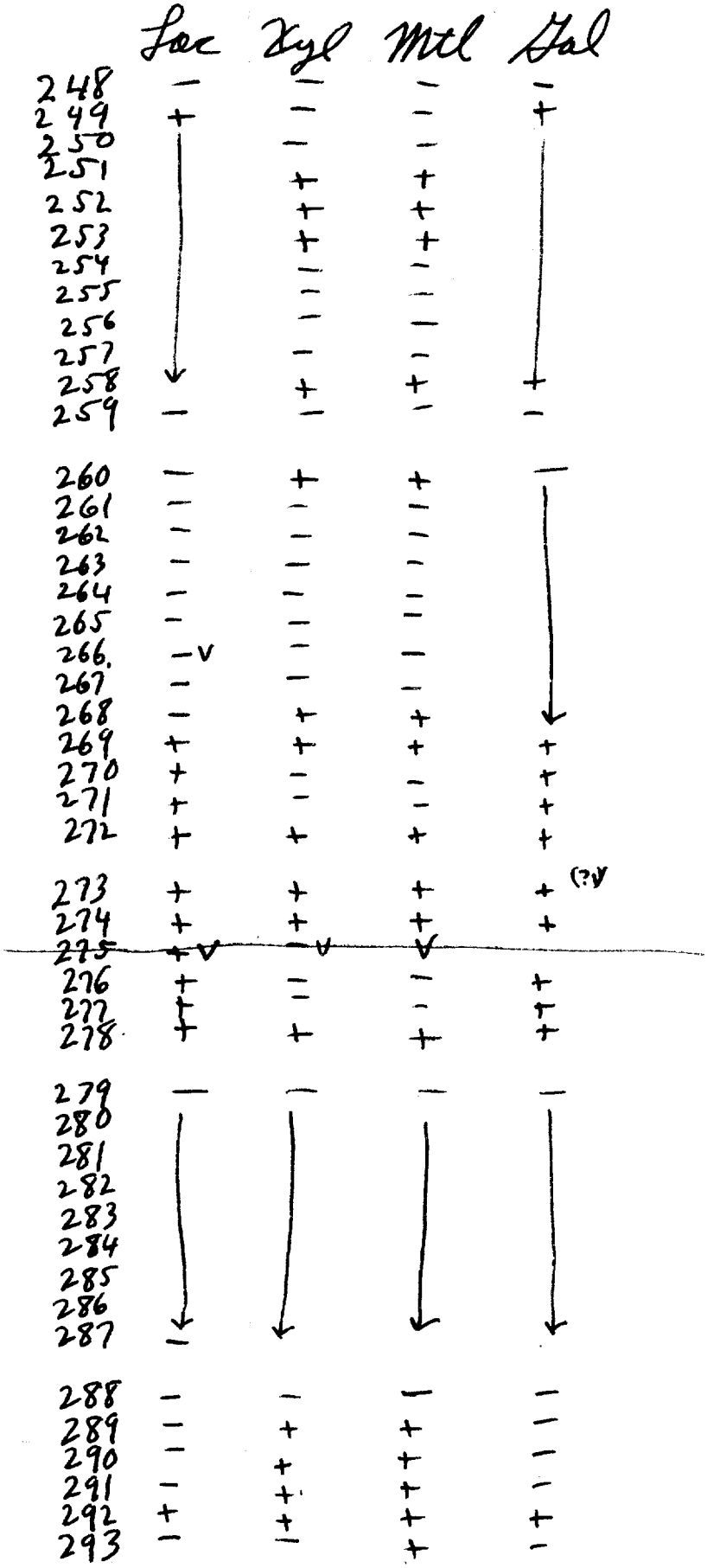
200
201
202

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
Xyl
Mtl
Gal
248
249
250
251
252
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284
285
286
287
288
289
290
291
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.

~~Interference of A and~~ Sp-19. Y83

Reconstitution of H1B6 segregants 451

~~3/18/49.~~

P19: Inoc. 1 ml each of an 18 hour culture of W418 and 677 into 1 tube YE broth.

Plate out 10^{-4} and 10^{-5} on EMB lact. Incubate 48 hrs. Count colonies. Actual value $\times 10^{-2}$.

Initial, at 10^{-5}:

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

Final 2P20:

	19	13
	16	9
	18	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. $\chi^2_4 = \frac{1}{27}\left(\frac{25+4+16+1+9}{16}\right) = 63$

$= 2.3$ $\quad p = 0.6$

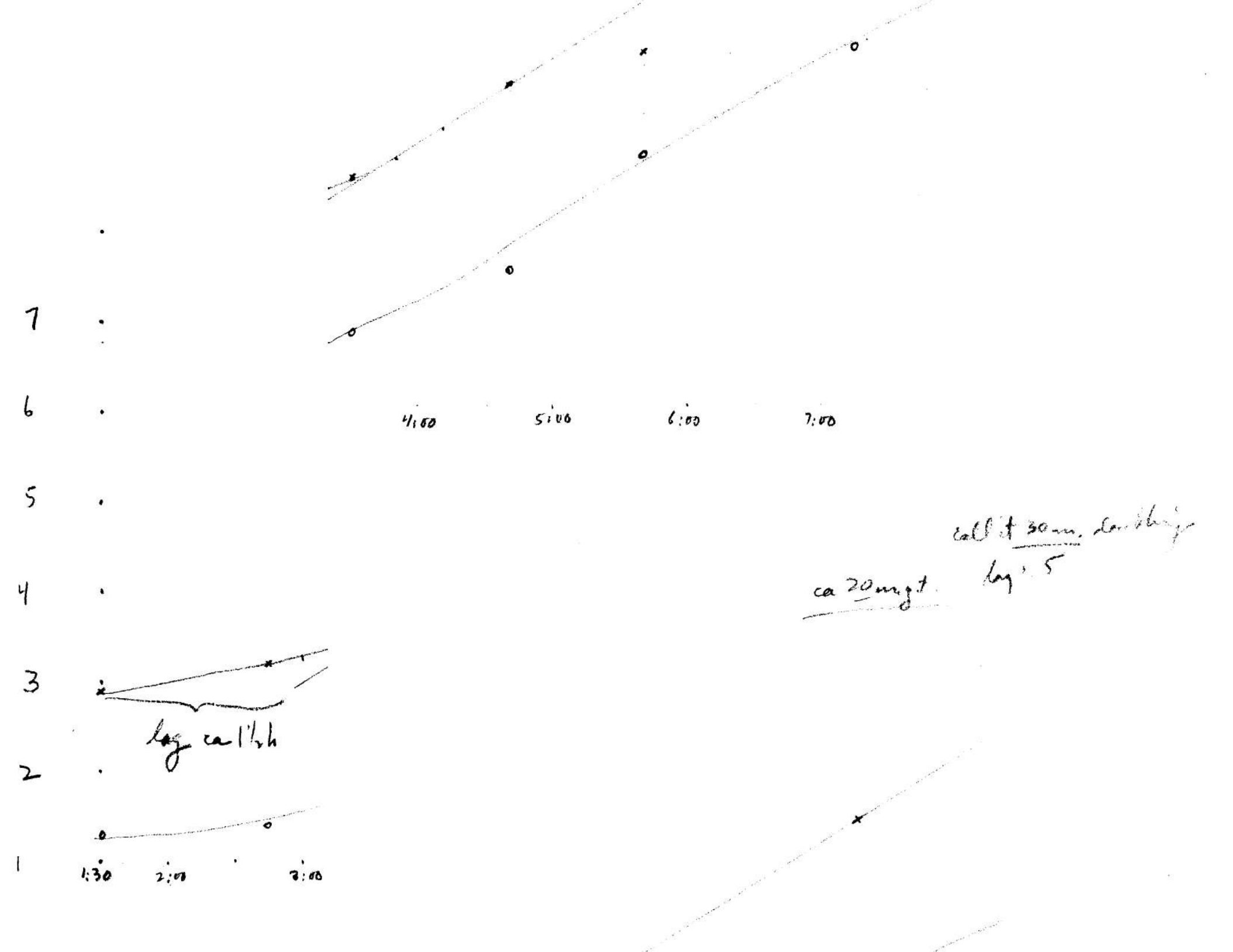
agreement in segregation: $\chi^2_4 =$ $16/17 + 16/15 + 16/12 + 16/13 +$
$36/12 + 36/12 + 4/14 + 4/16$

$= 11.11$

$p = .025$ 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 !!

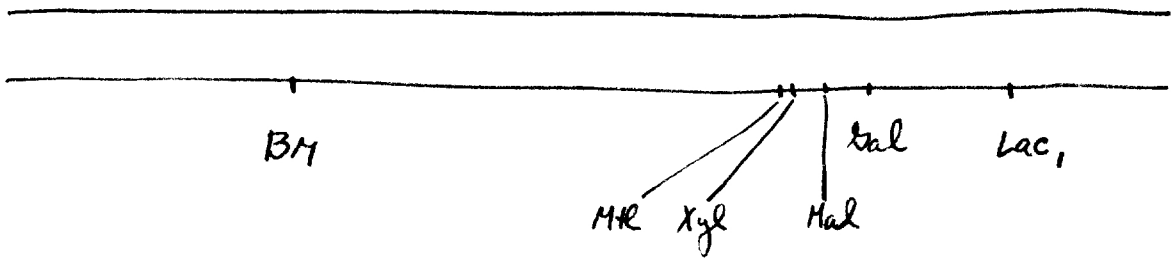
10 Lac- all $T5^S$! }

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	++	++	++	++		

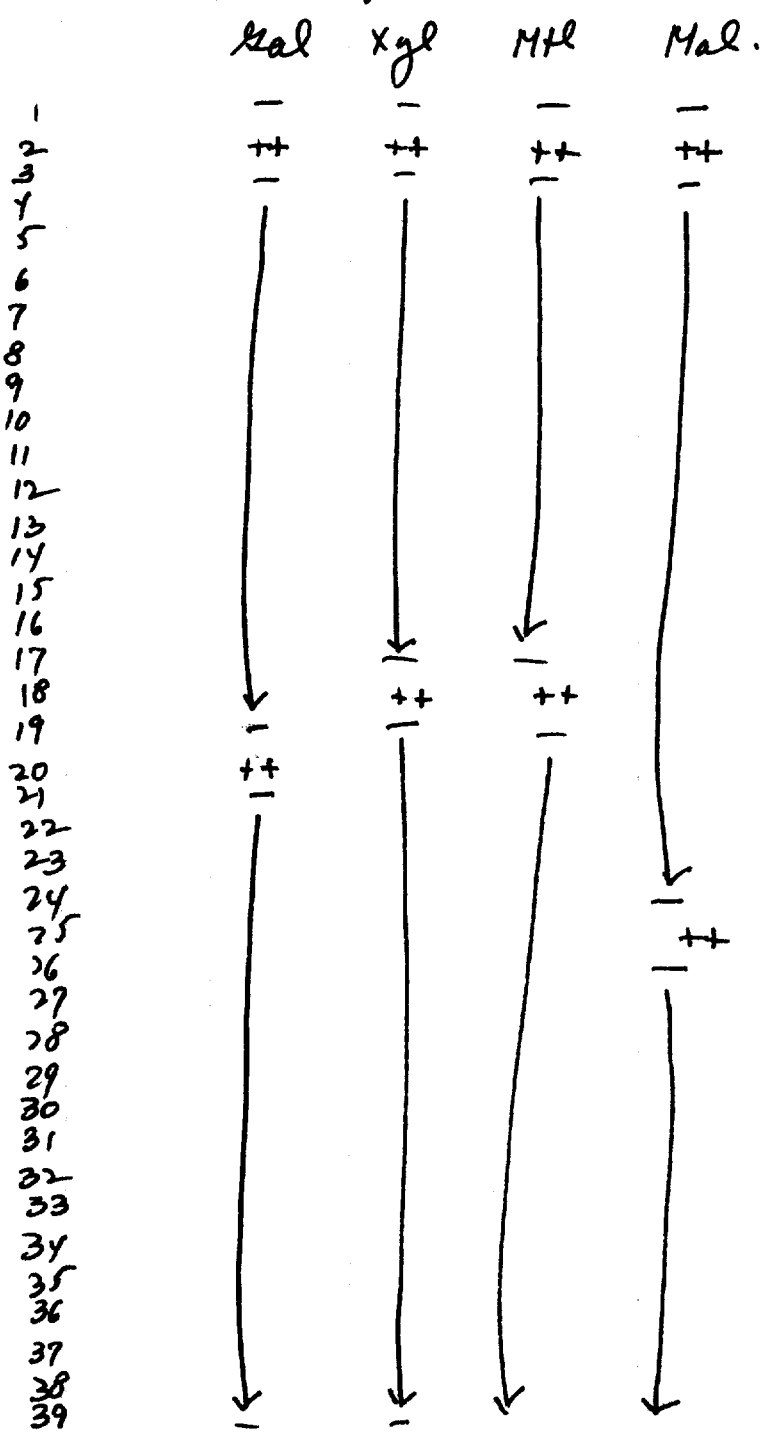
Lac⁻ Gal⁻ linkage test.

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 imperfect 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.