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.

May 24, 1948.

On EMS-lac B,

(1) W-108 × Y4/0. Cross n.g. W-108 check streak very large in lac+. (ca 1:4)

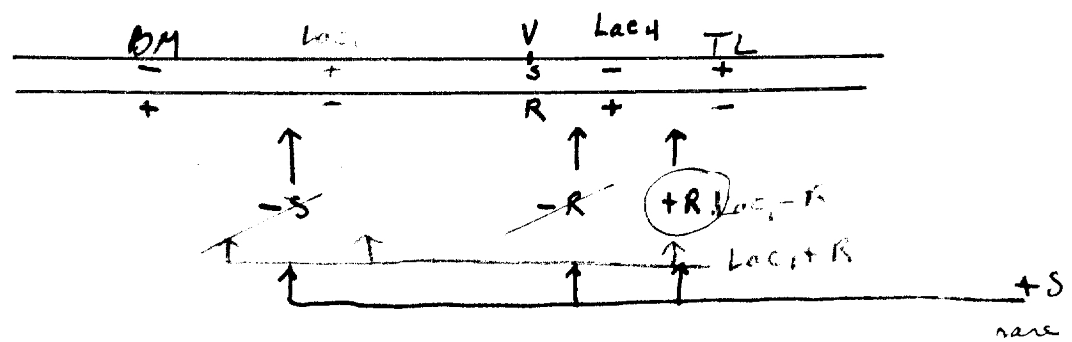
(2) W-67 × Y4/6. Only an occasional + colony. None on 3 glucose plates.

5+ tested All V_1^R.

What linkage relationships are indicated if the Lac4- are merely not recovered? The combinations are:

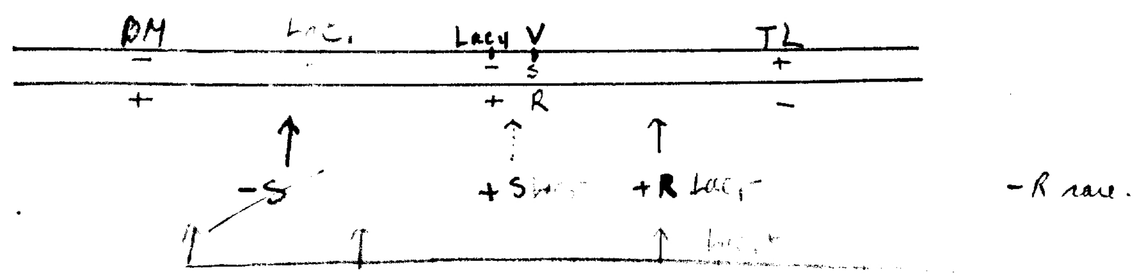
BM Lac+ V_1^R TL. × Lac- V_1^S. Lac4 may simply be closely linked to V_1 or situated so that a triple interchange is required to give a Lac+ V_1^S combination, e.g.

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.

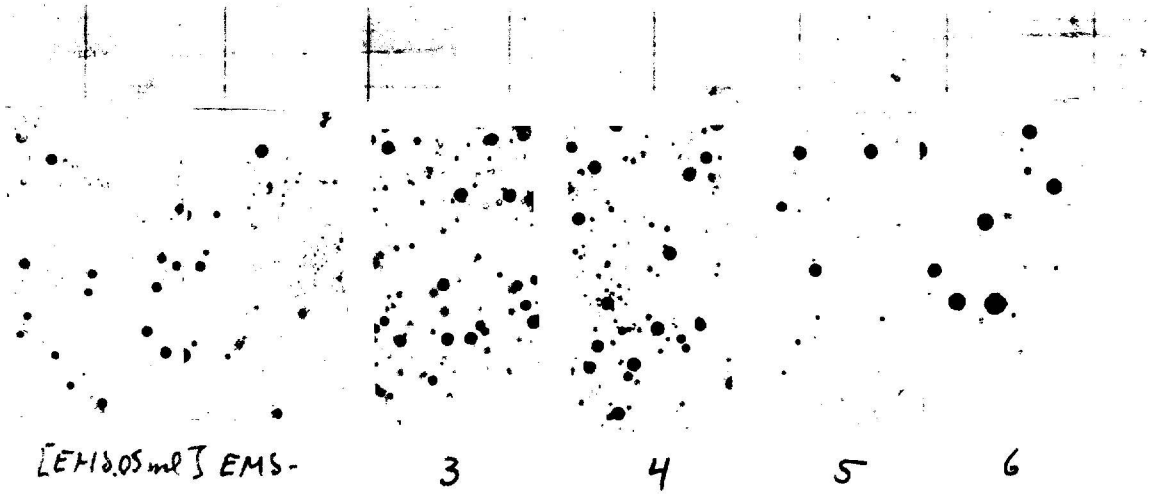
9A 26.

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)

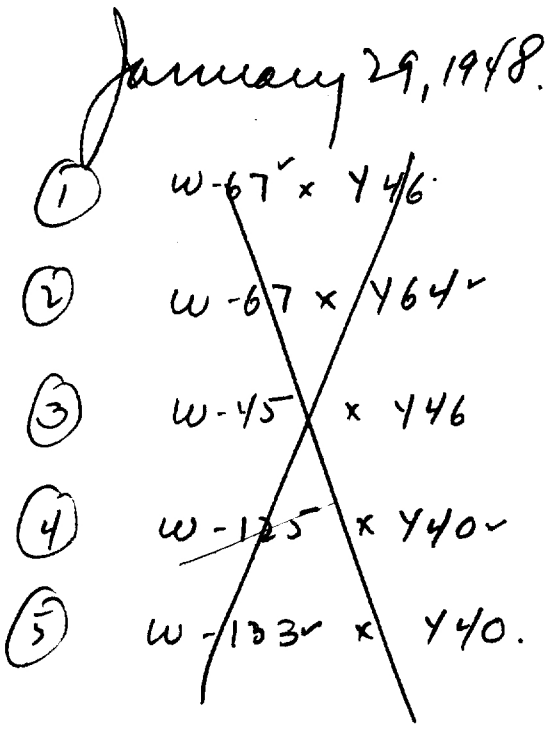
+	−
6	25
4	29
3	4
4	20
17	78

] 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 S.O. on glucose agar.
25	—	++	—	++			SW-7
26							
27							
28							
29							
30	—	++	—	++			SW-8

(2–21: v. rough)

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

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

	① lys+arg	② meth+cys	③ leuc, isol, val.	④ phal, tyros, tryp't.	⑤ [illegible], threo, [illegible], prol.	⑥ serine glycine alanine hydroxypro	O	HC	Adenine AA?
SW-3	±	±	±	±	+++	±	±	+++	
4	±	±	±	±	+++	±	±	+++	
7	—	—	+++	—	—	—	—	++	B-V ±?
8	—	—	—	+++	—	—	—	++	

S-21	YNA	Y.Extr.	N2 Case	Pur+Pyr	H.C.+Vits.	O	
5	—	++++	—	—	—	—	
6.	—	+±	+	—	++		Vits. [illegible] V.
S-21.	+++	+++	+++	+	+++	+++	

more AV.

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

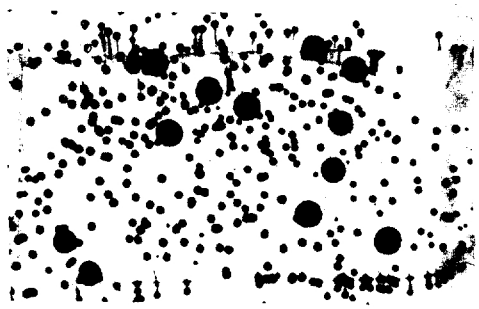
(8) W145 x ~~Y46~~ 58-161.

~~(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-

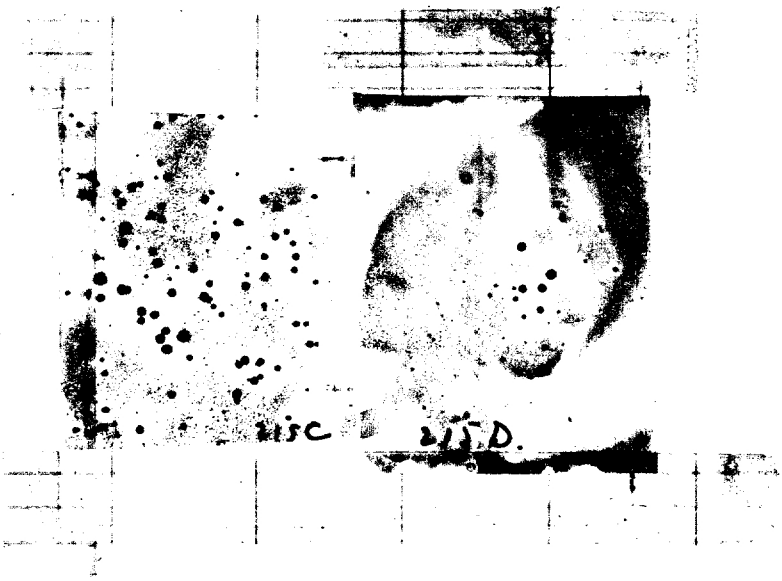
D. Ca 50/plate 26+:16-

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. }

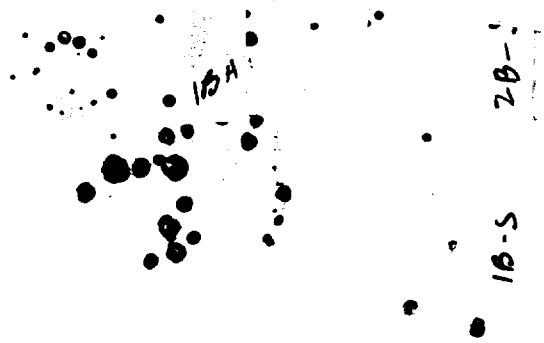
30 − 1+ lac S. }
21. 3+ } Conclude: Shaking is certainly deleterious to crosses!

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 "