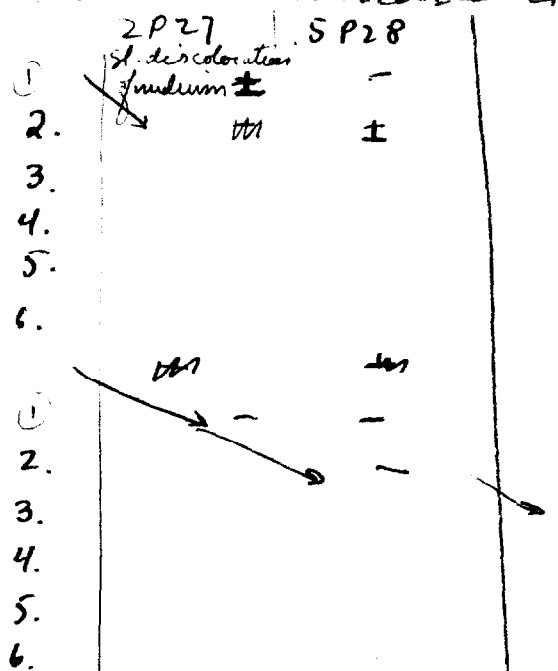
Selection for Sucrose + mutants.

Oct. 26, 1947.

Prepare 10ml. 2% sucrose 1% peptone, ~~0.1% y.e.~~ with Bromcresol Purple Indicator. Incr. as indicated, in series.



A. Y53. 10/26/47. (1). SP.

B. 58-161. (1) 10/26/47. SP.

(-)
IA3 was incr. into IA4 P30 and left overnight at room temperature. Later turned alkali. Not Sucr +

P4. (IA4) was +. Streaked out on sucrose EMB. $\left.\begin{matrix}25^\circ \\ 37^\circ\end{matrix}\right\}$ both Sucr -

Y105 —

Y106.

Abandoned A6.

Selection for α-methyl glucoside mutants. 2

Oct. 26, 1947.

1% α-methyl glucoside (EK) broth, autoclaved together. BC Penicillin.

	2P27	5P28.
A. Y53. ① 5P	— ↓	– – ↓
B. 58-161 ①. 5P.	— ↓	– – ↓

growth only fair at 24hrs

abandoned A 6.

Oct. 26, 1942.

Glucose + 2% peptone. Vary glucose in methyl-red indicator to establish the critical level. K-12. 5P26. In duplicate.

Glucose: 2% 1% .5% .2% .1%.
Methyl red destroyed.

3P27. Growth + fermentation ++. No color changes. Test pH in 1 series.

pH:
acid to MR ++ ++ ++ ± -
" " BCP. ++ ++ ++ ± -

Use glucose .3%; Bran Cresol Purple. Prepare series of tubes ē 2% peptone. Incr. each with K-12. P28.

11A29. pH B. = ca. 6.8. pH. A. < 5.2.

(B) (-glucose).

(A.) (2) 1-10.

(1).

Add ca 8ml B to A1. pH ca. 6.0.

Retain sample in refrigerator for comparison to detect alkali production.

(A1). A30. alkali produced. Transmit to A2.

(A2) A3. Alkali produced Add to (A3) i.e. mix cultures.

(A3). A6 alkali produced.... Start A4

(4) A10 alkali produced.

end. Expt.

Mustard gas: Maltose Mutant; Galactose tests

10/25/47.

Harvest Y53 from 2×25 ml/125/f. YP broth and from 3 × 10 ml YP agar slants 20 hrs. old. Suspend in 10 ml M/10 phosphate buffer at pH. 6.0. Add 20 mg HN2 nitrogen mustard [$(Cl\,Et)_2 NCH_3 \cdot HCl$] in 10 ml buffer. Let stand 30 minutes. Sediment cells and replace with peptone 10 ml. 1% Inoc 1 ml samples into 25 ml YB broth and incubate to prepare inoculum for mutant detection. Spread .1 ml ea check on survival → ca. 10^4. Estimate.

$pS = \log 10^{10}/10^5 = 5.$ Many mucoids noted!

	Plates	Colonies/plate.	Total colonies.	Suspected mutants
10/28/47.	40	250	10,100.	-1 not Mal-
				~~-2~~ (mucoid). —
				-3

	T1	T4	T6	T7	Lac	Nutr.	
-1 (W1)	S'	R	S	S	—	T-L-B, ±	∴ mutant!
-2	R'	R	S	S	—		
-3 (W3)	S'	R	S	S	—	?	Mal- "papillae on test streak. Reisolate.
Y105	R	R	R?	R	+		
Y106	R	R	R?	R 'plaque	+		
K-12	S	R	R?	S	+		

(plaquing)

10/29/47. Spread similar populations on galactose EMB plates. 11AM.

(colony dimorphism noted • S/R?)

Of ca. 40 × 250 = 10,000 colonies, no galactose - were noted. However, one colony which was unusually light purple was noted + picked for further study = 4-4.: Not Gal-.

Oct. 28, 1947. Test the cultures indicated on the following EMB:

	Y53	Y87	58-161	Y53	Y81	58-161	
Galactose	++	18 hrs. ++	++	→			✓ *** same pelted!
Rhamnose	−	−	−	−	−	−	+ + + ✓
Inulin	−	−	−	−	−	alk	
Melibiose	+	+	+	+	+	+	✓ *
a-methyl glucoside	−	−	−	−	−	−	
Cellobiose	−	−	−	alk	alk	alk	✓
Dulcitol	−	−	−	−	−	− ppt.	− ± ± **
Sorbitol	++	±	+	++	+	+	✓
Sucrose	−	−	−	alk	alk	alk	
Ethyl Butyrate	inhibited			→			produce alk
Lactose	−	−	+	−	−	++	

on $CaCO_3$ (.1%) peptone agar:

Lactose Minor clearing around lac+ colonies. Not definite enough!
Ethyl Butyrate (add "sterile" to hot medium.)

Cellobiose α-methyl glucoside Sucrose Melibiose

W-1
W-3.

* Streaks not uniformly dark: lighter in centres. However, this is rather a thin plate.

** Streak out the papilla.

P 31. Y53 is Dulcitol negative; Y87 + 58-161 Dulcitol +.

[dulcitol is related to galactose!)

~~W-1~~ W-1. Melibiose slow +
10/31/47. α-methyl glucoside −
Sucrose −
Cellobiose −. Papilla (?) on 58-161 and Y87?

Streak out again

Carbon source utilization.

T(m) + Indicated Carbohydrate + BM for 58-161
G = glucose ½% (½%)) + TLB for Y53...
g = glucose .05%

16 hrs. 37°.

	O	G	g	Maltose,	+d.m.g.	d.m.g.	+g	+G.	Cellobiose	+g	+G
W-1	−	++	++	+	+	±	++	++	±	++	++
Y53.	−	++	++	+±	++	±	++	++	+	++	++

	Sucrose	+G	+g.
W-1	±	++	++
Y53.	±	++	++

	−BM O	+BM O	G	Lac	Suc
58-161	−	−	+++	++	±
Y87.	−	−	++	±	−

48 hours:

	O	b	g	M	M+d.m.g	d.m.g	+g	+G	C+g	C+G	Suc	+G	+g
W-1	−	++	++	++	+	±	++	++	+	++	++	±	++	++
Y53	−	++	++	++	++	±	++	++	+	++	++	±	++	++

	BM	Gl	Lac	Suc
58-161	−	++	++	±
Y87	−	++	+	±

Use .05% in testing sugar utilization in future growth experiments.

P 29. Inoc JS 8-161 into YP broth. 2 P 30 Harvest and suspend in 1 ml
M/10 Phosphate pH 6.0. Add 10 mg. to each of two tubes -
MNNG.

a. 2:28 PM - 3:02 + 8 minutes. 34 min.

~~b. 2:48 PM.~~ b: 3:02 - 3:17 + minutes 15 min.

at 3:02 PM Centrifuge 10 mins. Resuspend in peptone dilute peptone
Inoc 1 ml each into 25 ml YP. Spread .1 ml samples to assay killing.

Killing a) >> 10^4

b) > 10^4 survived. pS < 5. Spread out +

Retreat survivors also!

Spread on Lac, Gal. and Mal.

Galactose. 83 plates. Sharp division into large + small colonies noted. ca. 300 large + 900 small noted. Noted noted on Lac or Malt plates Lac + Malt plates are v. crowded ē "uniform" colonies - ca. 1000/plate ∴ are some cells inhibited by galactose?? Only large could be scored: 25 000 colonies. Ca 7 most likely possibilities. Also reisolate G^R and G^S colonies + test as contaminations? 7:1-7.

Lactose: 37 plates. Ca 800 on each plate scoreable = 30,000. Almost 1 Lac- per plate noted. 12 selected for further study. 7:11-30

Maltose (1/1000) 63 plates ca 55,000 colonies scoreable.

8 apparent Mal- noted. 7:41-50.

Total tests: 110,000

Reversion of Lac + Mal in W-1 and W-3.

Streaks out papillae of W-1, W-3 and YS 30 cultures on Lac and on

Nov. 2, 47 Malt. EMB agar. Note that on original plate, W-3 had some papillated, some non-papillated colonies.

W-1. Lac. All Lac -. Papillae in streak?
Mal. alkali. All Mal -.

W-3. Lac All -.
Mal All -!

} Hold for evaluating

Restreak A6.

W-1. Recorded for Lac found. Purify and number W-33.
W-3-(Malt) all - ; papillae are not Mal+!
W-3-(Lac) + and -. Purify. ✓ W34

Mustard killing : 58-161.

Add 10 mg mustard to 10 ml 58-161, assay = 3×10^9, in phosph. M/10 pH 6.0. Dilute 1:100 at intervals and spread (on sucrose EMB which is available). .1 ml. Colony count = survivors/ml $\times 10^{-3}$

0 time assay = 3×10^9

60 mins assay = 2×10^6. pS = 3+. for 60 mins.

Assume 10% survive for each 20 mins. at .1% in phosphate buffer. Use .2% and 30 mins. treatment.

Nov. 2, 1947. G^S G^R

P1 - Inoc small and large colonies of 58-161 on galactose EMB plates of Expt. 7 into YP 25 ml. Incub. overnight.

P2. Treat ē .2% HN_2 in phosphate buffer 40 mins. Add 1 ml susp. to 25 ml YP for further incubation.

Incubate G^S on left (sinistral) side of incubator.

G^R: Lac. 70 pl. x 20 col. → 1400 tests → 12 colo. from unth. inoc all lac +.

ca 50% of colonies are lac −. (to inoculum?).

Pick to sl. 10-1.

Test nutrition: BM vs

Pick colonies from a Gal plate inoc. with the untreated suspension.

Pick 3 colonies to slants W30, W31, W32.

Malt. 16 x 40 = 640 tests.

1:1 dimorphisms of very dark and less dark colonies. Both are + and have a sheen. (Corresponds to above?!)

Gal. 23 x 30 = 700. No mutants.

some contaminated

G^S. Lac. 65 x 100 = 6500. No mutants.

Malt. 17 x 80 = 1360 No muts.

Gal. 23 x 30 = 700 No muts.

Nov. 3, 1947. Characterization of Mutants of Exp 7.

A. Galactose mutants – 1st. streak on galactose EMB.

7-1. Majority of light purple colonies ◉ with a few typical Galactose +. Pick to glucose slants. BM W-2.

7-2. Gal ± as in 7-1. No Gal+ BM W-4

7-3 do. BM W-5

7-4. Majority of alkaligenic, v. small colonies. Streak of heavy streak, a few Gal+, larger colonies. Test as Lac: Lac- alkaligenic. throw out.

7-5 as 7-1. BM W-6

7-6. Do. BM W-7.

7-7. As 7-2. BM. W-8.

B. Lactose mutants.

7-11. Typical Lac –. n.g. in 16h. not B-M-! W-9

7-12. Do. Papillogenic W-10

7-13 Do. Pap. W-11

7-14. Do. + two Lac + colonies. W-12

7-15 Do. W-13

7-16. Do. W-14

7-17 Do. W-15

7-18 Do. Colonies smallest. Lac + in heavy streak W-16

7-19 Do. Weak utilization? W-17

7-20 Do. W-18.

7-21. Do.
1-22 do.
~~7~~-MALTOSE.

what is the nutrition of these creatures?

W-19
W-27

7-~~31~~.	Majority white light purple. Some Mal+.	BM	W-20
7-42.	Mal+.		
7-43.	As 41.	BM	W-21
7-44.	All Mal+.	BM	W-22
7-45	do.	BM.	W-23
7-46.	do.	BM	W-24
7-47	do.	BM	W-25
7-48	do.	BM.	W-26.

Test nuts. by comparison of T(B+M) & T(0).

58-161.	$\mathcal{G}^R$	W-28
	$\mathcal{G}^S$	W-29

Galactose Inhibition

Nov. 3. Dimorphism on galactose was noted in 7.

G^S + G^R were streaked out on EMB.

	G^S	G^R
Lac	++	++
Gal	++ only sl. smaller than.	++
Mal	++	++
Sule.	± (slow)	± (slow).

The galactose effect was not reproduced here, nor in the plates streaked from cultivated G^S, G^R, in expt. 10. These were, however, a different batch. Tested on:

galactose 1%	peptone 1%
galactose 1%	NH$_4$ amine 1%
galactose 1%	N Z Tone 1%.

Dulcitol.

Nov. 3 '17.

K-12 ferments dulcitol only weakly. Inoc into broth + compare. By 16 hours, dulcitol broth is vigorously fermented by 58-161.

A. from broth
B. from plate (a slow colony). } Both form only "weak" colonies.

Streak A. again. - slow + as before!

?? Reduction of Methylene Blue??

Galactose media.

Nov. 4, 1947.

EMB - Gal 1% - agar 1½%

A. Peptone 1%

B. " 0.3%

C. N-2 - Annie "B" 1%

D. N-2 - Tara 1%.

Streak out G^R + G^S colonies. P4.
A5.

A No dimorphism

B large + small colonies. Not as marked as C.

C large + small colonies.

D. no dimorphism.

4
Nov. 19, 1947.

Mutant Rem. Treat a single colony culture of 58-161. E HN2 .2% for A) 5 mins. B) for 30
Lac mutant plating. Lo. W-1 for galactose mutant plating.
Incubate 24 hours before plating per convent tulinagine

A. 58-161 (treated) - 50 pl x 70 cols. on Lac = 3500 tests

No mutants

B. W-1. 35 pl x 100 cols on Gal = 3500 tests

No mutants.

Reduced sugar utilization.

Nov. 4, 1947.

See 14A. for Treatments.

Add ~~flate~~ ca 10^{+9} cells tsaurois of mustard) to:

			A5	A6	A7	A9
58+161:	1.	100 ml T(m)	+ (BM.)	±		
	2.	100 ml T(m) + glucose .05%		+++		
	3.	do. cellobiose		±		
	4.	do sucrose		±		
	5.	do sucrose 200 ml.		±		
	6.	do. sucrose 200 ml.		±		
	7.	do. d-methylglucoside.		±		
w-1.	8.	do. sucrose TLB, 200 ml.		±		

No change

ditto

abandon experiment on Nov. 9.

Nov. 5, 1947. U.-V. Killing Rate.

Hanovia Lamp. 6" from plates. Spread .1 ml (10^8 cells) of 24 hr cultures of 58-161 on EMB lactose plates. Irradiate as indicated.

t.	colonies
0	$>10^5$
5 sec.	$>10^3$
10 sec.	112
15 sec.	ca 100.
20 sec	50
30 sec	28
1 M.	20
2 M	7
6 M	3.

There is certainly a break in the killing curve between 5 and 10 seconds, or else, the survivors of higher doses represent large changes.

10 secs. is a convenient irradiation time which has a $pS = \log 10^8/10^2 = 6$.

One "weak" lac colony noted on 10 second plate.

Streak + compare with subs.

β-methyl glucoside

Nov. 4, 1947.

Sample from H A Lardy. (mp 106-108°)

1) 1% in EMB.

W-1	—	A6. dark spot - Restreak. →
Y53	—	
Y10	—	all —.
58-161	—	
W-22	—	

P5

2) .05% as C-source in T(m) + BM. 58-161 mod. P5.

	A6.	A7	A10.
1) β-methyl gl.	—		+
2) α-methyl..	—		—
3) α + β	—		+
4) glucose	++		++
5) β + glucose	++		+++

The Lac- W-30.

Nov. 6, 1947.

W-30 represents a series of Lac- colonies which constituted 50% of the colonies found upon spreading mustard-treated 58-161 on Lactose EMB. 12 colonies derived from the untreated inoculum were all Lac+. The inoculum was derived from a single large colony (3 R) of a type constituting about 1/4 the colonies formed on a galactose-N2 dennis B-EMB plate (see. Expt. 7) In Expt. 10, the ratio of Lac+ : Lac- was:

	+	-	
plate 1.	18	10	
plate 2.	10	4	
plate 3.	8	6	
plate 4	12	16	
	48	36	84.

A large fraction of both these types was found on each of the 70 plates of the experiment. (10)

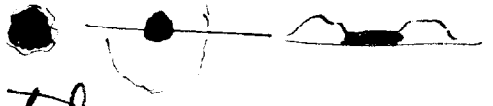
A). Pick 5 colonies from plate Expt. 7 in attempt to reproduce the expt.

B) Pick + streak out 5 Lac+ colonies from Expt. 10 to determine stability of these Lac+ : all plates show all Lac+ ~~-~~ !

c) Set up selection Expts. between these Lac+ and Lac- in broth.

On the Lac plates, W-30 forms broad flat colonies. These seem to engulf Lac+ colonies which they may contact.

On maltose two colony types are seen. Initially, they differ only in that some are less intensely colored on EMB than the others. Later they develop large beads of gummy material which projects from the surface.



On galactose no peculiarities had been noted.

C. Selection Expt.

A. Broc Lac+ colonies 2P6 into YP broth 25 ml.

B. Do. + 1 loopful of Lac- (W30) suspension.

Streak out duplicate lac plates for initial assay.

A: all colonies lac+ (Lac- < 1/100)
B: ditto.

		P7.	
C. Streak out	A	A 6: all+	all+
D.	B	A 6.: all+	all+.
		A10	
A2	Streak out P7.	all+	all+
B2	" " .	all+	all+

The combination: W-30 and the population of Lac+ from one of these plates does not seem to satisfy selective hypotheses. The Lac+ may be resistant to the (hypothetical) inhibition. 58-161 (18-A) should be used instead.

D. Selection Expt. 11/10/47. Mix 1 ml broth culture of 18A1 with .05 ml similar suspension of W-30 from streak in YP broth. Streak out initially, etc.

			A 11
(A).	18D-A1	Streak 18A1 on EMB-Lac	all+ all+
	-B1	18A1 + W-30 on EMB-Lac.	ca 100:1 +:-
			A12
N12	A2		all+ all+
	B2.		ca 20:1, +:1
A12	B3		ca 20:1 +:1

This selection expt. does not explain original finding.

Nov. 6, 1947.

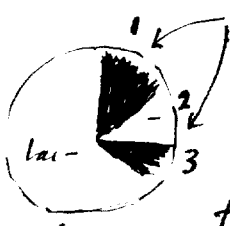
Spread .1 ml 58-161 culture on EMB-Malt and Lac plates.
Irradiate each for 10 secs. ± H. Smith's Hanovia lamp.
ca. 1 ko / plate

Lac (10) Nothing noted on 9 plates. See below for 10th. *

Malt (10) No mutants or sectors noted.

~~No mutants or sectors noted.~~

* A colony was found of the following appearance.



This suggests further a delayed effect of the mutagenic agent.

Picks from each of the 4 distinguishable zones to YP Broth + Incubate. When grown spread on ~~and streak out.~~

1	W-35	W-38	38	Lac EMB.
2	W-36	W-35	35	(A10).
3	W-37	W-37	37	
4	W-38.	W-36	36.	

1. Ca 50:1 Lac+ : Lac-. No evidence of sectoring of the +.

2. Ca 50:1 Lac- : Lac+ . No sectoring

3. Ca 100:1 Lac+ : Lac. No sectoring.

4. All Lac-.

Streak out and transfer to slants.

Reversion of Lac - 1453.

Nov. 6, 1947.

P3. Streak out Y53 on EMB-lactose - 3/10% peptone.

A6. Papillae well developed. Pick papillae to small H_2O and streak out.

#P8. All of 20 plates pure Lac+ exc. for 1 which was streaked from a mixed colony. ∴ Lac+ is a stable reversion in most cases.

Nov. 8, 1947.

Spread 10^8 cells of a single cell culture of 58-161 from YP on maltose + lactose EMB plates. Irradiate each plate ca 10 secs. c̄ Hanovia u-v lamp at 6". Score at 36 h.

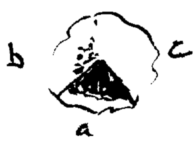
Maltose. 24 pl x 60 cols. = 1440 colonies tested. All colonies +.

Lactose 59 pl. x ca. 150 scoreable colonies = 9000 tested.

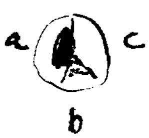
12 plates showed 14 likely colonies. Some of these are clearly simply mucoid.

4 clearly sectored
3 not sectored
5 mucoid
2 ??

①, ② Mucoid.

3. 

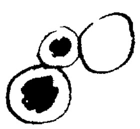
4. Small white colony ✓

5. 

Streak out 3 comps.

6 M

7.  ✓

8.  ✓

9. M

10 

✓

11. 

✓

12.  → no Lac. on streaking

13. 

all ± c̄ mucoids

14. O mucoid.

3a 1:1 +:− (+) w50
3b almost pure(−) w48
3c 1:1 ± and − (−) w49

4. pure −. w39.

5. a. 20:1 Lac+:Lac− Puffy + : w41.
b. 2:1 + : − →
c. pure − . w40.

7. pure − w42. (slight utilization?) ✓

8. 1:1 +:− The lac− is w43.

10. a. 10:1 +:− +/w44
b pure − −/w45

11. a. 2:1 +:− (+)/w46

b. pure − w47.

12. all +. Not mutant.

β-~~pheno~~methyl glucoside

Nov. 10, 1947.

Compare 58-161 and 17-1.

	glucose	β-Me Gluc.	cellobiose	—
(mglucose) 17-4.	± +++	− −	+ ±	− −
(mβ-methyl) 17-1.	++ +++	− ±	± ±	− −
(Hettche Veg.) w-20.	± +++	− −	− −	− −

✓

24hrs., 36h. — def. diffuse meltose content

A15. do. only glucose is +++. others from − to ±.

∴ 17-1 is only slow utilization.

5 A16: P17

	β-Me Gluc.	Cellobiose.
17-~~4~~1	±+ ✓	± ✓
17-4	± ✓	± ✓
w-20.	± ✓	± ✓
17-1A.	± +	±

Transfer 17-~~4~~1 to similar series → much slower on β-methyl than on glucose.

U-V. cm.

Nov. 14, 1947.

Irradiate 50 ml of 24 hr. ~~W-37~~ W-37 conc. to 10 ml. with washing. 3 ml / 25 ml quartz flask. 40 secs. at 6 in. from Hanovia Lamp, with manual twirling. Spread .1 ml on EMB.

30 Lac, 20 Gal plates. All too dense (ca. 10^5 - 10^6 / plate).
(Autoabsorption rather marked!)

1 ml sampler, irradiated 5 secs moc into synthetic minimal.

- glucose
  sucrose
  α-methyl glucoside
  cellobiose

all neg. after 4 days.

Attempts at induced ultraviolet
of sucrose + other sugars.

Dec. 10, 1947.

Prepare suspensions, ca 10^{10}/ml, of Y10. Irradiate 3 ml at a time 5 secs. in a quartz flask rotated at hood of Hanovia uv lamp. ½ ml inocula into 50 ml / 125 ml flasks containing (1 m) + sugars + .05% (except ~~fructose~~ .15%) ± glucose .005% (g.) or .4P10.

		9A11.	2P11.	P611	9A 12	P13	P16.
1.	Sucrose		±				
2.	"		±				
3.	" g.		++	-+No+	* No+.	-	(+)
4.	" g.		++	-			-
5.	Raf.		±				
6.	Raf.		±		* No+.		
7.	" g.		++		++		
8.	" g.		++±.	* No+	+++		(+)
9.	Cellobiose		±				
10.	" g.		++	- * No+	* No+.	do. W55.)	
11.	d-Me gluc		—	+No+.		(+)	-
12.	" g.		+				-
13.	Trehalose	+++	——				
14.	"	+++	——				
15.	Glucose	+++	+++				✓
16.	g.	+	+				✓
17.	—	±	—	barely.			

Experiment terminated 12/24, without the recovery of any plus-variations in this series. Y55 (Salicin-plus mutant) should be tried on the beta-glucosides.

* Streaks out on corresponding medium.

[.005% glucose is apparently an excessive "boost". Use .001%. I. Also these sugars were evidently somewhat broken up by this prolonged autoclaving.

November 21, 1947.

1... Streak out presumed + and - derived from 58-161 on EMB-βΦgal. et

	βΦgal	galac.	galac+ΦOH	
a)	++	++	no growth	WY52
b)	-	++	no growth	WY53. (58-161 purified re βΦgal +)

(Note: this is failure to ferment, not growth inhibition.

2... derived from Y10.

segregation of Bϕ, Lac.
WS2 × YS3.

November 21, 1947.

Stocks which are Bϕgal + Lac+ and Bϕgal- Lac- are available. If there are three alleles:
Lac$^{\phi}$+, Lac + and Lac −, only the parentals should be recoverable. If there are two loci, the type Lac+ Bϕ- should be found in this cross. It can be controlled by testing the cross Lac+ Bϕ- × Lac − Bϕ- which should not segregate for Bϕ. For additional segregating characters, M+I may be used.

P21 Broc cultures inf to 25 ml/125 YP broth. Incubate overnight at 37°. A22. Transfer 5 ml ea. to new 25 ml YP. Incubate 9 AM − . Wash, etc., mix in T(0) and T(B$_1$) plates.

A. WS2 × YS3

B. WS3 × YS3

A24. Suspend in 1 ml. H_2O and streak out on Lac EMB to obtain single colonies of Lac+ and Lac- segregants.

Segregation of Lac, Bϕ.

November 24, 1947.

A. WS2 × YS3 (Lac+ ϕ+ × Lac− ϕ−)
B. WS3 × YS3. (Lac+ ϕ− × Lac− ϕ−).
Bϕ+ Bϕ−

Streak out prototrophs on EMBlac agar and separate Lac− from Lac+ in pure form. Test them on ϕgal.

A (0) 8: −

A (B1). 20 Lac− } all appear to secrete Bϕ+ on mass streaks.
7 Lac+. } Streak out 3+ and 3− on a Bϕg plate.

B (B1). 16 Lac−
1 Lac+

B 0) 1 +
2 −

ind. parentals.

	Bϕ	Lac	
WS3	+	+	
YS3	+	−	!
Lac− segs.	+	−	
"	+	−	
Lac+ segs.	+	+	
"	+	+	

If is possible that ϕgal− do not adept on βϕgalactoside but that lactase splits the glycoside! N. B.

Assumption that YS3 is Bϕ+ Lac− is incorrect.
All parentals must be retested.

Complementary genotypes. >3

Nov. 25, 1947. Y87 Y10

Plan. Cross $B-M-T+L+B_1+Lac-V_1^R \times B+M+T-L-B_1-Lac+V_1^S$

and recover B_1-Lac- segregants. Plate these colonies into BMTL lactose agar to suppress the parental and major recombinant type. The only types which could survive are B_1+Lac+ which includes the complementary genotype ~~B+M+~~ $B-M-T-L-B_1+Lac+V^?$ and also possible reversions of $B+M+T-L-B_1-Lac+V^S$ in re B_1-. This procedure affords at least some chance, however, of recovering the complementary type by selective means. 20 colonies plated.

#11, 13, 14, 15, 17. are Lac− (i.e. 5/20). Throw out other plates.

Streak these out.

1	T	
2	T	
3	T	
4	T	
5	T	
6	separate colonies	
7	separate colonies.	
8	T	
9	T	
10	T	
− 11	S.C.	1 colony.
12	T	
− 13		1 colony.
− 14	S.C.	1 colony. Pick.
− 15	S.C.	>10 colonies. Pick 1-5.
16	T	
− 17		3 colonies. Pick 1-3.
18		>10 colonies. Pick 1-5.
19	T	
20	T	

		BM	TLB$_1$	BMTLB$_1$
1	11-1	−	+	+
2	13-1	+	−	+
3	14-1	+	−	+
4	~~15-1~~	−	+	+
5	15-2	+	+	+
6	15-3	+	+	+
7	15-4	+	+	+
8	15-5	+	+	+
9	17-1	+	−	+
10	17-2	+	−	+
11	17-3	+	−	+
12	18-1	−	+	+
13	18-2	−	+	+
14	18-3	−	+	+
15	18-4	+	+	+
16	18-5.	+	+	+

B−M− probably are reversions of Lac; TLB$_1$+ maybe B$_1$− reversions of the TLB$_1$ parent. Use maltose instead which does not seem to allow reversion!

Streak out and test single Lac+ colonies for nutrition and phage. compare by segregants.

No complements found.

Comparison of various grades of sugars for EMB test.

November 20, 1947.

	Mal+	Mal-	Lac+	Lac-
EMB +1%:				
Lactose c.p.			[+++]	- alk+.
Lactose U.S.P.			+++	± alk-
Maltose, c.p. (Paragon)	+++	- alk++		
Maltose, c.p. (E+A)	+++	- alk+		
Maltose, purified (Merck)	+++	-	alk+	
Maltose, technical (E+A)	+++	±	alk±	

Larger colonies than c.p. lactose. Probably minimal amounts of monosaccharide.

24hr. readings.

+++ denotes good-sized colonies with deep, uniform purple-black coloration and a green metallic sheen. ± is faint pink coloration, suitable for scoring.

- denotes pale or translucent colonies. alk refers to development of blue coloration

Technical grade sugars therefore, seem to be suitable for preparation of EMB plates. Hereafter unless otherwise specified, EMB plates for mutant detection will be made up from Lactose USP (milk sugar) Mallinckrodt and Maltose (Malt Sugar) Technical, E+A.

Lucis, program, appar. follow:

	(c.p.)	(Merck USP.)
Maltose	.03	.002
Lactose	.002	.001

Adaptation Expts: Prelim.

Nov. 18, 1947.

Cells grown in lactose, β-φ galactoside + glucose are sedimented and washed. Resuspension ca 10^{9-10} cells/ml. Cells diluted to comparable concentrations. Add 1 ml cells to 1 ml 4% sugar + .01 ml M/5 phosphate buffer pH 7.0. Add .03 ml BCP .15% purple as indicator.

Made up 11.15 AM. Acid production as a + ... +++ scale.

		11:30	11:45	1:30	A 19.
Glu /	glucose	−	−	−	+++
	lactose	−	−	−	+++
	β-φgal	−	−	−	−
Lac /	lactose	++	+++	✓	+++
	βφgal	−	−	−	−
0φ /	lactose	−	−	−	++
	βφgal.	−	−	−	−

Urease in coli

3,

Nov. 20, 1947.

Prepare media with peptone 1% agar 1.5%, Phenolphthalein .01% ± glucose .2%, ± urea 2%.
After autoclaving, phenolphthalein turned slightly. This subsequently disappeared.

	A21	A22	A24
–	growth, no color	"	turning pink
Glucose	" "	"	"
Urea	Growth inhibited	growth, no color	"
Urea + Glucose.	Growth inhibited.	" "	"

This does not seem to be a satisfactory method for demonstrating urease.

Nov. 26, 1947.

Make up media containing ½% Na Formate, 1% peptone, 1½% agar and various indicators, ± glucose .3%

1. EMB. glucose a) [illegible] 24h. 36 hr.
glucose + formate b) [illegible] light lavender.

2. Phenolphthalein .01%
formate a) [illegible]; growth inhibited somewhat.
— b) [illegible], good growth.

3. Bromcresol purple. Add AcOH to medium until turned acid.
glucose a)
glucose + formate b)
formate c).
} no growth (pH?)

EMB seems to be the most suitable, esp. glucose + formate.

Methyl green sulfate - lactose:
Lac + colonies green, diffusing into agar
Lac − colonies translucent & light blue.
n. satisf. because of diffusion

EMB + sugar 1%: Iron streaked - out 58 161.

gentiobiose −
β methylglucoside —
α phenyl glucoside + uniformly.

Colony formation on synthetic agar.

~~2~~
52

Nov. 25, 1947

T(m) agar + various concentrations of sugars. Add BMTLB.

	24h. 58-161.	36h.	24h. Y87.	36h.
Lactose:				
.1%	as below.	2 mm.	microscopic pinpoint; papillae.	
.05%	small, dfinite.	1-2 mm.	microscopic pinpoint (.1 mm)	
.01%	pinpoint.	1 mm.	no visible colonies; micr.	

.1% is a satisfactory level of carbon supplementation.

later, Y87 shows continually forming papillae on all plates.

On .1%, Y87 forms distinct colonies certain proportions of which contain revertants. .01% is also suitable.

November 25, 1947.

Cross W52 × W-1 on O, B, agar.

B-M-T+L+B,+Lac+Bϕg+Mal+ × B+M+T-L-B,-Bϕg-Lac-Mal-.

Came up very slowly and in small numbers. Segregants not used in view of 27b.

~~Use for maltose segregation~~:

Nov. 26, 1947.

Streak out 58-161 on EMB agar: .3% W2 Aminé B, 1.2% galactose A 26.

A Definite colony dimorphism as previously described. : S R. about 1:1

B. Streak out components and mixture on Galactose EMB. A26.

W-28 + W29. .

Reversion? of C-2 mutants.

Nov. 29, 1947.

Plate 24hr. YP cultures into agar supplemented as indic. 10^9 per plate

Y138: T(0). No colonies.
Y138: Arginine: 1 colony?
Lysine: No colonies.
Arg + lys. No colonies. Not turbid!!!

Y142. T(0). >30 colonies.
+ isol + val. "
+ arginine + isol-val. >100 cols. Only sl. turbid.
+ arg. turbid.

Y138 + Y142 ... 0 >30 cols.
A. turbid. colonies form.

Check the requirements of these strains!!

Check C_2 mutants.

11/29/47.

		T(0)	T:	T:	T:	T:	
	Y						
1.	114.	0: −	iso −	val −	i+v. +	+++36	48 hrs. OK!
2.	117	0: − ±36	arg. +++			+36	adapted.
3.	120	0: − ✓	val + +++36			OK!	Try crossing with 138 or 139, or make mutants from this strain.
4.	121	0: − +++36	cyst +++			adapts.	
5.	132	0, −	arg. −	gly −	arg. −	no growth, 36h. ✓	AB Both A+AG +++ Recheck Req.
6.	133	0, ±	arg ± +++36	lys ± +++36.	arg +++	adapts.	
7.	134	0:	arg	thre	arg.		
8.	137	0:	arg	trpp	arg		
9.	138	0: −	arg −	leuc −	arg leu +++ OK.		all OK.
10.	139	0: −	arg −	hist −	arg hi +++ OK.		T(0) OK others adapted.
11.	142	0, − +36	i+v − +++36	arg +++	i+v	+arg. +++.	all +++. Requires arginine only! adapts on [illegible] +½!

First readings at 14h., 2d at 36, 3d at 48. Inc. at 37°.

Y142 is very adaptable. Y138 + Y139 are fairly stable, especially Y138. do. Y120. and Y114.

Utilization of starches.

Dec 2, 1947

.05% in T(m) (BM) and 1% in EMB.

A Amylose (Clinton – from K.P.L.)
B Amylopectin (do.)
C Waxy Starch, soluble, from Brink.
D. Glucose.

P 11. Continued, slow utilization of amylopectin noted. to "++" compared to +++ for glucose.
v. slight utilization of ~~B~~ W$_x$ noted.

P16. Continued increase in turbidity. Density ≅ eq. ~~.1%~~ .01% glucose

P24 Utilization apparently complete. Rate measurements were exceedingly crude. Waxy starch was not utilized to nearly the extent that amylopectin was. This should be repeated for confirmation. Save flasks of amylopectin culture.

Exp. terminated this date.

Jan. 7, 1948. Compare molds from B with V55 inoculum on T(m)/BM + following:

		A10	A17	Iodine color A17
Ap. .05%				
Amylose .05%	α	±	+	faint red-violet.
	β	–	±	blue
Waxy starch.	α	++	+++	No color
	β	±	±	~~blue~~ blue
	α	+	+	Light Red
	β.	–	–	~~blue~~ Undetermined.

SC 86.

all starch utilizations are correlated there. Possibility of adaptation rather than mutative utilization not excluded. Compare c inulin on EMB!

Synthetic EMB Medium.

Nov. 29, 1947.

Medium,	per l.	/200 ml.
Na Succ. $6H_2O$.	5	1
Lactose	10	2
$(NH_4)_2SO_4$	5	1
NaCl	5	1
$MgSO_4$	1	.2

EMB; Agar

(Phosphate is in EMB mixture).

OK! Compare K-12 +++

B_1-lac- -- (+ B, added).

Dec 1, 1947.

W-1 x W-53. T-L-B,- Lac-Mal-Sø+ x B-M-Lac+Mal+Sø-

a) T(o) plates.

	Mal+	M-
Lac+	2	15
L-	2	44

b) T(B,) plates.

	Mal+	Mal-
Lac+	1	10
Lac-	.2	47

Total:

Lac+	3	25
Lac-	4	91.

123.

As %

	M+	M-
L+	2.4	19.7
L-	3.1	71.6

Total Lac+ / Lac- = 22%

	M+	
L+	2.4	20.3
-	3.2	74.0

Lac+ = 22.7
Lac- = 77.2

Mal+ = 7.6
Mal- = 94.3.

∴ Mal is v. clearly linked to B-M. Evidently not to B, in view of homogeneity of distributions.

probably between B and Lac. This leads to an excess of the triple type, M+L+. check on each purported example here of M+. Check L+. Scores count.

Dec. 3, 1947.

From same cross plates as 41, streak colonies on maltose agar + count, pick out M+ for lac characterization. (TB, plates).

M+	M−
1	38
0	47
1	50
1	27
3	36
4	43
3	31

15 / 285. 272 / 287.

Mal + = 13/285 = 4.6%.

Test all Malt + on lactose:

	Lac +	Lac −
M+	10	5

Summary of Lac distribution among Mal+:

+	−
3	4
10	5
13	9 / 22

Total distribution:

	M−	M+
Lac −	272	15
Lac +	116	7
	388	22 / 400.
	94.5%	5.5%
Lac −	74.1%	2.2%
Lac +	20.4%	3.3%

From same plates as 41, segregate Lac+ and Lac- and streak an isolated colony on βφgal agar, EMB.
at 24 hours:

	Lac+	Lac-
βφ+	20	36 +1
βφ-	0	~~1~~ 0

20. 37

The parents were compared by streaking from YP broth and, unfortunately, are not comparable. Neither W-1 nor W-53 was readable at 24h.

~~Isolate all segregants to small plants~~

Parents are also both βφgal+ and cannot be distinguished. A modifier may enhance βφgal-ase in ~~Y53~~ W-52.

To summarize, all available Lac+ are βφgal+

The "Lac-" of Y53+der., Y87+der., W30, W40 and W42 are βφgal+; The "Lac-" of W35;36, W43, W45, W48, W49

Maltose segregation. 41d.

Cell suspension stored 2 days in H_2O at room temperature was plated on T(0) and T(B.) as well as EMB.

On EMB, None of 171 was Maltose +.

On 3 comparable plates only 2 possible Malt.

On T(0). None of 139, streaked to Maltose, was Lac+.

(Check for B. interaction again.

On EMB, Lac segregation was: Plate rather crowded.

+	−	
40	66	Some colonies were noticed to be sectored!, as
16	37	if complementary or supplementary types were
56	103 /159	present.

In this sample, therefore, only 2/>600 was Mal+. Compare with above!

12/ /47

Lac- Bϕ- Lac+ Bϕ+

W45 x Y10.

T(0)

T(B,). Streaks to synth Lac(B,). →

Lac+	Lac-
68	9
58	3
126	12. / 138.

Lac- 8.6%!

Few or no Lac- noted on EMS-lactose crossing plates [Spiegelman phenomenon?].

This is a much lower proportion of Lac- than hitherto found.

Streak Lac- and Lac+ to Bϕgal

	Lac-	Lac+
Bϕ +	0	40
Bϕ -	12	0

[Check for alleleism with Y53.]

Suggests identity of Bϕ and Lac loci.

Cf.

Maltose segregation:

A. Y+0 x W-1 Lac + Mal + Lac - Mal -

~~B. W-20 x Y64. Lac + Mal - x Lac - Mal +~~

all plates too crowded.

On EMB nearly all Mal -. < 1:100 Mal +. These can be picked out more readily than in the previous cross. However, the plates are too crowded to be very useful. Use T(0) etc. plates to confirm ratios.

Y120 × Y133.

12/4/47.

See 43 for cells.

(1) Incubate at 37° only (plates).

Y120 10^{-7} colonies

Y138 No colonies — two plates

Y120 + Y138. As above

~~Add 1 loopful to 4 flasks for further incubation~~.

Y120 is too unreliable for sex tests

12/4/47.

Inc. Y120 into YP. Incubate 3 ml. suspension in gyratory flask. PS.

A6. Inoc. 10^{-7} dilution into T(Val) plates (detection).

A8. Layer 1% Y.Ex., 1% N2 Fone, 1% Hyac on plates.

15 plates. Sample counts:

58
72
71
54
70
54
60

7|419 =60 average

(3) small colonies recovered.

-1 Not mutant, though inhibited by isoleucine

-2 Contaminant

(3.) see ff.

Test Jan. 6. 1948:

		Value +	3 do.
1.	—		—
2.	NA		—
3.	EA		—
4.	N+EA		—
5.	MC (GBI)		±
6.	N2 Case		+
7.	Y. Ex.		+++

Acc. 5 [illegible], Calc. mg. on nits.

Allelism of Y45 Lac− and Y53 Lac−

December 4, 1947.

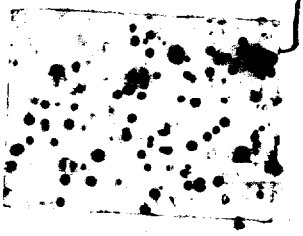
Y45 × Y53. On T(B,) and EMS Lac(B,)

On EMB. Ca. 1:6 Lac+ : Lac− !!

[This suggests faulty identification of Y45 as Lac−
1) May be Lac+
2) May be $Lac_2^- Lac_1^+$]

~~Reddish~~

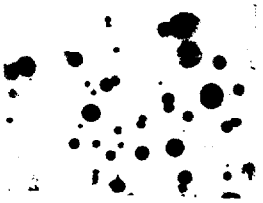
+	−
ca. 16.	40



Yields seem to be higher on EMB. Come up with varying lag.

From T(B,).

−	+
25	4
19	7
31	8
22	8
97	27 /124



W45 x Y53.

Repeat 46. Check parents: Both −. W45 Allealigenic.

Lac+ present in cross! Of 41 isolates from T(0), 8+ 33 −

Streaked out from T(B,) on Lac EMBagar to purify. also, 29− 4+

62− : 12+ /74

ca 5 : 1

On EMS Lac, most plates too heavy.

5 Thanium,

+	−
3	11
6	11
6	12
3	3
18.	37 /55

The EMS procedure seems to be biased for Lac+ compared to T(0) plating. It should possibly be improved.

1 Thanium.

9	20
14	31
12	35
34	56
69	142 / 211

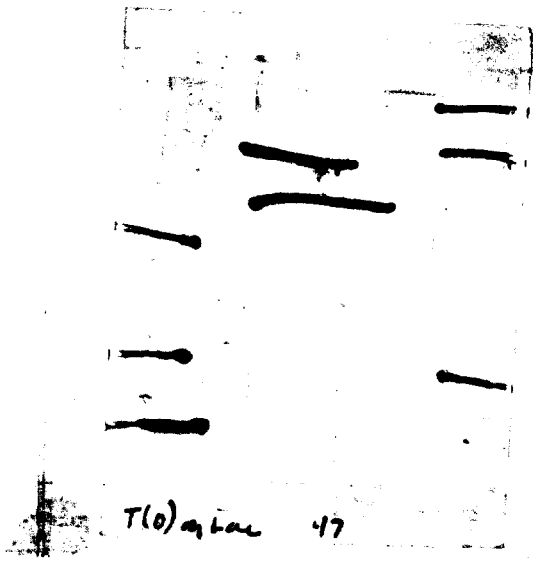
Read from paper impression slips.

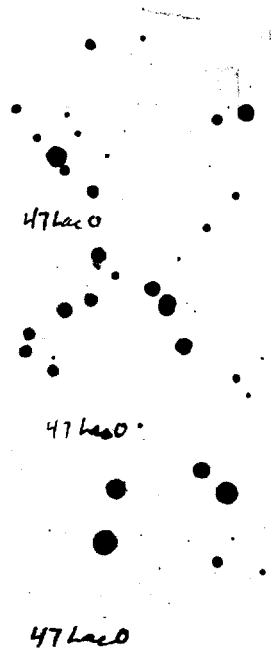
of Same average ratio, however 67% Lac −, whereas the random isolations give 80% Lac −.

χ^2 for difference is, approximately, at the 5% level.

8	33	41
14	28	

$\chi^2 = \text{ca. } \frac{36}{14} + \frac{36}{28} = 2.5.$


T(0) a Lac 47


47 Lac O
47 Lac O
47 Lac O

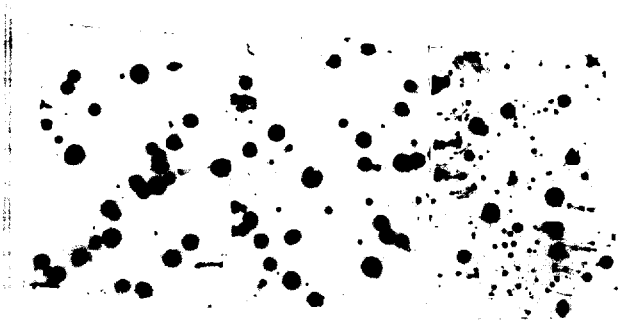

B1
B1

Maltose Segregation

W-21 × Y64 B-M-T+L+B+ Lac+ Mal-V_1^S × B+M+T-L B_1, lac- Mal+V_1^R

An EMB., nearly all Mal+. << 1:100 Mal-

Cf. 43, where cross where Malt+ is very rare.



Dec. 8, 1947.

a). Raffinose 3%, Melibiose 1% + Salicin 1% EMB. Streaked 58-161. Dec. 7.

		A9.
R	− to ±	
Sal.	−	~~++~~
Meli	++	Colicin therefore split glucose α-galactoside but not sucrose α-galactoside!

b). Same sugars, .05% in T(m) + BM. 48 hours reading.

		A9.
R	±	
Sal	±	++)
Meli	+++	
Glucose	+++.	

"Streak to Salicin EMB and inoculate second tube of T(m) + Salicin. + and − colonies seen. Selection for Sal+ has therefore been successful. Sal+ is W-55

Test 453, W-45 on melibiose: both +++.

E-M-S- Modifications.

EMS: old formula, + : , streak K-12. Read at 48-72 hours.
(succinate)

		Growth	Color.
—		±	—
K Glucarate	.1%	+	±
	.2%	++	+
	.5%	++	++
	1%.	++	+++
Glucose	.05%	+++	—
	.1%		±
	.2%		+
	.5%.		+++

Glucose .05% May be useful. Try with Na formate equimolar, or perhaps with K-saccharate.

Supplementary Recombinants.

Dec. 9, 1947. See 51a

Y40 x W-1.

Plate v. dilute on EMB lactose (B_1). and on EMS Mal ± B_1.

Look for sectoring. Count only clearly scored in uncrowded portions. Yields much higher on M'. from 20-100/plate.

	+	−	sectors.	
M(0).				
1.	2	32	0	
	1	38	0	
	4	32	0	
	3	25	1	v. clear sector. (1)
	12	117	1	(2)
	22	244	2	288.
2. M(B_1).	15	285	5	

seems to be fairly frequent among Mal +. However, plate is too crowded for accurate estimation.

3. Lac B_1. Yields lower than on Mal. (Mal contaminated with amac., etc?)

	+	−	sec.	
1	1	9	0	
2	17	32	0	
3	16	16	0 2?	Not clearly duplex. Maybe all Lac+.
	17	27	1?	Almost certainly not contam.
	6	33	0	
	6	18	1	v. clear
	12	21	0	
	9	14	1	v. clear (+)
	9	16	0	
	0	3	0	
	99	189	5.	

Streaks out mosaics. Test Remainder of population to get complete score on Lac, Mal + V,

Supplementary Recombinants — Maltose Segregation.

Dec. 9-14, 1947.

Y4/0 × W-1. Lac, Mal T1^R segregation.

Plate very dilute on EMS agars. & looks for sectoring (A) Scored by inspection of plates.

		Mal+	Mal−	Sectored	Sum
1. M(0).		22	244	2	268.
2. M(B,)		15	285	5	305.
Sum.		37	529	7	573
Mean.		.067	.92.50	.012	100.00.

About 8% of colonies carry Maltose +.

		Lac+	Lac−	Sec.	Σ
3. Lac(B,).		99	189	5	293
	$\bar{M}$	.338	.646	.017	

(B) Sample colonies from Lac to Mal & Mal to Lac.

B.

		Lac+ R	Lac+ S	Lac− R	Lac− S.	Σ
From 1. to Lac.						
1	Mal+.	4.		12		16
2	Mal−	45	1	66	7	119 ←

Phage scores probably unreliable from appearance of secretors. Not too good a fit with 3:1. 39% Lac+

From 2. to Lac.						
3	Mal+	10		6		16
4	Mal−	No tests.				
		Mal+ R	Mal+ S	Mal−R	Mal−S.	
From 3. to Mal.						
5	Lac+.	1	0	66	3	70
6	Lac−	0	2	89	21	112.

Compare R/s with B2!

Scores on 51 organisms.

Maltose, agar, minimal. Phage scores uncertain.

Maltose +. 12 Lac − V_1^R
4 Lac + V_1^R.

Maltose +. B, agar. 10 Lac − V_1^R.
6 Lac + V_1^R.

Maltose +. 22 Lac −
10 Lac +

Maltose −

Lactose B, agar.

	Malt+ R	Malt+ S.	Mal− R	Mal− S.	
Lac+.	1		14	1	
			7	1	
			9	0	
			~~10~~	~~0~~	
			2	0	
			8	0	
			9	1	
			7	0	
	1	0	66.	3	70
Lac− →	0	2	89	21	112.
			7	1	
			8	1	
			6	0	
			6	3	
		1	4	1	
		1	7	0	
			2	0	3 0
			15	3	15 4
			8	8	6 5

M –

Lac+ R	Lac+ S	Lac– R	Lac– S
12	5	5	
		2	
3		1	2
3		3	
5		9	1
6		9	
3		7	
4		4	1
4	1	7	1
2		10	
3		8	1
		1	1
45.	1	66	7

Phage sources unstable

Cumulative data on maltose:

a +:-	−	+
	272	15
	244	24
	285	20.
	801	59 / 860.

M+ = 6.8%.

Lac+:- in Mal +: ~~15~~ : 13 Lac+ : 9 Lac −
22 + : 10 −

35 Lac+ 19 Lac− / 54

The 2.4% triples compared to 4.4% singles imply a map distance ca. twice that found on the basis of the Lac, V data. A crossover in the Mal Region may, by interference (a) ~~coincide~~ favor additional crossovers to make unrecoverable that chromatid, or (b) augment the relative frequency of triples.

Dec. 9, 1947.

Spread W-1 and T1 on EMB-Maltose plates to select for spontaneous T1 resistant mutants.

Numerous, well-defined smooth W-1/1 found.
Streakout one such colony to provide (W-54) derivative. (1)
Test 70 others. all ~~#~~ resistant to T5.

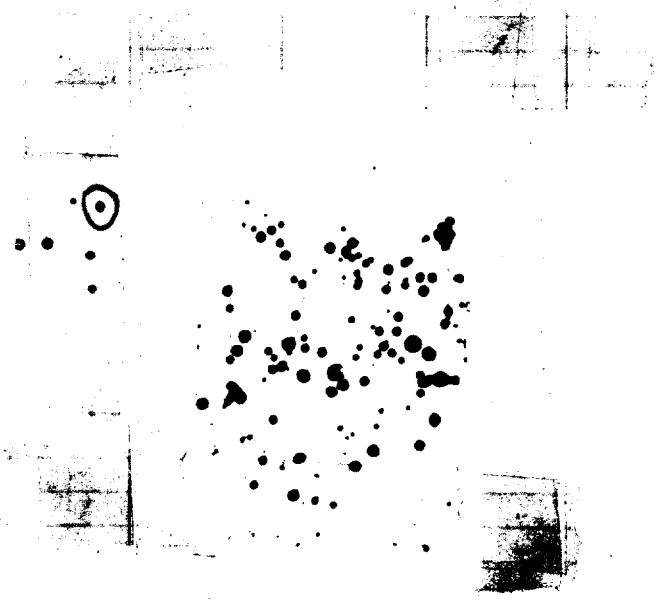
Dec. 9, 1947

W-45 (Lac_2^- B_1^+ Lac_1^+ × Lac_2^+ B_1^- Lac^- $Mall^-$)
×
W-1.

Plate dilute on B, EMS Lac.
Numerous Lac+.

+	−	
32	68.	/100

plates stiff scatter crowded in some cases.
Some probably valid sectoring noted.



Streak out Lac− on Lac agar to look for reversible Lac− types.

Pick single colonies to maltose plates to score Mal and to provide cells for reversibility test.

of 42 Lac− tested, #41 Mal−
1 Mal+

Test all of these for Lac reversibility on lactose .05% medium.
All marked reversible turbidities with heavy inoculum. Test by loopful streak on Lac EMB. all+ except number 5.
Keep as (53-5) If this is irreversible, regard as Lac_1^- Lac_2^- and test by recombination tests.

W-1 53-4 results!

Streak out, on EMB Lac all produced papillated colonies, although only one rec. colony of W-45 was papillose.

53-4
53-5
53-6 – note that two colonies were non-papillogenic. Retest!
Y53
W45

Dec. 9, 1947.

Irradiate .1 ml Y40 per EMB plate, 20 plates 10 secs. u.v. Hanovia lamp

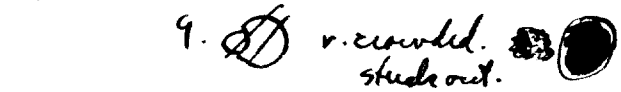
Irradiation by D.G.

Lac. 59 plates. ca 200 readable colonies/plate = 12000.

1. Crowded plate  maybe juxtaposition. 8. 

2.  v. clear sectoring.

3.  debris.

9.  v. crowded. streaks out.

4.  small colony.

5.  v. small crowded plate

6.  both pure white. separated.

7.  v. small.

Mal. 45 plates. = 9000

11.  Tiny sector, scarcely distinguishable.

12.  [redacted]sector "ws" at best. Streak out entire colony

13.  clear, small sector. Streak out vicinity of sector.

14. sector indistinct

15. 

16.

17.  sector indistinct.

18. Intact white.

19. Intact white

20. Intact white

21. 

22. Like 12.

23. 

24. O intact white

25. 

Classification of Mal + Lac mutants.

Dec. 13, 1947.

11. Dark + dull blue colonies noted. Streak separately in effort to find destinction

12. Some colonies light brown in transmitted light. As above.

13. Mostly – W56 Some + W-57. Also some v. light ± Restreak these.

14. Some definitely ± compared to stand. Re.

54-15 Remic.

54-16 Remi.

17 No marked variation.

18-20 Remi. 18: W60
19: W61
20 W62

22. As 11.

23. as 11.

25. Mostly –. A few + in sectors. – W58
+ W59. Restreak.
+'s in form of sectors.

Dec. 15, 1947.

54-11 ~~Restreak~~. Reject.

54-12. No difference noted.

54-13. 3 types noted. – W56 } Repeat comparison.
+ W57 }
± W63 }

54-14. a) Mal ++

b) Faded in part of streak. Reject.

15. a Turned Blue. Restreak c = Mal- b like a. restreak. W71.

16. Restreak.

21 Restreak

22. Restreak.

23 Restreak.

24. W64.

25 W.59 (Mal+"). Vc sectors. Pick b slant.

---

1. + and - colonies. – W65
+ W66

2. 2+ 2- sets. – W67
somewhat mutually – W68
contaminated. + W69
+ W70

3. Indistinguishable pairs

4. ~~Test~~? + and – – W72
+ W73.

5. ~~Test~~? + and – – W74 + W75.

~~6. a + b. W76 W77. May be sibs.~~

7. + and – Mostly –. – W76
+ W77

8. Restreak.

9. all +.

Dec. 16, 1947.

-16 two types of colony.
a. + W79
b. - W78

22- a. + W81
b. - or ± W80 + weak? Definitely less than c.

8 - a Mostly + colonies. W84
b. = Apparently - colonies } Pick all of these W83
c. " " ". W82

23. Indistinguishable.
c. Indistinguishable

15. b. = Mal+. W85.
a. + and -. Probably mixture. - W71a.
c. = W71

Lac − alleles by Lac reversion.

Dec. 8, 1947. and earlier.

Streak out various Lac- strains on synthetic Lac agar to select for reversions. Streak from reversion colonies directly onto β-D galactoside and read after 48 hours. Original responses from previous data.

		or.	a (Reversions)	b	c	d	e	f	g	h	i
1.	Y53.	+	+	+	+	+					
2.	W35	−	±	±	+	±	±	+	+	±	±
3.	W30	+	+	+	+	+	+	+	+	+	
4.	W36	−	±	±	±						
5.	W40	+	±	±							
6.	Y87.	+	+								

Isolate on Lac EMB the W35 series.

2: a. Only an apparently "weak" positive on lactose. Keep.
b. Lac ++.
c. Mostly ++. Some Lac −.
d. Lac ++
e. Lac −
f. Lac ++.
g. Lac ++
h. Lac −
i. Lac −.

Types.

	βϕ	Lac
a :	±	−
b	−	+
c	+	+

Compare:

	βϕ	Lac	
58-161	+	++	
W-35	±	−	
a	±	±	
b	±	++	
c	+	++	
d			like b
f.			like c
g.			like c
~~h, i.~~			

Keep on slants.

Also compare c W-35 on Lac ~~[illegible]~~.

Dec. 15, 1947.

Y132. in Arginine T(0) + : Incr. P15.

		A16
1.	—	—
2.	—	—
3.	NEA	—
4.	EAA	—
5.	N+EAA	—
6.	HC	—
7.	Y Ex	+++
8.	Vits	—
9.	HC + Vits	—
10.	Glycine	—

Yeast Extract Mutant?? try N2 case, Nucleic acid.

45-1, 2, 3 in: Valine T(0) +

	1	2	3
1. —	±	not coli	—
2. EA (circled)	+++		—
3. NA	+		—
4. Vits	±		—
5. Pur+Py.	± (maybe pur)		—

No growth on any of these! see 46. W-1.

1st reading A16

No mutant. Grows on T(0), + all T(+ ess. amino ac.) except isoleucine which seems to inhibit it!

U-1A. on T(0) + Inc. at 22-28°. (surface of water bath at 37.

	A16	A18
1. Y. Ex.	—	++
2. Vits.		++

N-1 on T(0) +

		A16
1.	—	++
2.	~~2.5~~ .25 ug/ml CAB (2-chloropab from Strandskov)	+ ~~++~~
3.	do. + methionine 10γ/ml.	+++
4.	do. + pab .1γ/ml	+

YNA. Preparation. Dissolve 25 g. Schwarz Nucleic acid in 125 ml H_2O + 11 ml 28° NH_3 water in 500 ml flask. Wire stopper and autoclave at 15 lbs.

Maltose mutants on trehalose

Dec. 15, 1947.

Test the following on trehalose E17B. 16 4.

		Tre.
Maltose + :	Y40.	+++
Maltose − :	W1	+++
	W3	+++
	W21	+ weaker.
	W60	+++
	W61	+++
	W62	+++
	W56	+++
	W64	+++
	W58	+++.

Trehalose is, therefore, attacked by Maltose negative mutants. Cross adaptation should be checked. ✓

		Tre	Mal
Dec. 18, 1947.	W-63	+	+
	W-71	+	−
	W-78	+	−
	W-80.	+.	+

all maltose-negative mutants so far found are Tre- +.

Nutrition of Y132.

Dec. 17, 1947.

T(0) +.

		A17 (16h.)	A18.
1. Y. Ex.	.5%	+++	✓
2. "	.05%	++	✓
3.	.005%	±	✓
4.	.0005%	−	✓
5. Y.N.A.	.5%	−	++
6. N2Case	.5%	±	+
7. —		−	−

N2Case is much less active than yeast extract.

YNA has some activity - only ca. .1 − .01 of yeast extract.

Try Casein, Acetate, other protein hydrolysates, e.g. gelatin; lactalbumin; fat-soluble incl. oleic acid.

T(0).

1. −
2. Y. Ex. .1%
3. N2-Tone .5%
4. N2Case .5%
5. N2AmineB .5%
6. N2AmineA .5%
7. Casein .5%

Suspend 10g. Y. Extr. in ca 30ml $CHCl_3$. After 1 hr. filter. Evaporate $CHCl_3$ from extract and take up in # 20 ml H_2O. Do. Residue, taking up in 200 ml H_2O.

Lac-1 and Lac-2 mixtures

Dec 18, 1947.

Make up 10 mm tubes of lactose 1% BCP broth.

	Add .5 ml inocula of :	6 P 16	Set up 2P16 10 A 17	48	A18	
1	W-45	-			++	*
2	W-45	-			++	
3	W-54	-			-	
4	W-54	-	The Same		-	
5	W45 & W54	-			++	*
6	W45 & W54	-			++.	
7	K-12	///				
8	K-12	///				

Therefore mixtures of Lac-1 and Lac-2 are unable to utilize lactose, although recombinants are able.

* Streaked on lactose. Probably revertants
Mostly + colonies. Streaked out to get W-45^R for allelic tests.

Dec. 17, 1947.

Harvest ~~x~~ W-45 (Mal+ Lac-1+ Lac-2-) ~~x~~ and W-54(Mal-Lac-1-Lac2+ V_1^r) from fresh YP cultures, and mix at a conc. ca 10^{10} /ml each in water. Store over night in refrigerator. Dilute to 10^3 / ml. and spread .1 ml on EMB-Lac (NZCase) plates to ~~detect~~ possible Lac1+Lac2+ recombinants.

12/18 PM. 111 plates x 357/2 totalling ca. 40,000 colonies examined. None were Lac+. This is a control on the reversion of both Lac-1 and Lac-2 The recombination rate under these conditions is apparently too low.

Nutrition of Y132

Dec. 18, 1947.

Inoc. into T(A) ∠

	A19.	P19.
1. Y. Extr. .5%	+++	✓
2. Y. Extr. .05%	++	✓
3. YX Residue .5%	+++	✓
4. YX Residue .05%	++	✓
5. YX Extract .5%	±	bacteria in initial extract.
6. YX Extract .05%	–	✓
7. Gelatin Hydrolysate .5%	±	+
8. Tomato Juice .5%	++	✓
~~9. Casein .5%~~		
10. NZTone .5%	+++	✓
11. NZAmine A .5%	+	++
12. NZAmine B .5%	±	±
13. YNA intact (NaNucl.) .5%	±	±
14. YNA hydr. .5%	–	–
15. YNA hydr. .5% + YX .5% test for inhibition.	+++	+++
~~16. xxxxxxxxxxxx~~		
16. --	–	–
17. NZ case .5%	±	+++ (adaptation? or auxin?)
18. Oleic acid .001%. Free acid still at surface.	± - +	turbidity not due to bacteria.

[Try Tween, Na oleate, etc. (Acid extract of fresh yeast!)] [Maybe in NZ case?]

10g. Yeast Extract Difco extracted with 40 ml. $CHCl_3$ in flask. Separate, evaporate extract and take up in water. Expressed in terms of original yeast content. (Very little material was extracted, perhaps 1-5mg. at most.)

W5-3 in T(V) +

		P19
1. –		
2. YX .2%	++	++
3. HC+V	+	++
4. NZ case	–	++.
5 YNA		

2-Chloro-4-aminobenzoic acid
Inhibtion and resistance mutations

Dec. 15, 1947.

Prepare plates of T(0) agar with 25 mg% CAB. Do. (0) agar.

Spread ca 10^2 cells of K-12 on both, incubate 72 hrs.

A) T(o) agar: 400 colonies noted

b) CAB: ca. 42 colonies noted. However, direct microscopic observation and smear impressions show a large number of "micro-colonies", probably equivalent to the difference between CAB and T(0) plates. Each colony contains, as a guess 10^4 - 10^5 cells.

(This suggests that Strandskov's observations can be accounte d for on the basis of spontaneous mutation and selection among the relatively large numbers of cells in the micro-colonies.)

Dec. 16, 1947.

Harvest from YP and cross W-55 x W-54, heterozygous for Lac_1, Mal, Sal,B_1 as well as V_1^r. Cross on EMS-maltose with .002% glucose added. $+B_1$.

A20. a) Estimate frequency of maltose+, and of sectored colonies. Score only those where the sectors could be scored accurately.

Proportion of Mal + (including sectors). Count sectors as 1 + and 1 −.

	+	Sec.	−
1	1	2	57
2	4	0	139
3	3	1	117
4	2	0	77
5.	7	2	132.
	17	5	512

$\frac{Mal+}{Total} = \frac{17+5}{512+5+17} = \frac{22}{534} =$

4.2%.

Proportion of sectored to plus colonies: (Score under conditions stated above)

Plate	Sec.	+	#	Sec	+	#	Sec	+
# 14	2	6		1	5			
	0	3		2	3			
	0	2		1	4			
	1	6		0	3			
	1	1		1	1			
	0	2		2	1			
	0	4		0	2			
	1	1		1	4			
	0	2		3	3			
	0	1		1	7			
	1	1		4	1			
	0	3		1	2			
	2	4		0	4			
	2	4		1	3			
	1	3		0	2			
	4	2		2	2			
	1	4		2	3			
	1	3		2	3			
	1	1						
	0	4						
	2	2						
	1	3						
	0	2						
	0	1						
	2	3						
	1	1						
	1	2						
	1	1						
	2	2						
	0	2						
	0	1						

52. 130 / 172.

30% of the Mal+ colonies also have a Mal- segregant.

a20

Score Mal- segregants re Lac and V_1. Also Score Mal+

Mal-	Lac+ V^R	Lac+ V^S	Lac- V^R	Lac- V^S
	0, 1,	10, 9,	5, 4	4, 2
Mal +	Lac+ V^R	Lac+ V^S	Lac- V^R	Lac- V^S

(Not scored well on Lac) too heavily contaminated with parental to score on EMB. Recover Mal+ from these plates & test on EMS.

Obtain new sample of Mal- from cross plates.

Pick 57 apparently sectored colonies to water N20. Store in refrigerator for later separation. Streak out on Mal.

Scores on Mal p/m components of maltose sectored colonies.
Lac p/m and V_1 r/s

Colony	Mal p	Mal m
1	ms	ms
2	ps	ps
3	ms	ps
4	ms	ms
5	ps	ps
6	ms	ps
7	ps	ps
8	ms	ms
9	ms	ms
10	mr	ps
11	ps	ps
12	ps	ps
13	mr	mr
14	ms	ms
15	ms	ms
16	ps	ps
17p	ps	ps
18	ms	ps
21	ps	ps
22	ps	?s (m)
23	ms	ms
24	ps	ps
25	ms	ms
26	ms	ms
27	ms	ms
28	ms	ms
31	ms	ms
32	ps	ms
33	ms	ms
34	ps	ps
35ms	ms	ms
36	ps	ps
37	ps	ps
38	ms	mr
39	ps	ps
40	pr?	ps
41	ms	ps
42	ps	ps
43	ps	ms
44	ms	ms
45	ms	ms
46	ps	ms
47	ps	ps
48	ms	ms
49	ps	ps

Scoring very clear except where total lysis may have obscured fermentation reading in 22

Totals: 45 tests.

	Mal +	Mal −
− S	23	~~20~~ 22
− R	~~20~~ 22	
+ S	20	
+ R	1	

	Mal +	Mal −
− S	22	21
+ S	20	22
− R	2	2
+ R	1	0

M+ ↓ / M− →	−S	+S	−R	+R.	Σ
−S	17	4	1	0	22
+S	4 ~~20~~ ~~18~~	~~4~~ 16	0	0	20
−R	0	1	1	0	2
+R	0	1	0	0	1
Σ(M−).	~~24~~	~~20~~ 22	2	0	45.

Compare − and + only.

	M− (L−)	M− (L+)	
M+ (L−)	19 [12]	5 [12]	24
M+ (L+)	4 "	17 "	21
	23	22	45.

$\chi^2 = 16.2$

$p \ll .001$.

∴ There is a definite correlation between the Mal− and Mal+ components of sectors in re Lac segregation. Recover pairs to Mal plates.

(Y54 x Y55.) . Mal-Lac,- V_1^R x Mal+Lac,+ V_1^S.

Lac, V_1 scores of intact colonies:

A) Mal +

−R	−S	+R	+S.	
5	4	0	7	
2	7	0	9	
3	4	0	10	
2	4	0	1	
12	19	0	27	/ 58.
21	32	0	47	

B) Mal −

5	7	1	10	
7	5	0	10	
5	6	2	5	
4	7	0	10	
4	3	0	11	
25	28	3	46	/ 102
35	39	2	64	

37. 47 3 73 160

[23.2] [29.4] [1.9] [45.7]

$\chi^2_2 = .85$. $p = .6$

Compare with Table 5 of Kimura's paper. Ratios were.

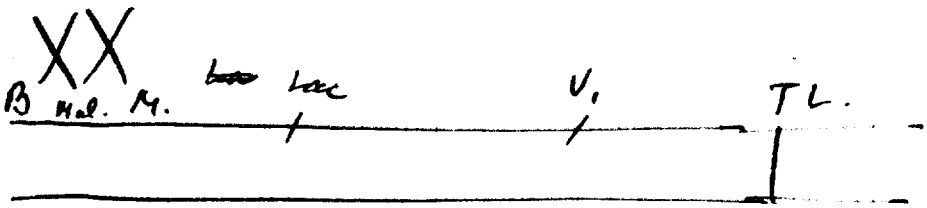
Is this medium keto-sufficient?

Mal must be between M and B. (no interaction with Lac).

Compare with sectored colonies:

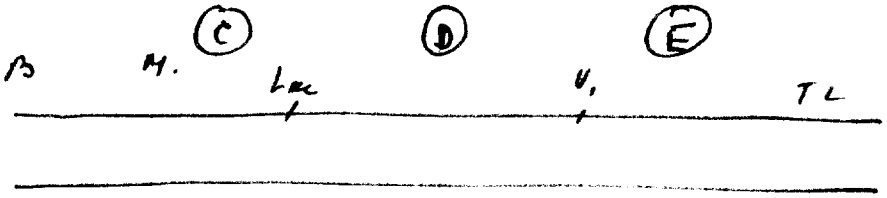
Intact.	37	47	3	73	160.
Sectored	46	43	1	42	90.

Note: Absence of -R and deficiency of +S.



a) a double crossover in Bal region does not interfere with chromosomes to the right - homogeneity of Mal+ / Mal-.

b.



If a double takes place, it tends to be both in region D or both in region C. Very few doubles are recovered involving region E.

Mutagenesis with GE 100W AH-4 lamp Unit.

Killing curve.

Ded. 24, 1947.

Spread .1 ml. young (ca. 10^9) 58-161 culture on EMB-Lac plates. Irradiate at 25 cm.

Lamp borrowed from Stauffer. operated in horizontal position, with 10 mins. warm-up allowed. This lamp has section of glass cut out to allow unfiltered uv radiation.

	Time	Surv.
	5s	~37L + 33S. = 70. ca. 10^4
*	10s	ca 100L + ca. S. 200.
*	15s	32L + ca 100S. = 130. Including white colony. Streak out + test with T1
	20s	17L + 26S = 43
	30s	13L + 9S = 22

Are these curves really non-linear? What about resistance of residuals?

Large and small colonies noted. Restreak for mix. determination;

* Reject as probable contaminant: not lysed by T1.

The difference between large and small colony noted on the above survival plates breeds true on the first restreaking on EMB-Lac. Transfer to slants as 67-1 and 67-2 for the large and small respectively.

Dec. 25, 1947.

Use same cells of 58-161 as in Exp. 67. Expose .1 ml culture per plate to 15 secs. UV from GE AH-4 lamp, as in 67. EMB - Trehalose plates

A). 9 plates.

Most plates, due to faulty pouring, had pitted surfaces and were very unsuitable for scoring. 1 plate, prepared earlier was more satisfactory. This had many (5-10%) colonies which had radial striae suggesting numerous variations affecting intensity of fermentation. Hold plate in refrigerator for later testing.

1/3/47. No mutants found.

Dec. 25, 1947.

Set up as in 68. EMB Mal plates.

Ca. 11 x 200 or 2200 colonies. 1 sector noted. Pick to slant as 69-1 for later verification.

large and small colony types seen as above.

Jan 2/48 Streaked on EMB ~~Mal~~ Mal

all Mal+. No mutants

Dec. 26, 1947. Set up as 68. Lac-EMB plates.

Killing variable. Ca. 200 scoreable survivors per plate average 65 plates, or ca. 13,000 colonies examined. 5 possible sectors.

1 Mucoid

2 Not sectored

3.  No mutants. All lac+

4.  + and −. − W87 + W88

5.  No lac mutants. Many mucoid.

Pick to slants

Jan 2, 1948. Streaks out on lact EMB.

Dec 27 1947.

Grow W-55 (58-161 Salicin pos. mutat,) in YP broth, harvest and conc. to ca. 10^{10}/ ml. Irradiate 3 ml. in quartz flask ca 5 secs. rotating at ffont of Hanovia UV lamp, and inoc. ,5 ml samples into 50 ml T(m) with .05% sugar.

1. Unirr.

   A. Salicin(.1%) 3+
   B. Cellobiose -
   C. b-Me Glucoside 3+/

2. B. Cellobiose -
   C. b-Me-Glucoside 3+

Incubate 37°

Examine Jan 3 48.

Streak out the b-MeGl cultures on similar ~~xxx~~ EMB plates. This may be slow rather than mutative utilization ,a s has been observed before.

No rapid utilization indicated on EMB - b-me glucoside plates. Growth assumed to be due to slow continuous utilization.

Raffinose tests continued from previous experiments; some possible diversity in progeny of repeated selections. Compare streaking of a - and ± colony.

A8. (3da.) + types are somewhat more purplish than - . Buds time on this streaking and all colonies are, more or less, ± coreable. Inoc. into .15% T(m) - Raffinose to continue selection. No growth.

A15. 1B (Cellobiose) noted to have reached ++± while 2B is still ±. Streak out on EMB Cellobiose agar to isolate possible mutant.

A13 Inoc. melibiose Tp (BM) ± 58-161 to select more rapid Mel+ types. Streak out and compare with standard.

Jan 4 1948

OK. W-1 X

A 8 (proportion +).

Parentals.

1	W-56	-	All −.
2	W-58	+	ca 1:10 Mal+
3	W-60	+	ca 1:20 "
4	W-63 (Z?)	+	< 1:10. Parent Mal+.
5	W-71	+	ca 1:50
6	W-78	+	ca 1:50 Mal+.
7	W-80 ±	++	
8	W-20	+	ca 1:50

W-63 × Y53 all + Parent +.

W-80 × Y53 all +

([illegible] × Y53.).

a) W-1 & W-56.

b) W58, W60, W-20, W-71, W-78.

All + recombinants checked and definitely ++.

checked as parentals.

	−	+
W1.	OK.	
W56.	OK.	
W58.	OK	
W60.	OK.	
W71	OK. Numerous papillae. Streaked out single colony.	
W20.	OK. Many mycoid. W-20 may be faintly +.	
W78.	1 Mal+ colony / 200 Mal −. May not be purified. Streaked out.	

Jan. 5, 1958

Irradiate Y-53 on Tre-EMB plates, 10 sec. under Hanovia UV lamp. 10 plates.

10 x ca 150 = 1500 colonies.

2 colonies showed fairly distinct sectoring.

1. 

2. 

Restreaked.

① and ② both give two colony types:

a.  Extensive darkening; no sheen W-89 and W-91

b.  central spot only. (This may be due to hydrolysis.

W-90 W-92

2. colonies showed radial striations suggesting quantitative variation. This is correlated with green shiny and from colony, sheen and sheen-colonies noted. The original population is very variable in this character.

Test on Maltose:

89	++
90	++
91	++
92	++.

Retest on Tre EMB. (studying Trehalose by filtration).

Jan 5, 1948.

3P. Inoc. Y105 into YP broth to obtain cells.

~~Lamp Broke Down~~

Use Stauffer lamp. Irradiate 15 secs. (Y105 is apparently more sensitive to UV than is Y10)

Mal EMB 37 plates x ca 20/plate. = 700 colonies. ~~No mutants~~

Lac EMB 38 x ca 20 = 750 colonies.

1 white colony noted. Streak out on Mal and Lac

Mal - ok. Lac + and morphologically identical with other types.

W-94.

Nutrition of W-93

Jan. 9 1947

Inoc from fresh slant into:

T(Val) plus:

		48h.
1.	-	—
2.	HC	+
3.	Vits	-
4.	HCVits	++
5.	NZVits	+++
6.	HCV+ YNA	—
7.	Y. Extr	++++

~~xxxxxxxxx~~

Looks for sp. vitamin.

UV - Killing - Liquid suspensions.

Jan. 9, 1947.

Irradiate 5 ml standard suspension in water of 1/10.

Plate out .05 ml samples.

T. (secs)	S.	
10	3	Irradiate flasks at aperture of lamp with shelving by sand.
20	46	
30	1	
45	0	
60	0	
90	0	
120	0	
180	0.	

1/9/47 PM. Repeat.

~~10 s.~~

20 s.

~~30 s.~~

Y10 Mutation Rem.

Jan 8, 1947.

as 75. Irradiate Y10.

Maltose: 36 plates x 200 = 7,000 cols.

1. ● +and- Restreak to purify. −102 +103
2. ○ faint+. All−. Picked as W95.
3. ● +and−. + W97 − W96.
4. ◐ +and− − W98 + W99.
5. ◕ +and− Restreak to purify. 104 − 105 + 106 − 107 +
6. ● +and− "
7. ◕ +and− − W100 + W101

Rae: 36 plates. x = 7,000 cols.

1. ● +and− W108− W109a+
2. ⊙ Resuscitate
3. ◐ + and ± (◉). W110± W111+ See 197.
4. ● All +.
5. ● + and − W112− W113+

[Cross-test these].

Jan 9, 1948.

Lactose analogues 1% EMB

		b-Me-galact	b-N-butyl gala.	O-Cresyl-b-galac ·
Y10	Lac+	++	++	± v.slow pegillate similar to
Y53	Lac$_1$-	± - + slow	++	± O-phenyl.
Y35	Lac$_2$-	—	—	— strangers inhibitors
Y45	Lac$_2$-	—	—	—

The β-NButyl galactoside gives the most straightforward differentiation so far noted.

Sucrose & Melibiose & Raffinose.

	Raf 3%	Melibiose st.fil.	Sucrose.
" Raf ± "	±	slow ++	—
" Raf - "	∓	slow +	—
Y40	∓	slow +	—

Melibiose activity should be enhanced before attempting to test on raffinose!

Fructose sterile filtered.

Y40	+++
W-1	+++.

Cross-test Lactose mutants

January 4, 1948.

Inoculate YP broths with following:

Y53 (Lac_1-) and: Cross each on three plates.

A8. (progostion +)

1	W-30		
2	W-35	++	1/3.
3	W-40	+	Like 65. ca 1:100.
4	W-42	−	All − [1/>200 + noted J!
5	W-43	1/100 +	Like 65. ca or < 1:100.
~~6~~	~~W-44~~		
7	W-45		++ 1/2 − 1/3.
~~8~~	~~W-47~~		
9	W-48	1/>100 +	
10	W-65	−	All − [1 + ecdary!] 1:100.

Harvest and mix cells. Plate dilute on EMS-Lac(B_1).

∴ None seem to be allelic with Y53. Lac, −.

a) W35, W45 1/2 − 1/3 Lac + recombinants

b) W40, W42, W43, W48, W65. ca 1% Lac+ Recombinants.

c) Y53. (Y87?). Original data on Y87 were more limited than these.

Streakout all Lac − and Mal − mutants for recheck!

Maltose segregation: Effect of preadaptation on maltose.

January 8, 1948

Prepare inocula overnight in YP broth.

Y40 10 AM add 2-3 ml to YP-maltose (A,B) and YP-glucose (C,D) broths.

Incubate W-1 similarly in YP for five hours to 2 PM Cultures of Y-40 are actively producing gas at this time. Was and cross samples of A,B,C,D, with W-1. Plate on synthetic EM-Maltose(B_1).

Count Sectors as +.

A: (M2)

M+	M-	% +	
4	130		0
3	78		0
3	88		0
6	113		1
3	156		0
9	248		0
3	177		0
12	398		1
2	64		1
			2.

3.099%

B:

0	68		0
1	179		0
12	435		2
7	236		2
9	384		0
12	284		2
1	70		1
10	237		2
4	135		
46	2028	2.74	

2.218%

91	3480	3571	

2.548%

Conclusion: No effect of preadaptation.

	R			
Mall	8	5	5	0
	7	2	7	2
	15	7	12	2
	8	3	5	0
	4	3	11	1
	8	2	9	1
	8	5	6	0
	6	3	9	0
	9	5	2	0
	11	2	6	0
	54	23	48	2
	71	30	60	4

C: (G1)

	M−	M+.	S	
1.	3	207		
2.	2	47		
3.	2	109	2	
4.	3	135		
5.	8	267		267
6.	2	85	1	
7.	2	98	1	
8.	0	71	0	
9.				
	22	1019.	4	total: 1041

D: (G2).

11	269	3	
8	213	3	
3	108	1	
14	357	3	
5	165		
			1153.

41	1112	1153.
63	2131	2194.

~~2.727 % Malt.~~
2.871 % Malt.

Comparison.

Glucose.	~~58.6~~ 59.	2135	
	63	2131	2194.
	91	3480	3571
	154	5611	5765

3806

$\chi^2 = 16\left(\frac{1}{63} + \frac{1}{91} + \frac{1}{2} \cdots\right)$

$= .5$

Mean: Malt+ $= \frac{154}{5765} = .027$ %

Y105 Mutation run: Lac attempts

Jan 12, 1948

Irradiate .1 ml per plate (LacEMB) 9 secs. under Hanovia.

71 plates x ca. 30 colonies or 2000 colonies.

3 suspicious colonies streaked out:

1:

2: No mutants

3:

Jan. 13, 1948.

Plate mixtures on Lactose-EMSB$_1$:

	Y87(Lac$_1$-)	W-45(Lac$_2$-)
Y53	(>1000) ✓✓	+++
W108	++ ✓✓✓	++ ✓✓
W-112	>1000 ± ✓✓✓ a	+++± ✓✓ b

see 81 ✓ are replicates

Lac$_3$- .

On Maltose EMSB$_1$:

	a W56(Mal$_1$-)	b W-60(Mal$_2$-)
W-1	(>1000) ± ✓	+ ✓
W95	? ±	++
W-96	±, +	++, ✓
W98	± (1:1000)	+±
W100	±, -	+ ✓
W102	±	+±
W104	±	+±
W106	±	+± many satellites.

Mal$_1$- : W-1, W-56.

Mal$_2$ - : W-60

Mal$_x$ - all others.

+ = 1:100

++ = 1:10

+++ = majority or same order magn.

Parentals checked. p = papillation in heavy streak.

Y53	- p
W45	- p.
W108	- p
Y87	- p no or
W112	- few p.
W102	- p.
W56	- No p.
W98	- p.
W96	- p.
W95	do.
W100	± p

W78. Slow but ++ ultimate

W60 - No p.

W20 slow ++ ultimate.

106 slow + p.

104 slow + p++

W71 ± p.

Jan 10 ff 1948

Test strains indicated on T(m) plus .05% substrate.

A. Inulin	W-55	Ap~~f~~ (39)
P12 (48h)	-	-
	—	—
B. "Bacterial Dextran" Lot L-10 from K.P.Link	—	—
	—	—
Inoc. P12		
C. "Soluble Starch" as above,		
A14	±	++ → dodine color red-violet.
A17	±	+++
425		

A25 —

W55 Hm+ seems to accumulate a red-staining "dextrin" from Amylopectin and soluble starch, but utilizes <u>amylose</u> completely. "Saccharifying amylase ??"

Lac - Cross Tests.

Cross available B-M- Lac - mutants with TLB, Lac, and Lac3 testers, ~~W3~~ W112 and W-108

	A ① W-112	B ③. W-108.
Y87.①		
W31	n.c. N.C.± 'col.	no.col. ✓✓
W35		+ ✓ ++
W40.		+ ✓ ++ ✓
W42		✓✓
W43.		++
W45①		++ ✓
W 48		n.c. ✓
W55.		++ ✓
W67	-? + ✓ + and intermediates?	+ ✓ also intermediates ??
W72.	n.c. + should be scratched.	n.c. ✓ + ++ sm. colo. (poor plate).
W74	++ ✓ —	n.c.; ✓ n.c.✓
W76	+ ✓ ✓✓	++ ✓ —
W83	+ ✓ +	++ ✓ — —
W87	+ ✓? +✓	n.c. ++ ✓
		++ ✓

Jan. 16, 1948.

410

Suspend cells from slants. Spread on Lac EMB (ca. 100,000,000/pl) and irradiate ~~3 sec~~ 15 secs. under Stauffer's lamps. as supra.

x . = colonies.

Run n.g. Evidently, wrong cells (mixture Lac + / Lac -) were used for irradiation.

TP from W94.

Jan. 17, 1948.

Grow 12 l. W94 in N-Case 1%, Glucose ½% (ster. sep.) and $K_2HPO_4 + KH_2PO_4$ (3:1) .4%. 1 5 gallon Pyrex carboy 24 h. at 37° with aeration.

Collect 53 g. paste in Sharples. Resuspend in .9% NaCl 2 litres and recover 39 g. washed paste.

Mix paste with 2 parts pyrex and 9 s. citrate saline and crush in portions in a Pyrex cone mill, ℞ assistance RH Barnes. Resuspend in 200 cc citrate saline (.1m each). Sediment glass & debris and collect supernatant juice. add 2 vols alcohol and store in refrigerator. To 100 cc portion. (A).

To remainder, (40 ml.) add 1/30 v. chloroform & ~~1/30~~. 1/10 vol. BuOH (B)

Mix and store.

D18 (A) Decant and reject supernatant from A. Sediment and redissolve in 50 ml .1M NaCl. Add 2 vols 95% alcohol in a sterile flask. Repeat. → 3.9 gms. alc.-insol. paste.

(B). Reject gelled $CHCl_3$-BuOH-proteins. Sediment and decant supernatant. Retreat with $CHCl_3$ overnight. Repeat twice.

Store bulk of extract A. in 95% alcohol.

Suspend 1 gm. paste A in 20 ml NaCl. Add 5 ml aliquots to sterile test tubes and add 10 ml alcohol to each (use acetone for B4). allow to stand for stratification, sediment and replace alcohol with sterile saline, 10 ml. These will contain 1 gm paste / 40 ml saline.

Sol. "A" 90A

B. Third "Swagging" → almost clear, opalescent. y.g. liquid. Remove from residual $CHCl_3$ and ppt. with alcohol 2:1 as above. Sediment and wash with 95% alc. to remove exc. $CHCl_3$. Resuspend sediment in 10 ml H_2O, add 5 x alcohol. Ppt. fibrous. Lift out with glass rod and resuspend in .1M NaCl → clear but str. opalescent solution. Repeat with remainder of sediment. Gave very little fibrous sediment, considerable granular which is thrown out. Final suspension presumably polymeric NA. in 10 ml NaCl. ~~"Sol. B"~~. Sediment with 5 vols. alcohol in sterile tubes, and resuspend in sterile NaCl, 40 ml. "Sol B." 90B.

Note N24, 1 tube of (B) ppt'd with 2 vols. alcohol. No fibrous ppt. formed suggesting depolymerization.

TP Activity.

January 19, 1948.

(Add 1 ml. 90A + B. resp to 10 ml YB broth tubes (5 ea.).
Use 2 for sterility tests. Inoculate each of the other three with 48 hr. culture Y138. Also 3 tubes of C smears for no-treatment controls.

Read A 20.

1	A1			all Mal +	
2	A2			all Mal +.	(A phage plaque ?)
3	A3			all Mal +	
4	A St	turbid		Some everything	Not coli
5	A St	turbid.		~~No colonies~~ Some everything.	Not coli.
6	B1			All Mal +.	
7	B2			all "	
8	B3			all "	
9	B St	Turbid!	str. out	Cont. Not coli.	
10	B St.	Clear	str. out.	— No colonies.	
11	C1			All Mal +	
12	C2			"	
13	C3.			"	

Streak out all tubes on Mal ~~and~~ EMB.

↳ ~~Streak out~~ Test on ~~Mal~~ Y138

	O	A	L	
1-1	0, 0, 0	0	11, [illegible]	
1-2				
1-3				
2-1	0 0 0	0 [illegible]	5, 1	
3-1	0 0 0	0 0	15, 15	
4-1	heavily loaded with actinomycete contaminant			
5-1	heavily contaminated.			
6-1	0	0	34	
6-2	0	~~3~~ 0	3	
6-3	0	0	0	
7-1	0	3	30	
8-1	0	0	45	
9-1	Loaded with "mucoid" contaminant.			
9-2				
9-3				
11-1	0	0 1	16	1
11-2	0	0	1	
11-3	0	0	0	
12-1	0	1	32	26
13-1	0	0	46	

There is no evidence from this experiment of transformation of the A- or L-loci either by the crude extracts or by the fibrous material of "B".

Replate cells in series 1 in A + L agar.

[illegible] later.

Preparation of I.P.

Jan 23, 1948.

Grow W-94 "anaerobically" in 12 l. N2 case medium, 2½.
37°. Yield: 17 g. Sharples paste (⅓ aerobic yield).
Suspend in 170 ml NaCl (physiological) + blend ē. 2 ml toluene.
Let stand 4 hours, sediment & ppt. supernatant ē 2½ vols 95% alc.
V. little sediment formed. Separate + store in 70% alcohol. (C)

Specific reversions of W108.

Jan 27, 1948

Streaks out the following "reversions" of W108 on the homologous medium, as indicated, to purify.

From glucose. EMB plates of 93. – to lactose & maltose.

	L Y10	M Y10.
1.		
2.		
3.	Test 31 "reversions" on glucose plates on	
4.	lactose and on maltose.	
5.		
6.	All 31 glucose-reversions are also lactose & maltose +.	
7.		
8.		
9.		

plates M1, M2, L1, L2

From Lac + Mal EMB. Streaked out to Lac + Mal + Man.

10 Mal + are Lac +
6 Lac + are Mal +.

L, M, Man = ½ Man + also Mal + and Lac +.
3 Man + are "weak", fourth is "strong".
Purify + compare ē Y10.

From 93 Broth. 108 M / M & L resp.

From 93 T(m). Maltose 108 M (Tm) / M + L resp.

→

All reversions are non-specific for glucose, maltose + lactose

Nos. tested:

Glucose	31
Lactose	6
Maltose	13
Mannitol	4

54 tested altogether.

Select. are as W-108 R = W116.

Characterization of W-108.

January 28, 1948.

T(m) T+B. + .05% = W108 (autoclaved together).

	W108	Y/O.
glucose.	−	+++
d-ketogluconic	−	+
hexose diphosphate.	++	++.
" + glucose		+++.

The HDP was prepared from the Schwarz Ca salt product by adding excess oxalate and neutralizing with NaOH. The solution contains exc. oxalate, which is evidently not inhibitory considering the control. In autoclaving, the HDP solutions turn quite yellow so that breakdown must be suspected. Repeat expts. using filter sterilized HDP.

Test Proteus X-19 on HDP. Add to T(m) + nce:

	A29.	A2
glucose	−	++
fructose	−	−
HDP.	−	++

Jan 29, 1948.

S. dublin I IV g,p ; — Arab - B_1 -

X

S. paratyphi A. I II XII a ; — Ar+ B_1+ Meth - Tryp -.

on arabinose minimal medium.

Mix sep. + together into YP broth. (1) S1 (2) S37 (3) S1 + S37 X

(A). Plate .1ml washed samples of 16 hr. cultures on arabinose T(m) minimal.

1. S1 12 cols. 4. S1+S37. 2. S1 smears on ~~agar~~ arab. minimal.
2. S37 —
3. X ca 10-20 cols.

(B). Do.

1. S1 0 2 large 1 sm. many small,
2. S37 0, 0
3. X 0, 3 sm. cols., 10 cols., 0, 0
4. S1 + S37 3 c., 10-20 c., 100 c., 100 c. 100 c. many small.

Read 2/4/48.

(4) may represent a cross. Addn'l differentiating characters needed to eliminate S1 reversion.

Jan. 29, 1948.

Test 93. W108: glu+ and tre+ on glucose & trehalose EMB.

1. Glu+ On EMB, all white colonies with no glucose + trehalose
2. Tre+ In T(m). Both grow rapidly on glucose, fairly quickly on ~~glucose~~ trehalose, T+ both.

Streak from Glucose plates to EMB glucose.

(1) —
(2) — in 24 hours.

Take 99-1, impure, as W-117

W-117 is either an aerobic oxidizer of glucose or else a slow fermenter.

Compare on glucose and on K gluconate:

W117:	EMB:	
glucose	+	weak +. Use these colonies for pure W-117
Maltose	± - +	
Lactose	−	
K-glucon.	+++	

January 29, 1948.

Remove most Ca from crude preps. Ca Maltobionate + Ca Lactobionate
Expd. by KP Link by Bromine Oxidation. EMB tests.

Streak out, on ~~Lba~~. Lba:

Y10	–	No papillae noted.
Y87	–	Colonies markedly papillate 2–5 / colony. Streak out *
W45	–	Occ. papillae 1–2 / colony
W108.	–	Tiny but fairly numerous papillae!

* → Lba – and Lba + types. Purify and describe as W115 Test on lactose:
Thus wild type Lac+ is Lba –.

Maltobionic Acid:

Y10	–	No papillae noted.
W60	–	
W56.	–	
W108	–	Numerous small papillae (3-6 / colony).

W60: may be very slow +.

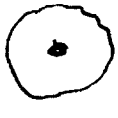
On second day, the original papilla restreaked on Lba did not remain + but all colonies were faint purple. On lactose W115 is +++ but, apps. still Lba. –

Streak out papillae again. ~~Jan.~~ 2/1/48.

All Lba – negative! (What are the papillae??)
(Acid?)

58-161 } incc. into Lba minimal. No growth.
Y10

The following sectors gave + and − colonies.

		Mal	glucose	T1	W −
41.		+	+	S	139
42.		−	−	S	164
43.		−	−	S	165
44.		+	+	S	140
45.		+	+	S	141
46.		+	+	S	142
47.		+	+	S	143
48.		+	+	S	144
49.		−	−	S	166
50.		A few lac+ in heavy streak.	−	S	167

Jan 31, ~~1948~~ Feb. 1, 1948.

182 plates x ca or > 500 colonies readable per plate, average 410.

= ca. 100,000 colonies.

Most mutants are intact colonies rather than sectors. Streaks out on EMB Lac.

Inoculate 10^8 cells/plate 75 secs. under Watson's low pressure Steri-lamp. Killing very variable. Apparently smaller proportions of sectors among mutants 1-39 intact white colonies. Test when pure:

	Maltose	Glucose	Gal.	TI	W-
1.	−	−	±		146
2.	+	+	+		120
3.	−	−	±		147
4.	+	+	+		121
5.	−	−	±		148
6.	+	+	+		122
7.	−	−	++		149
8.	+	+	+		123
9.	+	+	++		124
10.	+	+	Gal-		125
11.	−	−	++		150
12.	−	−	Gal-		151
13.	+	+	+		126
14.	−	−	±		152
15.	−	−	Gal±		153
16.	−	−	±		154
17.	+	+	++		127
18.	+	+	+		128
19.	−	−			155
20	−	±	±	S	145
21.	+	+			129
22.	+	+			130
23.	+	+			131
24.	+	+	++		132
25.	+	+	++	S.	133
26.	−	−	±		156
27.	−	−	±		157
28.	−	−	±		158
29.	+	+		S.	134 168
30.					168 134

	Maltose	Glucose	TI	W-
31.	+	+		135
32.	+	+		136
33.	+	+		137
34.	−	−		159
35.	−	−		160
36.	−	−		161
37.	+	+		138
38.	−	−		162
39.	−	−		163

Sectorial:

40.

41.

42.

43.

44.

45.

46.

plates too dry for TI det.

39a:

Record for Gal tests

LM	Val.
1	±
2	++
3	±
4	++
5	±
6	++
7	++
8	++
9	++
10	−
11	++
12	− thin
13	++
14	±
15	− th
16	±
17	++
18	++
19	− th
20	++
21	− th
22	++
23	++
24	++
25	++
26	++
27	±
28	±
29	±
30	++
31	++
32	++
33	++
34	− th
35	±
36	±
37	++
38	− th
39.	±
41	++
42	±
43	
44	++
45	++
46	++
47	++
48	++
49	±
50	± (th)

Dna (gluconic ac.) Mutation Rem. 104.

Feb. 3, 1948.

Y10

A). 10^9 cells per plate 3 mins. under Watson's Steilamp.
6 plates x 500 = 3000 colonies.

B). 10^8 cells. 75 secs stei lamp. ca 1/3 unreadable.
40 plates x 500 = 20,000.

No very clear-cut colonies or sectors. Streak out suspicious colonies.

1 slow on gluconic from A. (intact colony). W169.

2. Gluconic − from B.

1. intact W170

2. (S) + W171
− W172

Compare:

	glucose	galactose	Gluconic	Lactose	Maltose	Arabinose	T1	
W169.	−	±	±	−	−	±		S
W170	+ v.s.c.	++	++ v.sm. col.	++	++	++		S
W171	++	++	++	++	++	++		S
W172.	++	+ and − (diverse).	−	++	−	++		S

4 x h

W169 is lactose slow or negative.

W172 is mixed! Dna − Maltose − Galactose ±?

Repeat these tests!

W145. ++ ± − − − S

W117 x Y40.

W108 - reversion or glucose mutation

Feb. 2, 1948.

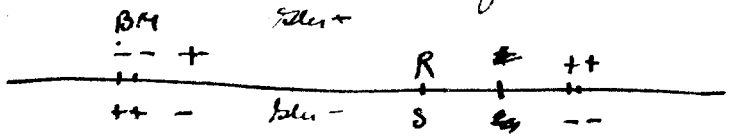
On EMS-glucose. Cross W117 (W118 glucose partial reversion) x Y40 (wild standard). and look for glucose - recombinants.

Feb. 5, 1948.

Glu+ easily distinguished from residue of Glucose- or ±. Two classes of latter cannot be directly distinguished on the EMS-glucose cross-plate. Majority of colonies Glu ±.

Check out most likely Glu- on Glu EMB and compare with W108 and W117.

Glu+	Glu ±
7	189.



∴ ~~Glu- is located near Tl. Most Glu+ should be~~
∴ Glu is located near BM. (in neighborhood of Mal$_1$).

Check by distribution of V_1^R/S

Glucose ++. V_1^R	V_1^S
13	5
5	4
16	4
14	5
12	5
58	23

Glucose ± V_1^R	V_1^S
1	0
16	3
8	1
11	6
14	5
50	15

This is essentially similar to behavior of Mal$_1$ (W-1).

Glucose- and Glu ± are difficult to distinguish. Among ca 2000 colonies, pick the most likely - types and compare also with W108, W117 and Y10:
~~23~~ examined — 4 glucose - found. These are quite distinguishable from Y117. ∴, presumably a suppressor mutation can take over the functions of Glu$_1$- . ~~Evidence~~

(over)

Purify the four glu- recombinants and compare with Y10 and W117 and W108 on glucose EMB.

	24h.	48h.
Y10	+++	✓
W117	—	++
W108	—	—
-1	—	—
-2	—	—
-3	—	—
-4	—	—

l-Arabinose mutations rum.

Feb. 4, 1948.

Irradiate 58-161 on Ar. EMB plates.

20 plates. Colony dimorphisms as on galactose noted.
× 300 = 6,000 cols.

(A). Take Ar^S and Ar^R and test on Ar, gal. plates.
Same differential as glucose, Arabinose + galactose!

(B). 3 possible mutants noted.

		Ar.	Gal.	
1.	intact	slow		W-174
2.	"	–		W-175
3.	v. tiny colony.	+ and –	+ and –	W-176, 177

Galactose mutations rev.

Feb. 5, 1948.

Y10. 50 plates × ca. 150 scorable colonies → 7500 colonies.
3 suspicious colonies streaked on gal EMB.

1.  + and − W-180
2. do. + and − W-181.
3. 0

Feb. 6, 1948

58-161 (S and R) irradiate 10^8 cells/plate 85 seconds. Watson's lamp.
on Lac EMB.
75 plates × 300 survivors = ca. 22,000 scorable colonies.

Picks. P7 & streak out. Following mutants obtained:

		W-	
Distinct Cols.	1.	182	–
	2.	183	–
	3.	184	–
	4.	185	–
Sectorial	5. 	186	–
Crateriform	6. 	187	slow – +++ in 48 hours.
	7. 	188.	–
	8. O	189	slow growing.

Retest:

	Lac	Mal	Gal	Glu	Sna	Xyl	Ara
182							
183							
184							
185							
186							
187							
188							
189							

Cross-test Lac Mutants.

Feb. 6, 1948.

Cross: ×	A: W-45	B: Y-87	
W-120	++ +	+(1/300)* OK	1 plate each. Each plate had at least 500 scoreable colonies unless spec
121	++ −	− Lac,−	
122			
123	++ +	− Lac,−	
124	++	− Lac,−	
125	++ −	+(2/50)* OK slow +.	
126	++ −	+(3/1000)* OK	
127	++	− Lac,−	
128	++ (−)	−	(No effect?)
129			
130	++ +	− (<100 colo.) Lac,− * OK :−	
131	++	− Lac,−	
132	+		
133	++ −	±(1+/1000)* OK	
134	++	− Lac,−	
135	++	− Lac,−	
136	++	− Lac,−	
137	++	− Lac,−	
138	++	− Lac,−	
139	++	− Lac,− ~~(Slow!)~~	
140	++ −	1/1000+ * OK	
141	++ ✓	−(slow±?) Lac,−	
142	++	− Lac,	
143	++	− Lac,	
144	++	− Lac,	
156	++ (+)	+(1/100) + OK	(See 115a)
~~145~~			

Note smaller * streaks of ++'s. and repeat cross.

February 6, 1948.

W-145 is Lac- Mal- plus+.

Cross with W45, Y87 to exclude alleleism and with (Y40) to determine whether one or more mutations are responsible for the Lac-Mal- state. Cross on Lac and on Mal medium.

W145 × Y87 → ++ Lac+.

W145 × W45 → No colonies ✓ (Hold). } on ~~lactose~~ EMS.

W-145 × Y40 on maltose → heavy growth in background; numerous +

(Plates may have had same pipette!) do. Lac.

Pick from Lacs to Mal EMS + vice versa.

Lac+ tested on Mal.:

Mal+	Mal-
98.	0 ~~+? to be rechecked~~.

Mal+ tested on Lac:

+	−
102	0.
200	0

∴ No Recombinants found in which Lac- was separated from Mal-.
in 200 tests.

Febr. 7, 1948.

A. On Glucose EMS:
W108 × Y40

B. W117 × Y40. C. (Feb. 8) W116 × Y40.

Both crosses give Glu ++ and Glu "−". Although, as a whole, the "−" colonies in B are darker than in A, they are not readily distinguished on this plate.
Pick "−" colonies at random from A and B and streak out on Glu EMB.

A. All − (15)

B. All ± (24).
+ after 2 days.

~~Streaked Lilly — from B. as before.~~

C.
200 Glu + colonies. No −

C-source utilization & selective reversion.

Feb. 9, 1948.

1 (m) + .05%: Mor. W-117 P9.

	A-11	P14
1. Glucose	+++ *	✓ ***
2. Lactose	++ *	+++ **
3. Maltose	++ *	+++ **
4. Ammonium Acetate	+++	+++
5. Sucrose	−	−
6 "	−	−
7 Raffinose	−	−
8 "	−	−
9 Cellobiose	±	+
10. d Meglue.	−	−

11. Lactobionate No growth. Granular sediment. −

* Streak out.

	Lac	Mal	~~Glu~~
1.		All −	
2.	+++ and + colonies.		
3.	+ and −	+ and −	

~~(Test Lac+ on Glucose.)~~

**

	Lac	Mal	Glu
1.	All − or −I	All − or −I	All + (117 type)
2.	++ and −	++ and −	+++ and + 117 (hard to score at 48h.)
3.	++ and −	++ and −	do.

Purify 3++ as W−

(See over.)

Evidently, selective pressure of glucose on W-117 is inadequate to force development of Lac_3^+ types. Lactose, ~~however, sucrose~~ and maltose, however, impose a more stringent differential so that the type $Sl_3 + Lac_3^+$ develops.

About 20 Mal+ and 20 Lac+ were tested on glucose. All +++.

Test Lac+/Mal and uv.

February 16, 1948.

From 112 *** plates, Lac+ colonies were streaked on Mal, and Mal/Lac.

of 30 Lac+ colonies, 12 were Mal ±. 1-12

of 27 Mal+, 8 were Lac-. 13-20.

Recheck and purify on Lac + Mal. First readings: 24 h.

	Lac	Mal	
1	+	−	
2	+	−	
(3)	+	−	W − 236
4	+	−	
5	+	−	
6	+	−	
7	+	− slow ±	
8	+ slow	−	
9	+	−	
10	+	−	
11	+	−	
12	+	−	
13	−	−	
14	−	−	
15	−	−	
16	−	−	
17	−	−	
18	−	−	
19	−	−	
20	−	−	

February 19, 1948.

P18 from W108 heavily ~~Lac~~ into T(m)+.

A. Lac B. Mal.

		P19	P20
P19.	Lac	+++	✓
	Mal	-	+++

Streak out Lac on Lac and look for specific reversions. Do Mal 2/20

	Lac+ on:	Maltose	Glucose	
112B1		69 -	85 -	These reversions are apparently Lac+ Mal- Glu- !
112B2		0 +	0 +	Most of the Mal- are faintly purplish.

Select 2 and streak out on the three media.

	M.	B.	Lac.	
1.	Smooth, faint pink 48h. + purple.	= 24h. No pink.	++	W-
W108	-	-	-	
W117	-	++	-	
2.	Rough, white	= 24h.	++	W-

After 60 hours, most of the 69 Lac+Mal- turned a fairly deep purple on maltose as if ±, but were glucose -. Pick to slants as W-251 and W-252

	Mal+ on:	Maltose	Glucose	Lactose		
(24h.)			71± 7-	65+ 2-		

Retest Sample of each group on each:

24h.
+24h.
48h.

1	+	+	-	-	-		W-327	M+B-L?
2	-	-	-	-	-			
3	+	+	-	±	+			
4	+	+	-	±	+			
5			+	-	+	+		
6	+	+	-	-	+	+	W-328	M+B-L+
7	+	+	-	-	+	+		
8	+	+	-	-	+	+		

Lac Mutation Run.

Feb. 12, 1948.

Y10 (S.C.I.) 10^8/plate. 80 secs. (Watson's lamps).
90 plates x ca. 800 per plate. 72,000 colonies.

Sectors: — W-

	1. 	190
	2. 	191
	3. 	192
	4. 	193
N.B. col. subs.	5. 	194
	6. 	195
	7. 	196
	8. 	197.

Also: 32 intact white colonies.

Lac test - crosses. Checks.

Feb. 10, 1948.

Y87 (Lac, −) × : on EMS' Lac

	Lac +	Lac −
1. W-120.	1	1000
	0	200
	1	1000
	0	1000
	1	1000.
	3	
	1	/ 750.

W120 Not Lac, −

2. W-125.	0	2
	6	9
	1	2
	1	2
	3	4
	12.	19

W125 Not Lac, −. Not $\overset{al}{=}$ W-120.

3. W-126.	0	30
	0	30
	0	40
	2	300.
	2	400.

W126 Not Lac, −

4. W130	0	100
	0	100
	0	100
	0	100
	0	100.
	0	500.

~~Helicum in doubt~~.

5. W-133	2	100
	0	200
	1	100
	1	100
	2	500.

Not Lac, −

Contd.

	Lac +	Lac −	
W-140.	0	100	
	1	200	
	1	200	
	1	200.	
	0	200.	
	3	900.	Not $\stackrel{al}{=}$ Lac, −
W-156.	0	100.	
	0	200	Probably $\stackrel{al}{=}$ Lac, −
	0	100	
	0	200	
	0	300	Phenotypically Y53. ✓
	0	200	
	0	200	
	0	300	
	0	300	
	0	1900.	

Lac Cross-Tests.

Feb. 12, 1948.

On EMS-Lac

A. W-145 × W-45

B. W-145 × Y87. (1 or 2 plates).

C. W-145 × Y40.

D. W-128 × W-45

E. W-128 × Y87.

E:.

Lac+	Lac-
-	100
-	100
-	~~300~~
-	100
-	200
-	~~400~~
	150.
	800
	400
	100
0.	2400
0	~~350~~
	~~2750~~.

∴ W-128 is (Lac$_1$ −). Not phenotype and compare with Y53.

0 Recombinants in 2750 tests.

A. 4 plates. No colonies!

B. 6 4 + the small
6 ?

On adequate incubation 8+ : 288 −
= 3% Lac+ recombinants.

C. ++ ++

D. 3 plates. No colonies. [What is wrong with W-45?].

Feb. 12

1.1% glucose

108 grown in YB Test m:

Set up 12 N	Glu	Glu+Gal	Gal	Sucra	Ara	Glu+Ar.	Su Gal	M. Gal.
230	+	+++	+	+++	+++	+++	−	−
430	++	+++	++	⌣	⌣	⌣	−	−

Reverted!? (W.117?). N.C.

type

Characterization of Mutants.

Feb 9, 1948.

	W-	Lac	Mal	Glu	Glucon	Xylose	Arabinose	Butyl gal.	Methyl gal.	GAL.
1	182	−	± +	++	+	+	+ ++	+ ++	± +	
2	183	−	++ ✓	++	+	+	+ ++	+ ++	− +	±
3	184	−	± ±	++	+	+	+ ++	+ ++	± +	
4	185	−	− − (−)	− + ++	− − −	− (−)	− +	− − (−)	− −	−
5	186	−	++	++	+	+	+ ++	+ ++	+ ++	
6	187	−	+ − −	− − +	− −	+	+	+ ++	± +	
7	188	−	± − (−)	− − (−)	−	+ +	− (−)	− ++	− (−)	
8	189.	−	− − (−)	− −	−	+	− (−)	− − +	− −	
9	108.	−	− −	− − ?	+	+	+ + ✓	− ±		
10	174	± + ++	± + ± (−)	− + ++	+	+	± dt+	✓	+ ++	
11	175	± +	− +	± ++	+ ++	+	− − ±	✓	+ ++	
12	177	± +	− +	± ++	+ ++	+	+ ++	✓	+ ++	
13	X ~~169~~ 112	− −	++ ++		+ ++	+		− ✓ (−)	− − (−)	
14	X ~~172~~ 143	− −	++ ++		+ ++	+		+ ++	− ++	
15	145.	− −	− − (−)	++	− − −	+	−	− − −	− −	
16	116	++ ✓	++	++	+ ++ ✓	+		+ ++	+ ++	
17	117	− −	− (−)	− + ±	− +	+		+ ++	+ + ±	
18	180	− ±	+ ± ±	++	− −	+		+ ++	+ ++	
19	181	+ ✓	✓	− ±	− −	+		+ ++	+ ++	
20	120	− −	− ++ ✓	++ ++	+ ++	+		− − (−)	− (−)	
21	125	− −	− ++	++	− − ±	+	+	− − (−)	− (−)	
22	126	− −	− ++	++	+ ++	+	+	− (−)	− (−)	
23	130	− −	− ++	++	±	+	+	+ ++ ✓	+ ++	
24	133	− −	− ++	++	++	+	+	− − ±	− (−)	
25	140	− −	− ++	++	++	+	+	− − (−)	− (−)	
26	156	− −	− ++	++	++	+	+	+ ++	+ ++	✓
27	121	− −	− ++	++	++	+	+	+ ++	+ ++	
28	123	− −	− ++	++	++	+	+	+ ++	+ ++	
29	128	− ∫	++	++	++	+	+	− − (−)	− (−)	
30	142	− ∫	++	++	++	+	V	+ +	+ ++	

Bac 6P9

− 8A10
− 2P10
− 6P10
− 9A11

Note 108 on Butyl-β-galactoside. Try W-108 on galactose and on glucose + galactose!

Lac Crosstests:

① BM mats × W-126.

Feb. 14, 1948.

		On Lac EMS'	W-126 ×	
	W			
1.	35		++ ✓	
	40 ~~no ++~~		±1/1000, 3/1000.	
	42 " "			
2.	43 "			
3	45		++ ✓	
4	48		0/500 0/600	allel.
5	65		2/500 1/400 ±	
6	67		0/600; 0/600.	Allel.
7	72		+± +±	
8	74		2/400; 3/200 +	
9	76		1/500 2/500 ±	
10	83		0/500; 0/500	Allel.
11	W87.		3/600, 2/600. ±.	
	182		1/600; 5/500 +	
	~~Y53~~ 183		3/600; 2/600 ±	~~W-126~~.
	186		1/400, 3/400 +	
12.	182 × ~~W6~~ Y53		1/600 0/600. ± ?	
13.	183 × Y53		0/600; * 0/500	
14.	186 × Y53		0/600 ①1/600 *	are these ++'s artifacts?

Streak out parentals + the sole +'s.

* Streak out. 186B: good ++. do. 182B.

W-83; W-67; W-48 may be regarded as Lac_4 –

W-35, 45 and 72 are probably Lac_2 –

W-40, 65, 74, 76, 87, are probably additional loci.

Feb. 14, 1948. Test on EMB:

~~76. 54. Y40 UV — } Lac sectors~~
~~77 54 Y40 ++ ± } —~~

	Glu	Gal	Sna		Me Gal	Bu Gal.
W-108	—	+± *	++		+	+++
Y53	+++	+++	+++		++	+++
W117	++	+++	+++		++	+++
W45	+++	+++	+++		—	—
W128	+++	+++	+++		—	—
Y10	+++	+++	+++		+++	+++
W145.	+++	++ *	—		—	—

* peculiar brilliant purple shade. Bleached in mass streak

108 on galactose is enzymatic.

● Streak out W-108 on glucose and galactose:

Glu All −

Gal Two types of colonies: ① Fairly strong Gal +
② Stained in center, clear periphery of colony.

Galactose is utilized by W-108. Maybe two colonial types.

Repeat, 2/15, 2/17.
Y108 is Glu − Gal + !

2/17/48. Gal Sna
W-2 on EMB. +++ +++
may be a little slow ⊙ ●

Characterization:

	Glu	Gal	Sna	Lac	Mal	
189	++					
190	++	++	++	−	++	
191	−	+	+	−	−	108 type
192	++	++	++	−	++	
193	++	++	++	−	++	
194	++	++	++	−	++	
195	−	+	++	−	−	108 type
196	++	++	++	−	++	
197	++	++	++	−	++	
198	++	++	++	++	++	
199	++	++	++	++	++	
200	±	++	++	+	±	
201	++	++	++	−	++	
202	++	++	++	−	++	
203	−	+	++	−	−	108 type
204	−	+	++	−	−	108 type
205	++	++	++	−	++	
206	++	++	++	−	++	
207	−	+	++	−	−	108 type
208	++	++	++	−	++	
209	++	++	++	−	++	
? 210	++	++	++	++	++	
211	++	++	++	−	++	
212	++	++	++	−	++	
213	−	+	++	−	−	108 type
214	++	++	++	−	++	
215	++	++	++	−	++	
216	++	++	++	−	++	
217	++	++	++	−	++	
218	++	++	++	−	++?	
219	−	+	++	−	−	108 type
220	++	+	−	−	−	145 type
221	++	++	++	−	++	
222	++	++	++	−	++	
223	++	++	++	−	++	
224	−	+	++	−	−	108 type
225	++	++	++	−	++	
226	−	+	++	−	−	108 type
227	−	+	++	−	−	108 type.
228	++	++	++	−	++	
229	−	++	++	−	±	108 type
[illegible]						

Feb. ~~22~~ 16, 1948

A. W-45⁻ × W-

		A	Megalac.*
1	190	++ ✓	
2	192	++ ✓	
3	193	++ ✓	
4	194	++ ✓	
5	196	++ ✓	
6	197		
7	201	++ ✓	
8	202	++ ✓	
9	205	++ ✓	
10	206	++ ✓	
11	208	++ ✓	
12	209		
13	211	++ ✓	
14	212	++ ✓	
15	214	++ ✓	
16	215	++ ✓	
17	216	++ ✓	
18	217	++ ✓	
19	218	++ ✓	
20	221	++ ✓	
21	222	++ ✓	
22	223	++ ✓	
23	225	++ ✓	
24	228	++ ✓	

B. Y-87 × W-

B	
0/100	LAC, -
+/1 col.	X
0/20	~~LAC, -~~
0/200	LAC, -
0/100	LAC, -
0/100	LAC, -
1/100	LACx
0/100	LAC, -
0/100	LAC, -
0/150	LAC, -
0/600, 0/700	LAC, -
* 0/200 0/500	LAC, -
0/200 1/200	X
2/400, 0/50	X
1/300 1?/300	X
0/200 0/200	LAC, -
0/300 0/200	LAC, -
3/3+ 7/10+	X
0/500 1/200	X
0/100 0/100	LAC, -
0/500 0/300	LAC, -
0/700 0/500	LAC, -
0/600 0/200	LAC, -

W-188 * W-108.

+ and - colonies found. W-188 is Glu$_2$ -
Some intermediates possible. Streaks out

All Lac, - except: 192, 193?, 201, 212, 214, 215, 217, 218, 221. of these, 192 + 218 are in one group, the remainder in another

mglucose. 3da.
W188 3-4+/200 -. Cross results uncertain. Needs purification.
No intermediates noted on purification of suspected prototrophs. (Change due to dying out + colony darkening.)

* Test streaks vs Megalac. EMB 2/23/48.

Lac Mutants Cross-Tests

Febr. 16, 1948.

	W--	Cross on EMS-Lac B_1.	A x W-45 (Lac_2)	B x Y-87 Lac_1	C x W-67 Lac_4	
1	120	++ ✓				
2	122	+++ ✓	ϕ/300 3/400	0/500 0/400		
3	125	++ ✓	++	++ ✓	Lac 6	
4	132	++ ✓	0/600 0/600	0/600 0/600	All. Lac, and Lac$_4$	
5	133	++ ✓	3/400 6/600	0/300 ~~2/600~~	"Not Lac, or Lac$_4$"	
6	140*	++ ✓	0/200 0/200 ✓	0/500 0/400	either (1) or (4)	
7	145	+++ ✓	++ ✓	+++ ✓		

* By mistake, 144 was given instead of W140. Cross was therefore attempted with cells scraped from stock slant of W140.

132 and 140 both gave no Lac + either $\bar{c}$ Lac, or $\bar{c}$ Lac$_4$

133 gave Lac + $\bar{c}$ both. 120 & 122 are Lac$_4$

February 17, 1948.
R
58-161 > .C-1.
95 plates × ca. 200 (v. uneven) = 19,000 colonies.

			Glu	Gal	Lac	Mal	Gua
Retest on EMB Streaks. 2/18.	W-237.	◐	++	++	−	++	++
	W-238	◔	++	++	−	++	++
	-239	○	~~+~~ −	N.G.	−	N.G.	~~+~~
	-240	"	++	++	−	++	++
	241	"	++	++	−	++	++
Slow +	242	"	++	++	+	+	++
	243	"	[illegible] −	++	−	−	++
	244	"	++ ++	++	−	++	++
	245	"	[illegible] −	− !	−	−	++
	246	"	++ ++	++	−	++	++
	247	"	++ ++	++	−	++	++
	[illegible]				[illegible]		
	248				−		
	249				−		
	250				−		

Types: Lac − 237, 238, 240, 241, 244, 246, 247

Glu − 239, 243, 245. See p. 129

Lac, inter-allelic changes

February 10, 1948.

1. Streak out W-128 on ~~Methyl~~ Methyl 3-d-galactoside and on lactose
A 18 All - ; No papillae.

A 20 all - No papillae.

P 22 Do.

Heavy inoc. into T(m) + Lac + fungal.
2/20 —
P 22 — —

W-128 is completely stable.

(2.) Streaks out W-138 on lactose & compare with ~~to~~ Y87; Y53.
[Esther says W-138 is slow +]

A18. All -. No papillae
Y87 is papillate

[redacted]

A 20. — Not slow +. No papillae.

A 21 — Slow + ! lactuals.

Dehydrogenase Media.

2/18/48.

Make up ½% Ca (casamino acids) in .2% aq. Ammon. Molybdate. + Agar

A.- B. Add 2% Sodium Succinate.

After autoclaving, A is blue; B is lt. yellow.

K-12. N.G. on A. Colonies on B.

Analysis of W-236.

W-236 x Y40. On EMS.

Ca. 5-10% Lac- Therefore W-236 is not Lac$_1$+. Call the gene "reverting" in W108 – W117 Sl_1+ (Suppressor of Lac$_3$). Call the differential between Y40 and W-236 Sl_2+. If Sl_1+ ≠ Sl_2+ then some of the Lac+ recombinants will be Glu- and v.v.
Compare on Lac + Glu S.:

(A) { 9 cultures were Lac – (±?) but glucose +.
10 cultures were Lac + Glu +

(C) 23 cultures were Lac – Glu –

(B)

Streak out samples of each type on Lac + Glu EMB.

		Lac	Glu	
1	L-/+	+±	–	This type suggests that the mutation differentiating W117 from W236 is at a distinct locus from the one between W108 and W117
2	L+/+	++	++	
3	L-/-	–	–	
4.	L+/-	–	–	

Cross-Test Glu Mutr.

	A-W108 × A	~~B-W153 × B~~
1. W239	++	
2. W243	++. Also ± and − (on cross-plate. ± not verified)	
3. W245.	++	

Knockout parents.

108 R/S variation; 1 ± / > 200 + revertants in streaks.
239 < 5% reverted. Natural colony − darkening around +
243 all − OK. Thin colonies.
245 50% reverted.

Crosses in conclusion!, etc.
243?

Gal Mutation Run.

Y10 80 secs Watson's Sterilamp.

70 plates x 200 cols. = 14,000 scored.
Very few entirely – found.

 ++ and – 254.

 ++ and – 255

0 – (and ++) 256 ±

Also, 9 cultures recovered which are not = but ± :

24h. ⊖ Pick 2 for study: 257
258

58-161 80 secs. Watson's steinlamp

50 plates × 200 = 10,000 scored.

W-253

About 10 others picked were not mutant. Pick to gluconate broth.

Lac Mutation Rem.

February 21, 1948.

4/10 80 secs. Watson's Lamps EMB.

100 plates x ca. 1500 cols. = 150,000 (very rough est.) colonies

68 mutants = 1/2000

	Glu	Mal	Lac	Sac	Sorbitol	Mgal.	
259	+	±	+	+	Slow Lac	−	
260	−	−	±	+	108		
261	−	−	±	+	108	+	
262	+	−	+	+	Lac	−	
263	+	−	+	+	Lac	+	
264	+	−	+	+	Lac	+	
265	+	−	+	+	Lac	+	
266	+	±	+	+	Slow Lac	+	
267	−	−	±	+	108	÷	
268	−	−	±	+	108	÷	
269	−	−	±	+	108	÷	
270	−	−	±	+	108	÷	
271	+	−	+	+	Lac	+	
272	+	−	+	+	Lac	+	
273	+	−	+	+	Lac	+	
274	+	−	+	+	Lac	+	
275	+	−	+	+	Lac	+	
276	+	−	+	+	Lac	−	
277	−	−	+	+	108	+	
278	+	−	+	+	Lac	+	
~~279~~							
280	−	−	+	+	108	+	
281	+	−	+	+	Lac	+	
282	+	−	±	−	(145)	−	
283	+	−	+	+	Lac	−	
284	−	−	±	+	108	−	
285	−	−	+	+	108	÷	
286	+	−	+	+	Lac	−	
287	+	−	+	+	Lac	+	
288	+	−	+	+	Lac	+	
289	+	−	+	+	Lac	+	
290	+	−	+	+	Lac	?	
291	+	−	+	+	Lac	+	
292	−	−	+	+	108	−	
293	+	−	+	+	Lac	+	
294	+	−	+	+	Lac	+	
295	+	−	+	+	Lac	+	
296	+	−	+	+	Lac	+	
297	−	−	±	+	108	+	
298	−	−	±	+	108	−	
299	+	−	±	−	(145)	−	
~~300~~	+	−	+	+	Lac	+	
301	−	−	±	+	108	±	
302	+	−	+	+	Lac	+	
303	+	−	+	+	Lac	+	
304	+	−	+	+	Lac	+	
305	+	±	+	+	Slow	+	
306	±	−	+	+	Mel − Lac −	+	
307	−	−	+	+	108	+	

W− : 259 v. slow ±

262 ●○●

264 slow??

274 ↓○

280 + diffuses.

Non-sectorial Mut. Catch.

		Glucose	Maltose	Lactose	Galactose	Gluconic	Hegal	
308		−	−	−	±	+	108	+
309		+	+	−	+	+	Lac	+
310		+	+	−	+	+	Lac	+
311		+	+	−	+	+	Lac	+
312		−	−	−	±	+	108	+
313		+	+	−	+	+	Lac	−
314		+	+	−	+	+	Lac	+
315		+	+	−	+	+	Lac	+
316		+	+	−	+	+	Lac	−
317		+	+	−	+	+	Lac	−
318		+	+	−	+	+	Lac	+
319								
320								
321								
322								
323								
324								
325								
[redacted]								
[redacted]								
248	1	+	+	−	+	+	Lac	+
249	2	−	−	−	±	+	108	+
250	3	+	+	−	+	+	Lac	−
~~253~~	4	− ✓	− ✓	+ slow-variable.	− ✓	+ ✓	Glucose	+ + + −
254	5	+ ✓	+ ✓	+ ✓	− ✓	+ ✓	Gal − !	−
255	6	+ ✓	+ ✓	+ ✓	− ✓	+ ✓	Gal − !	−
256	7	+	+	+	−	+	Gal −	−
257	8	−	−	−	±	+	108	+
258	9	−	−	−	±	+	108	+
259.	10	+	+	slow	+	+	Lac slow.	+
S. para A								inhibited
Landau 27								−
dublin 37								−
E. coli ML								

Lac Mutation Runs.
Spontaneous control.

February 23.

Dil. 1/10 suspension used in 132 to 5×10^{-6}. Use 1 drop (= .05+ cc) per Lac EMB plates. 20 plates.

ca 800/plate = 16,000 Test all suspicious cultures.

10 examined. No mutants.

Cross-Test Lac, & Lac4

February 24, 1948. EMS-Lac 8 plates each.

A.	Y53 ×	Y87.	0/400	0/400	0/2600.
	Lac1-	6-11-T+L+	0/400	0/200	B-11-T+L+
	from T-L-	Lac1-	0/400	0/400	0/400
B.	Y53 ×	W67	1/300,	0/200,	0/300 0/300
	Lac1-	Lac4-	0/200	0/200	0/300 1/300.
C.	W128	× Ø87	0/300	0/400	0/200 0/200
	L	Lac1-	0/400	0/400	0/200 0/300
D	W128	× W67	400 0/200,	0/100,	0/200, 0/100
		Lac4-	0/200	0/200	0/200 0/100
E	W120	× Y87	0/400,	1/300,	0/400 0/500,
	Lac4	Lac1-	0/500	0/300	2/300 0/500
F	W120	× W67	0/400,	0/400,	0/100, 0/200
	Lac4	Lac4-	0/300	0/100	0/100 0/200

0/2400

0/1300

3/3200

0/2000

Parents:

Y53	0/>>1000. +	Lac.	Y53, Y87	(W128)
W67	"			
Ø87	"	Lac4	W67, W120,	(W128).
W120	"			
W128	"			

W128 may be a deficiency for both loci, or a double mutant. Donor heavily into T(m) + Megal!

Methyl galactoside: [illegible]

February 23. Short streaks on 1% Megal EMB. 50/plate.

1	W-45	–
2	W-35	–
3	55 a	?
4	55 b	~~?~~ –
5	55 c	?
6	122	~~?~~ –
7	124	+
8	127	+
9	131	–
10	132	–
11	134	–
12	135	–
13	136	+
14	137	–
15	138	–
16	139	+
17	140	–
18	141	–
19	~~[illegible]~~ 143	–
20	144	±

W-190 series.

21	190	+
22	192	+
23	193	+
24	194	–
25	196	+
26	197	+
27	201	+
28	202	–
29	205	+
30	206	+
31	208	–
32	209	+
33	211	+
34	212	–
35	214	–
36	216	-
37	217	-
38	218	+
39	~~[illegible]~~ 219	~~[illegible]~~ -
40	222	-
41	223	+
42	225	+
43	228	+

Check Strokes.

Feb. 2[illegible], 1948
Streak out NA strokes on glu + Lac EMB:

	Lac	Glu.
W- 108	all - papillating	
188	1+/1000 - (pap.)	- pap.
239	colonies large, smooth, ±	v. small colonies. (bacch?)
243	++	all ±
245	large +, small -	all -, 2 colony types
251	+++	- glossy.
252	++ rough	all - rough
327	++ rough.	all -

253: slow + on glucose, magenta for lactose response. pH effect??
papillating v. strongly oxyglucose

Specific Recessives.

February 24 ff. 1928

1. W-35. Resuscitate 55-a + 55-b; Retest on lactose, Megal.
   55a (+) Relabel W-
   55b (+) " W-

2. Test W-253 papillae from glu, gal + mal on all three media + ē T1.
   5 + on all. T_1 - Insective.

3. Incr heavily into T(ns) + .05% sugar

Incr. P14.		Lac	Mal	Megal	RGma.	Sucrose.
24h.	W.45			−/=/−		
48h.						
P29.						
	W.145	± ++ all-	+++ all-		− +++ ++ clusters Lac, Mal.	
	W.243	− +++ Mostly +	− +++ Mostly +		W327:	− − −
	W.125	+++* +++ all + clusters Megal		++* +++ all + vs Megal.		
	W-128	=	=			

4. Test W45/Lac papillae on Megal. 9 +. 0 −

a) 37 125 Lac+R. test on Megal. All +.
16 Megal+R tested on Lac All +.
1 128 Lac+R " " Megal +.

33 W.145 Gma+R on Lac All +
29 " " " Mal " +

15 W.188 Glu+R on Lac All +
42 W.243 Lac+ on glu all +
53 " Mal+ on glu all +.

Specific. Reversions.

Test W-120 papillae on Megal. Megal - streak of on Lac.

6 all - (apparently) 2 + and -. Test ± on Megal.

Both are Mg +.

No specific reversions noted.

Nitrogen sources

Febr. 25, 1948.

Prepare, −N, per l:

glucose	1
NaCl	5
$MgSO_4$	.1
K_2HPO_4	3
KH_2PO_4	1

and autoclave 50/125 flasks.

Incr K-12 lid. suspension into:

			A27.	P29
P25.	A.	—	—	—
	B.	NH_4Cl 5% (.2%) 2.0 cc	v. dense +++	++++
	C.	Urea 20%, ster filt. (.2%) .5 cc	±	±
	D.	Glycine, 15% (.5%) 1.5 cc	++	++.
	E.	Asparagine, 5%. (.2%) 2.0 cc	++±	+++++ ! v. dense !

Final solution is ca. N/15 N.

This medium seems to be satisfactory for crease plating.

Cross-Test Lac Mutants.

~~124~~ 138

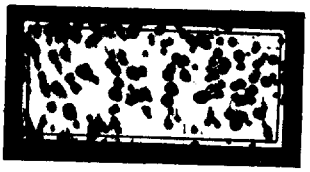
Reversions.

February 27, 1948.

~~+ x W-45 B. x Y-87~~

(1) Test ē W-45 for Lac-2 the fungal-ait of the current lac-series. 3 plates each.

276	+++ ✓ ✓	None of these are Lac_2-.
283	+++ ✓	
286	+++ ✓ ✓	
287	+++ ✓ ✓	
313	+++ ✓ ✓	
316	+++ ✓ ✓	
317	+++ ✓ ✓	



(2) Test 327 & 329 with Y10 & W236 on Lac & Mel for suppressors.

327 x Y10 on maltose Synthetic. 5 plates. No colonies!

Lac EMB 1 plate No colonies.

(3) 329 x 236. (W-35 Lac+ Rev. Mg- x W108 Sl.+ Lac_3-).

Many ++, --.

329 x Y10 on Lac. Apparently all +.

5 x 500 = 2500 colonies tested. Therefore, there are at least 3 alleles at the Lac_{35} locus: +, -, and a = Lac+ Mg-.

[Test 329 for mutation to +, and W-35 for relative frequencies of mutation to other states.]

→ Test prototrophs on Mg S. 71 all +! Should have been ca. 8% -.

B. globigii

138a

Feb. 25, 1948.

"Constant" yellow strain from P.W. Wilson.

Preliminary irradiation: 1 drop broth culture / ~~Nagler~~ GA plate.

40 secs. T

80 secs. T

120 secs ca 1000

108 x 243, 188.

Feb. 25, 1948.
On glucose - EMS.

W-108 x W-188.

Yield very low. 0/5. 0/5 0/16 Total: 0/41.
0/9 0/6.

W-108 x W-243. 0/13 0/7 0/10 ①/13 ①/50.
0/7.

The + recovered might be a reversion. Cross should be repeated on a large scale.

Test Reversions of Lac –

March 1, 1948.

Cross with Y40: 4 plates each. Lac S.

A3. W-235. Two classes noted: ++ and ±. (Allele?

P3. All ++. –: 0/200, 200, 200, 200. ~~Streak out ++/±~~

W-233 –: 0/300 0/200 0/400 0/400. ? Streak out.

W-232. 0/200, 0/300 0/300 0/300.

W-234. 0, but hold. 0/1000 –

W-231 0/200 /300 0/300. ? S.O.

W-33. 0/150 0/150 0/200 0/100 ✓

W-34. 0/1000, 0/300 0/300.

W-327 × [redacted]. Y40. T–L–L_3–B+M+ × T+L+L_3+B–M–

~~T+ ??/ B+M+ Lac~~

Lac S: 17+ : > 100 –. ∴ L_3 is linked to BM [redacted]

Mal S: 7 plates: ca 300/plate. Some probable Mal– noted.

Streak out. ✓ 9 Mal– recovered. Test these on glucose. All –.

Extract Lac$_2$ - .

Cross W-45 × W-34 on Lac, Mal's + Leucine or + Threonine.

Lac : leucine. Very few Lac - only one recovered.

Threonine ca 5% Lac - only four Lac - recovered

	T(0)	(B_1)	(T+L)	(T+L/B_1)
Streak out on EMB Lac + test purified clones: 1:				
2:	-	+	-	+
3:	-	+	-	+
4:	-	+	-	+
5:	-	++	±	++

Mal Mostly - L. agar needs doubling then T.

Test some + and - on Lac EMB. Fuit Runs. 7 found.

Threonine: Purify the Lac - 's.

Mal - 5/32 are Lac -

Mal + 2/16 are Lac -

		T/0)	B_1	T	T B_1
M+	1	-	+	-	+
M+	2	-	+	-	+
M-	3	-	+	-	+
M-	4	-	+	-	+
M-	5	-	+	-	+
M-	6	-	±	-	++
	7	-	+	-	+

141-G

See over

Test more Threonine Mal− segregants:

all Mal−.

Lac+	Lac−	?(Motility−).	
46	8		
42	5	3	
59	4	4	
50	3	8	61
42	7	4	
	4		

Streak out prob. Lac− and test nutrition.

		O	B_1	T	T B_1
	1. B_1				
	2. B_1				
	3. B_1				
W-338	4. (T B_1)	−	−	±	++
	5. B_1				
	6. B_1				
	7. B_1				
	8. B_1				
	9. B_1				
	10. B_1				
	11. B_1				
	12. B_1				
	13. B_1				
	14. B_1				
W-339	15. (T B_1)				
	16. B_1				
	17. B_1				
	18. B_1				
	19. B_1				
W-337	20. Prototr.				
−6	21. (T B_1)				
−6.	22. (T B_1)				
	23. B_1				
	24. —				
	25. B_1				
	26. B_1				
	27. B_1				
	28. B_1				
	29. B_1				
	30. B_1				
	~~31.~~				
	~~32.~~				

2/28	T B_1
1/28	Prototr.
25/28	B_1−

W-306.

March 1, 1948.

Streaks out on Mal, Lac EMB. P29.

3/2/48. Lac -, rather alkaligenes. No papillae

Mal - (faint slow purple); Numerous papillae. Test on Lac + Mal.

Papillae are alkaligenes. ~~Slow~~ Still all Lac - Mal -.
(111) (8)

3/3/48. W-306 x 58-161 on Lac's

3/4/48. Papillae noted in 306/L. Picks to Mal to check specificity. All seem to be Mal - or Mal ±. Streaks out on Lac. Test purified Lac^R + on Mal.

March 3, 1948.

W-306 x ~~W-46~~ 58-161 on Lac's.

T+L+ B-M-#Lac+ × T-L-B+M+Lac-

ca. mean Lac,.

5 plates.

Lac+	Lac−
11	14
9	8
~~4~~ 9	17
4	8
4	10
37	57 / 94.

Test Lac+, Lac− on Mal.

Lac+: ~~#~~ 1 Mal++ 29 Mal−

142-aa

test ~~#~~ by streaking.

1. Mal- Lac+
2. Mal- Lac+
3. Mal+ Lac+
4. Mal+ Lac+

142-ab. Lac− 2 Mal++ 27 Mal−.

Test ~~4~~ by streaking

1. Mal+ Lac−
2. Mal+ Lac−
3. Mal− Lac−
4. Mal− Lac−

∴ W-306 is a double mutant, $Mal_x^- - Lac_y^-$.

Hug Lac^R (Lac+ Mal−) as LV−

~~81 plates~~. March 7, 1948.

1 drop 10^{-5} dil. Y10/YB culture spread on each of 81 plates.

ca > 1500/plate. About 160,000 colonies scoreable

some plates > 2000.

9 suspicious colonies streaked out. All intact.

5 mutants recovered.

w- 331
w- 332
w- 333
w- 334
w- 335

[Compare with 68 mutants recovered from about the same number of colonies in Exp. 132).

Antisera to C1 + C2. 144

March 3, 1948.

Immunize Rabbits against Y105 + Y109.

Prepare antigens from broth cultures, wash in H_2O. Estimate cells.

386 F6	Y105.	$3/3$ 10^9
387 F6	Y105	10^9
383 F5	Y109	10^9
385 F3	Y109	2×10^9

All rabbits died in 12 - 20 hours. No post.

Fermentation tests.

3/4/48.

EMB:	W-108	Y10	W-118	W-119	
Mannose 1%	–	+++	+++*	+++*	
Sorbose 1%	*	–	–	–	v. poor growth (nils.?) 108 showed best growth.
Glucose 1%.	*	++	++	+++.	

* Indistinguishable. Compare Maintal.

Melezitose 3%	K-12	Y10	W-306	W-55	
	–	–	–	–	No detectable utilization

3/11/48. Ethylene glycol-β-glucoside (any more?)

18h.	K-12	+ weak	++
	W55	++	+++
	W-108	–	–
	W-145	+	++
	W-[illegible]1	+++	+++
	W-327	–	–
	W-328	–	–

	145	K-12	W-55	W45	W108	Y53	327
Methyl β-l-arabinopyranoside.		–		–	–	–	
Methyl α-d-xylopyranoside		–	–				
Methyl β-d-xylopyranoside		–	–				
Ethylene Glycol-β-d glucoside		±	++				
Melibiose.	±				–	±	–

328+!

weakly ambiguous!

Urease; Glycine utilization.

3/3/48.

See 137 for "N" medium. Inoc K-12 lightly or Y10 heavily into: P3.

		Inoc.	A5.	A7	
N(TLB,) 1mg	1. Y10	+	+	+	
"	2. Y10	+	+	+	
"	3. Y10.	+	+	+	
"	4. K-12	-	+	+	N from amino acids!
N(0).	5 K-12	-	-		
N(Urea).	6 K-12	-	÷.		
1mg NH4Cl	7. K-12	-	+++	+++	Urea not inhibitory.
NH4Cl.	8. K-12	-	+++.		

In following, omit glucose; add NH_4Cl for C-utilization: ~~K-12~~ + TLB, (Y10).

				P8	
11.	—	-	-		-
12.	.1% glucose	-	+++	+++÷	✓
13.	glycine	-	-	-	-
14.	asparagine.	-	+±.	+++	✓

Compare the N-utilization of glycine! (Acid acetic; glycollic acid!)

Fermentation Tests.

March 6, 1948.

EMB:

		Lac	Megal.	Mal	Sal	Sna	Glu.	
1	319	—	—	—	±?	-?	-	Growth limited.
~~2~~	~~320~~							
3	321	±	+	±?	±	+	-	(109)
4	322	—	—	—	±	+	—	(108) Megal -!
5	323	—	+	+	+	+	+	
6	324	—	+	+	+	+	+	
7	325	+	+	+	+	+	+	
8	326	-	—	—	±	+	—	Glu- Megal -! & 108
9	331	—	+	+	+	+	+	
10	332	—	+	+	+	+	+	
11	333	—	+	+	+	+	+	
12	334	—	+	+	+	+	+	
13	335.	—	+	+	+	+	+	

Mg: ~~W. 329~~ ~~W-330~~ ~~W-335.~~

			TRE 24. →	TRE 36h.	48.	
Tre.	W-1		+++	+++	✓	
	W-60		- .. ±	±	—	
	W-102		+++	+++	✓	
1st. rdg.	W-108		—	—		
	W-145		+++	+++	✓	
24h.	W-306		—	—	—	with +++ pap.
	W-327	108 Mal+	—	—	—	
	W-328	108 Mal+	—	—	—	
	W-117		—	—	—	

Repeat tests on purported negatives.

Select for specific reversions.

W-60

March 5, 1948.

Heavily inoculated: P7

W-243. Lac +++ * 99%+. Test on Glu, Mal. 60: Mal+. 34 Glu+ No.S.R.
Mal −
Glu −

W-145 Me-gal. ++ * Mostly weak+. Test on Lac. 16+. Test on Suc, Mal.

W-125 Me-gal. +++ * All+ Test on Lac. 10+.

W-120 Me-gal. ± ±

W-45 Me-gal. ± +++ * 41+/Lac all+.

P8. P9.
W-117 Tre. +++ * 85%+. Test on maltose 15 all+. Test these on glu+lac. All +, +.
±

W-60 Tre +++ * 60% weak+. Test on maltose. (6−).
Retest on trehalose: +±. S.O. ① on trehalose.

‡ W-117 controls easily distinguished from +'s., and between glu (±) and lac (−).

Papillae from 327, 108 on trehalose tested on glucose.
327: 4+, 2− } Retest on trehalose. 149-1-6
108: All−. } 149-7-10 (11, 12 S.O.)

When untested, no distinctive Tre+, unless Glu+, noted.

Test Recombination of C2 mutants.

March 6, 1918.

Prepare washed suspensions & plate .1 ml each on lac EMS '16.

	A 8.
1. W93	—
2. W138	—
3. W139	—
4. Y87 × W93	—
5. Y87 × W135	—
6. Y87 × W139	—
7. W93 × W138	—
8. W93 × W139	—
9. Y87.	—

No evidence of recombination. Mixed culture must be tried.

March 10-12, 1942.
Y10 x Y45.

A) T(B,) plates.
Streaks on Lac S again.

+	−	
18	2	/ 20

7 tested were all T_1^S as expected.

B). Lac S B, plates.
Hold. A 11.

+	−	
41	1	
72	2	
32	1	
145	4	149.

Recount A12.

LB,

+	−	
100	8	
121	13	
71	3	
117	9	
409	33	442.

Lac- = 7.5%

Compare with 8.6% of p. 42.

Lac (o)

+	−	
31	2.	
35	2	
66	4	/ 70

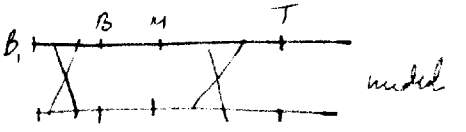
5.7%.

March 8, 1948.

Cross on Lac(o) Agar, W ~~22 324~~ 337 with the following:
3 plates each (.1 ml susp.)

W45.

No colonies.



W35 8 Lac – colonies all +old.

W72 3 Lac – colonies all +old.

Y87 9 Lac – colonies.

Crosses should be repeated.

Glucose - 1 - phosphate.

Make up T(m) · BHTLB, + equivalent of .05% glucose in 5cc volumes.
Inoculate legibly with: P10. [Filter - Sterilized].

		Y10	W-108	W-327
1. K. glucose-1-phosphate (Landy)	P11	—	—	—
	A12	—	—	—
	~~A12~~			
	A13.	—	—	—
2. Glucose.		++	—	—
		✓	—	—
		✓	—	—

Glutarate + Riboflavin as Recombinators. 15-4

March 9, 1948.

T (B$_1$) Y10 + Y87. Measured drops dilute suspensions.

A) + 4 ml H_2O/plate B) 4 ml H_2O + 700 γ B_2 + 35 mg Glutarate / 100 ml medium.

A. P10 (ca 364.) 33 / 7, 12, 4, 5 m = 8

B. 34 / 4, 11 (2 drops), 6, 13 m = 8½.

No pronounced effect of B_2 + glutarate.

More colonies may appear later). 12 appeared altogether.

See 155.

on EMS plates

March 8, 1948.

1. Y-87 X Y-10
2. Y-53 X Y-40
3. W-183 X Y-46

1A On EMS (−B_1) plates.

	+	−		−R	−S	+R	+S.
a) Readings from plates.	13	5	b.				
	13	5	S.O.				
	10	4	T_1-Lacs'				
	~~22~~	~~0~~					
	~~36~~	~~14~~	77.				
	~~63~~	~~14~~					
a' Repeat A12. :	~~64~~	~~18~~	~~82~~	52.	14	3	27	8
	36	18					

Sectors counted twice

X W45 according to label!

1B. On EMS(B_1) lac plates

a. direct counts.	17	4	
	16	8	
	16	3	
1S.	30	15	
	30	5	
1S.	16	3	
	22	11	
	28	11	
	19	3	
	194	63	257.
a' repeat A12 →	227	101	338

Total: 6 sectors.

1C. From T(B_1) plates.

See page following for raw data. Totals of all experiments this page are:

	−R	−S	+R	+S
S. 445	131	6	207	101
	(.294)	.013	.465	.227

Cf published results:

13 241

1a. Scored originally as Lac+.

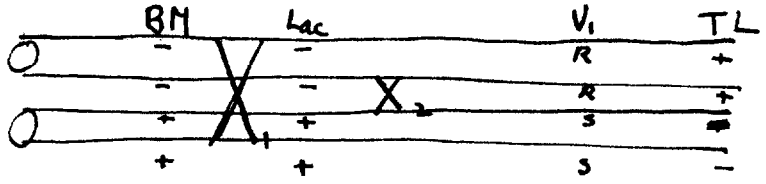
	−R	−S	+R	+S.	
	0	0	14	3	
	0	0	13	5	
As Lac−	14	3			
	14	3	27	8	52:
	.269	.058	.519	.154	

	+?				
1B. As Lac+	1	1	29	13	
	0	0	33	21	
	0	0	15	8	
	0	0	12	9	
	0	0	16	6	
	0	0	13	7	
	1	0	13	5	
	1	0	15	5	
As Lac−	49	1	0	0	
	39	1	0	0	
	~~91~~ 91	3	146	74	314.
	.290	.095	.465	.236	

1C. (plates = B_2, glut).	16	0	21	12	
	10	0	13	7	
	26	0	34	19	79.
	.329	0	.430	.240 ✓	

	131	6	207	101	445 ✓
	.294	.013	.465	.227 ✓	

A total of 6 sectored colonies were noted. These were purified and tested with T1. All 12 cultures were V_1^R.



No. X_1 $_{-R.}$ $+R.$ X_2 $_{+S.}$

In calculating p, the chances of X_2 being in Lac – V_1 :: V_1 + TL only should be considered. X_1 is almost completely fixed in region ① as –R. An expectation of 4:2 is not signi. different from the experimental value of 6:0.

Test on B_1 for requirement. A Lac – B Lac +.

	A	B
1.	B_1	
2.	+	B_1
3.	B_1	B_1
4.	B_1	B_1
5.	B_1	B_1
6.		

Also, test Y10 on pyrimidine + thiazole:

1. TL	–
2. TLB_1	+++
3. TL Py	+
4. TL·Th	++
5. TL·Th·Py.	++
6.	

Specific Reversion.

#64

April 8.

Streak out W-108 on EMB glucose, mannose, fructose. EMB

April 17. No papillae seen on streak plates.

March 31, 1948.

Test strains on lactose, epi-lactose, neolactose & galactosan received from N.K. Richtmyer. 1% - EMB (small plates).

	Str.	Lac	Neolac	Epilac.	Galactosan	[Megal].
1	+ K-12	+ P	− *	+	−	+
2	+ Y10	+	−	+	−	+
3	Lac$_1$ Y53	−	−	−	−	+
4	Lac$_2$ W45	−	−	−	−	−
5	Lac$_3$ W-108	−	− P	−	−	+
6	Lac$_4$ W-126	−	−	−	−	−
7	Lac$_5$ W-145	(+)	−	−	−	+
8	Lac$_6$ W-125	(+) !	−	±	−	−
9	Lac$_7$ W-133	− P	−	−	−	−
10	Sl, W-117	− P	− P	−	−	−
11	Sl W-252	+	±	+	−	+
12	Sl W-328	+	−	+	−	+
13	Gal- W-254	+	−	+	−	−
14						

[5/16/50 — Note that W 252 - isolated as neolactose pos!]

* Papillates to form shoving v. considerable utilization

Galactosan — all. Lactositol. All —

Neolactose all —

epilactose follows lactose.

Streaks out papillae of K-12 / Neolactose on lactose. Test colonies on neolactose. 8+ 3−. Isolate + as W. 341. Still Lac+

See over.

See 169

Inoculate 58-161[R] into 25 ml T(m) + Nolactose .25%.
+ galactosan

Delayed growth on nolactose.

Streaks out and test on nolactose EMB. 11- 0+.

Repeat streaking.

Synthetic Medium: Lactose

per 10 liter bottle. Use technical grade chemicals.

$NaCl$	50 g.
K_2HPO_4	30
KH_2PO_4	10
$(NH_4)_2SO_4$	50
Sugar	150 g. Sterilize separately.

Grow K-12 24 hr. aer., room temp., mixed, with lactose.
Collect 4 g. cells. Divide & incubate each portion for 3½ hours in 100 ml 1% peptone + 5% lactose or glucose. for adaptation.
Sediment after 3½ hr. + resuspend each in 50 ml M/100 Na citrate under toluene + autolyze! P8 – P10.

Autolysate volume after bacteria are removed is 50 ml each. The autolysates give very high blanks on Barford's reagent, so they cannot be directly assayed.

∴ ~~Assay~~ To 10 ml samples add 3.5 g AS + sediment. Assay ppt redissolved in 1/100 saline citrate. = cc $KMnO_4$.

G alone	< 1 drop.
.1 ml G + 10 mg lac	.90
1.0 " "	.41
L alone	< 1 drop
.1 ml L + 10 mg lac	.90
1.0 " "	.33
Lactose 10 mg.	1.14 [Blanks].
Glucose + galactose 10 mg.	19.06
" " 1 mg.	1.97
163 B2 + Lac.	5.42
" (blanks)	< 1 drop

Neither preparation hydrolyzed lactose beyond the blanks (ca 6%).

$\frac{5.42 - 1.14}{19.06}$ = ca 22% hydrolyzed in 20 mins.

W-125, W-145

April 2, 1948.

In neolactose tests it was noted that W-125 and W-145 were positive or slow positive on lactose. When streaked out again as controls on outcrosses, this was noted again, and suggests the need for reexamination.

Streak out on lactose EMB and compare:

W-145 stock slant < 1% Lac − colonies. - colonies quite small.

W-145, lyophil tube All Sca −, Mal −, Lac −. Recover to slant.

W-125. Numerous fairly good sized colonies that might be considered slow. Streak out must to good +.

[It seems that slow. 145 colonies near a + are more likely to be Lac + than those further removed. This suggests a pH or redox effect.]

Lactositol selection
Galactosan "

Apr. 9, 1948.

Inoculate 58-161 or Y10 heavily into T(m) TLB_1BM with 0.1% sugar.

25 ml.		24h.	48h.	
1. Lactositol	Y10	±	++	Apparently lactitol mutants can be selected for.
2. "	Y10	±	++	
3. "	58-161	±	+++	
4. "	58-161	±	+++	
100 ml.			A28	
5 Galactosan	58-161	—	—	Throw out
6 Galactose	58-161	+++.	+++	

A28. Streaks out 1 and 3. 1 was sterile. (3) gave 1 colony on lactitol which was +.

A29. S.O., side by side W-349 and 58-161.
W-349 is pure Lol+, but relatively weak; 58-161 is definitely −.

LACTITOL

EMB - 1% (from Wolfram, dihydrate)

K-12 —

Y10 —

Y53 —

W45 —

W-108 —

W-145 —

W-125 —

W-126 —

W-133 —

K-12 Nal+ — ~~Isla~~

58:61 Nal+ —

see p. 170 for selection of Lol+ mutants.

Lactose mutants.

W125, W145. Predominantly lac + or streaking.

W126 x ~~W10~~ 58-161.

	+	−
	26	16

lac − v. small colonies on EMS

W133 x 58-161

	+	−
not so small	33	53
	16	45
	49	128

W45 x Y10 >10+ : 1 −

W108 x 58-161. 3 types counted.

	++	±	−	
original streak shows not + but some variation.	8	25	31.	76.

see W-342 bb.

April 9/9/8.

410 S. typhi 4V Hanover.

l-Arabinose EMB. Ca 2000/plate unevenly spread + difficult to score.

11-30. 36 plates = ca. 70,000 colonies. 20 "mutants"

d-xylose EMB. 50 plates. ca 1000 scorable colonies per plate

1-10 ca. 50,000 colonies

10 "mutants"

W−		Xyl	Arab	Lac	Mal	Glu	Sna	Sal	TI
351	1	−		+	+	+	+	+	
352	2	−							
353	3	−							
354	4	−							
5	5	−							
	6	−							
	7	−							
	8	−							
	9	−		+	+	+	+	+	
L 360	10	−							
361	11	+	−						
	12	+	−						
	13	+	−						
	14	+	−						
	15	+	−	+	+	+	+	+	
	16	+	−						
	17	+	−						
	18	+	−						
	19	+	−						
370	20	+	−						
	21	+	−						
	22	+	−	+	+	+	+	+	
	23	+	−						
	24	+	−						
	25	+	−						
L	26	+	−						S
	27	+	−						S
	28	+	−						S
? ?	29	−	−	−	−	−	−	−	R
380	30	−	−	−	−	−	−	−	R

29 + 30 are probably contaminants, but nutrition should be checked.

Formate; Oxalate decarboxylase media

EMB ± 1% glucose +. Read at 24 h.

1. 2% F. no growth.
2. 2% + G no growth.
3. 1% F. Inhibited growth; some papillae?
4. 1% F G Small translucent colonies.
5. .5% F Moderate colonies translucent.
6. .5% F G large colonies. Milky or blue. ← good selection level.
7. .1% F Moderate colonies translucent.
8. .1% F G large, purple colonies.

9. 1% oxalate + .4% glucose
10. ~~1% oxalate~~ .4% glucose.

For formic "decarboxylase" selection medium, use .4% Na formate, 1% glucose EMB.

Retests; phage.

Apr. 29, 1918.

	Glu	Mal	Lac	Gal	Gna	Megal.	T I
182	++	+	−	+	+±		S
185	++	−	−	−	−		S
187	− thin	−	−	÷	÷		S
188	−	−	−	+ ±	+		S *
189	−	−	−	− p	+		S *
218	+ th	+	++	+ p.	+		S
239	−	−	−	−	− p		S
248	+ pap.	−	−	÷	÷		S
245	−	−	+	++	++		S
253	±.	+	+	+	±.		S
319	−	−	−	+	+		S
321.	++	++	++	++	+		S
108	− v.pap.	− v.p.	− v.p.	++	+		S

A.

These are suspensions from fairly old cultures.

* v. few plaques.

B

47
72
74
76
83
87
~~[illegible]~~
~~[illegible]~~
~~[illegible]~~

S.O. 321. on ~~glucose~~ lac

245 on lac [illegible]

218 [illegible]

182 lac [illegible]

185 [illegible]

108 S.O.

[illegible]

[Try O.P. effects on types thins on glucose I.
Many are "thins" on glucose than on desaccharides - e.g. 187, 218,

S.O. 249 on lactose 90%+. Purify a Lac− for test as Lac_3.

243 on lactose. All colonies are slow ++. Broad streak is −. One (−) colony noted. Purify.

245 on lactose. − acid very faint ± colonies predominate, with numerous papillae +.

S.O. − colony on lactose EMB: all − colonies.

Test:	Glu	Mal	Lac	Suc	Gal	Glu	
	108 pur	−	−	+++	++	−	W108
	245 Lac−	−P papillae	±	+	++	−	
184, 1-3.	243 Lac+	−	±	+	±	− th.	W381
	249 Lac−	−	−	+	++	−	
	243 Lac−	−	±	+	±	− th.	W243

Restreak purified W108 on Lac

249 is comparable to W108 and may be Lac_3−. 243 Lac+ may be a suppressor poor. Call 243 Lac− W243 as recovered, and 249 Lac+ = W381

Reconstitute all these strains.

W185, streaked out: Colonies small [illegible] slowly glucose. 95% +. [illegible] − retried.

Mannose All +.

Sorbitol All −

Fructose All +.

Recover ~~Roser~~ glu − and compare with + on extended series of sugars.

Reversals of W-245 177b.

May 5+ 1948.

Streaksout 177a, W-245⁻/Mal on Mal E 14B.
Pick 14 Mal+ colonies to Lac and Glu. at 37°.

a) All 14 are Lac++ Glu-

b) 3 Mal ± colonies Lac ± Glu -

1 Mal - colony Lac - Glu - as parent.

S.O. from a and b on maltose to purify. W397 + W398

Fermentation tests: EMB.

		Megal.
	K-12	+++
	W-108	+
	243	+
	260	−
	261	÷
A	267	÷ (variable)
	269	+
	270	+
	277	−
−	280	+
	284	−
	285	−
	292	−
	297	+ var.
B	298	÷
	301	−
	307	++
	308	÷
	312 *	
−	249	+
	257	−
	258	−
C	319	−
	322	+
	321	+
	120	− n ÷
	RS.1	+++
	RS[illegible]	++[illegible]
	453	++[illegible]
−	410	+++

		~~Megal.~~ Megal
1	112	+++
2	121	+++
3	276	−
4	283	−
5	286	−
6	287	+++
7	313	−
8	316	−
9	317	++
10	122	÷ (variable)
11	132	−

* 312 + 302 were found filled with water! Source? SO on glucose.

Glucose – mutation run

April 28-30, 1948.

58-161R. 135 plates × >100 scoreable colonies
= ca 15,000 total.

15 tiny colonies picked. None mutants.

No mutants from ca 6 other sectors.

Formate mutation Run.

Y10. Spread on Glucose 1%, Formate .4% EMB and irradiated as above. 46 plates × >500/plate = 25,000 colonies.
Due to crowding it is not certain how efficient mutant recovery would be. Test some representative colonies.

Formate mutants.

May 1, 1918.

Compare – (glucose EMB+) and + (–) colonies from formate-glucose EMB on
(a). Formate .5% N-Case Thalin .01% agar
+ (b) Formate – phosphate N-Case gas tubes.

	EMB.	(a)	(b)	(c) EMB/formate-glucose
1.	1–		+++	++
2.	1–			
3	1–			
4.	1+			–
5.	1+			
6.	2 –			++
7.	2+			–
8.	3–			+
9.	3+			–
10.	4–			
11.	5– (imp?)			
12	6–			–
13	6+			–
14	7–			–
15	7+			–
16	8–			

All cultures produce voluminous gas from formate broth

a) cannot be scored due to diffusion of alkali through agar.

Streak out 1, 4, 6, 7, 8, 9 12, 13 + 14, 15 on glucose EMB. Indistinguishable!

Test streaks on formate-glucose agar.

+ + = [illegible]

Transfer (6) to nutrient agar slant as W – 385

Some indicator media.

~~For formic~~

Test N-12 on:

	24h.	48h.
1. EMB – 2% Na glycerophosphate · 5H₂O.	Large – colonies.	✓
2. 1% Pectic acid, neutralized NaOH. N.S.	Agar very soft.	✓
3. Hydrolyzed casein (HC) agar.	Moderate colonies.	
4. HC – Succinate – Chlorophenolendophenol.	Moderate colonies. Agar was decolorized after autoclaving. Shows diffuse reddening around colony groups.	
5. HC ± succinate Cli " .	V. slight lightening around colony mass	
6. HC – Nadi.	No growth.	Spontaneous coloration in agar overlay lit. 1 cc. in U.V.
7. HC · Indigosulfonate .01%	Decolorized on autoclaving ± agar	
+ Succ.	Moderate colonies; no decoloration.	
– Succ.		
8. HC – starch Iodine.		
+ Succ.	Col. discharged on preparation (I_2 reduced.	
– Succ		
	large, slightly brownish ~~o~~ transp. colonies.	
9. Sorbitol 1%	++±	Not quite so intense + as glucose but unquestionably strong +.
10. Sorb. .5% + Galactose .5%	+++	No inhibition
11. (Lactitol)		
12. Galactose .5%.	+++. ✓	✓

K-12"; W-145; growth on synth. medium.

April 30, 1948.

Incc W-145 lightly into T(m) TLB, BM + .1%

	24h.	72h.
1. Gluconate	–	+++
2. Glucose	+++	+++
3. Lactose	±	++
4. Maltose	+	++

Incubate putter and examine for sp. reversions. S.O. P3 on homologous media.

58-161 into.

			24h.	
1. Na Glycerophosphate $\cdot 5H_2O$	0.2%		+++ Faint ± on EMB	S.O. 2p plate
2. Pectic acid; neutr. NaOH.			±	+ faint growth in synth.
		72h.		
EMB.	58-161	–	+	
	Y10	–	+	

P3.

S.O. 1, 3 and 4 on homologous EMB agar.

1. No acid production; colonies very substantial

3. Numerous + colonies. Pick to gna EMB

4. Maltose – all –

↳ 14 colonies all –. Purify on lactose EMB.

W-391

Glucose mutations.

April 29, 1948.

V/O 1 drop, etc. (Hanovia lamp 5 secs.) on glucose EMB.

Most of 5 L plates were heavily contaminated.

Select some likely colonies from 20 least cont. plates; ca 500 scorable colonies = 10,000.

3 Glucose −. Streak across T1. All V, S.

	Glu	Gal	Lac	Mal	Sna
1. W-382	− *	+++	+++	+ Pap.	+++
2. W-383	−	±	±	−	++
3. W-384	−	++	−	−	++

-382. Why papillae only on maltose? This appears to be the desired Glucose-specific mutant, for crosses with Gal −.

* produces acid strongly when left out at room temperature 2-3 hours! (Compare 340).

~~Streakout 382 and 340 on each of two glu plates. Incubate overnight at 37°.~~

See 185

184 fermentation tests. 185

5-3-42

Streaks out to form colonies of: (on EMB 1%):.

	Rhamnose	Glucose	Sorbitol	Fructose	Mannitol	Mannose	Galactose	Dulcitol	Mucic ac	Xyl	Ara
1. 254 *	−	++	+++	+− ✓	+++	+++	+++ ✓	++ ✓	+ and −	−	++ ✓
2. 108	−	++	−	− −	−	−	− − ✓	− +	−	−	+++ ✓
3. 185 D+	inh	inh	−	++ −	inh	++	− − ✓	− −	inh (1 col.)	−	inh inh
4. 185 D−	−	+	−	− −	−	−P	− −P ✓	− +	−	−	−P ++
				A6 P.	A6 P.			A6 P.			A6 P.
5. 249	−	+	−	* −	−	−	− −	− ±	++ -	−	− ++ ✓
				LP							A6 P. ++
6. 351	−	++	+++ ✓	+++ ✓	+++ ✓	+++ ✓	+++ ✓	+++ ✓	−	−	− A6 P ++ ✓
7. 361	−	++	+++ ✓	+++ ✓	+++ ✓	+++ ✓	+++ ✓	+++ ✓	−	−	− ++ ✓ ✓
8. 58-161	+	++	+++ ✓	++ ✓	+++ ✓	+++ ✓	+++ ✓	+++ ✓	−	−	+++ ✓ +++

Y10 - inositol −−

A6 some wwww +

P = papillae, presumably reversions.

* S.C. on hexadecane medium, A6

Lac_3 Crosses

May 4, 1948.

Cross the following on EMS-Lac-B_1.

1. W-108 x W-249 (A conc. susp) T-L-B_1-Lac_3 x B-M-Lac_x
2. W-108 x Y-40 x B-M- V_1^r
3. W-249 x Y-46 x T-L-B_1-V_1^r

P7.

① Yield very poor.
By plate.

	+	−	to retest
	0	1	
	0	1	
	0	1	
	0	0	
	0	0	
	0	1	
	0	1	
A.	0	5	
A.	0	3	
A	0	4	
A	0	2	
	0	0	
	0	3	
	0	1	
A	0	2	
	0	1	
	0	1	
	0	0	
	0	2	
	0	0	
A	0	3	
A	0	4	
	0	2	
	0	38	1

~~After several days incubation, some Lac+'s came up. Since these may represent diversions, do not use these plates~~

(2)

+	–
2 s	31
1 s	25
6	34
2	52
2	30
4	50
0	32
17	254 | 281

= 6.7% Lac_3+.

T-L-B_1-Lac_3-B+M+ } ×
T+L+B_1+ Lac_3+ B-M- }

Lac_3 is fairly closely linked to BM. (very near LacI)

Phage tests (on glucose plates).

Lac+:	6^R	2^s	8	All Glu +
Lac–:	48^R	13^s	61	All Glu –
Lac–:	51	12	63	
	99	25	124.	

% V_1^R = 80%

(3). Very poor yield on a rather dense background.

0	1
0	1
0	0
2	0
0	0
1	0
1	1
4.	3

May 3, 1948.

100 plates Glu EMB × 250/plate = 25,000.

17 tiny colonies streaked whole on glucose 3 – (1-3)

14 other possibles S.O. on glucose.

	4	O	mucoid
	5	O	+
	6	O	+
	7	O	+
(4)	8	O	–
(5)	9	O	–
	10	@	+
	11	@	+
	12	@	+
	13	@	+
(6)	14	O	slow?
(7)	15	O	– sm. cl.
	16	O	+
	17	O	+

1, 2, 4, 5 and 7 are T1 S, and probably mutants.

3 is a yellow chromogen } almost certainly contaminants.
6 a pink chromogen }

		W–				
1.	386	–	–	+ slow	+	++
2.	387	±	±	+	±	++
4.	388	–	–	– th.	–th [illegible]	–
5.	389	+	+	+++	+++	+++
7.	390	++	++	+++	+++	+++
		+++	–	+++	±	–

→ specifically bac +

Lac$_3$ Crosses.

May 5, 1948.

1. 108 × 58-161 on glucose ± B,
2. 249 × 108 on glucose B,
3. 382 × 249 on glucose, lactose
4. 382 × 58-161 glucose, lactose.

P7:

	+	−		
1 − B$_1$.	~~0~~	~~10~~		
	19	177		Too bad to be properly
	16	133		counted
	35	300	335	
+ B$_1$	21	163	184	Some colonies are darkened
	56	463	519	but probably not +

Second P10:

2. Yield negligible (ca^{c}l per plate)

3. (glucose) Yield negligible - all -
Lactose. All looks "+" after prolonged incubation. Score on glucose "T$_1$".

4. Glucose - unscorable - no yield
Lactose - all turned +.

Tetrazolium.

May 7, 1948.

(1). Make up varying concentrations of triphenyl tetrazolium chloride in nutrient agar and autoclave. Streak Y100 plates.

Per ml:

1 mg.	Medium faint pink; all colonies intense dup red
150 γ	Medium sl. tinged; isolated colonies deeply red with transp. margins.
50 γ	As above. Medium less tinged
30 γ	As above for isolated colonies; confluent growth colorless
10 γ	Color more limited in colonies and sl. less intense.

1 mg. level shows slight initial growth inhibition

Lac 3 mapping. May 10, 1948

(1). W-108 × Y40. n lac and glu EMS (NF).
(2) W-249 × Y46
(3) W-108 × W-249.

3:

	−	+
	24	0
	55	0
	9	0
	10	0
	31	0
	67	0
L	32	0
L	24	0
	22	0
	25	0
L	11	0
	31	0
L	26	0
	31	0
L	41	0
L	24	0
L	16	0
L	17	0
	5	0
Lac:	191	0.
Glu:	310	0
Total	501	0

Both are probably Lac_3 −.

501.

(2). Plates v. unsatisfactory. Overgrown or no colon. Some plates readable, esp. lactose.

+	−
18	2
2	
16	4
3	2
3	0
4	0
4	1
3	0
7	0
7	2
67	12	89..

This count unsatisfactory except to indicate more + than −.

(1) Lac.

−	+
53	8
45	13
24	3
39	10
14	5
44	16
29	3
31	4
35	8
42	11
42	9
39	8
75	7
512	105	617 = 17% Lac+. 83% Lac−

all scored (−) on glucose, probably due to uncertainty of medium. Test by streaking to fresh glu EMB.

Test Lac+ on Glu, T1:

R	S
22	2
17	2
13	4
52	8	60. = 13% among Lac+

Test Lac− on Glucose Tube T1

Test bac- segregants on T. (Glu or Gna EMS!)

R	S	
15	5	20
14	6	20
14	6	20
13	7	20
56	24	80 ✓

30% S among bac-.

The distributions is then: m.d. (calculated from III).

-R	.58	II	.67
-S	.25	III	.26
+R	.15	I	.16
+S.	.022	IV	
	1.00		109 [of 80 as previous estimates.

This gives a total for the V, segregation of 73% R; or 25% crossing over in region III which agrees very well with preceding data (v. thesis table 6) giving 27%.

YHO. -- + R ++

108 ++ - S --

Ia. II III

Estimating x from these data:

$\tanh^2 a$	$= .022 \times .15 / .58 \times .25$	$= .0238$	$\sqrt{.154}$	a
" b	$= .022 \times .58 / .15 \times .25$	$= .340$	.583	.67
" c	$= .022 \times .25 / .15 \times .58$	$= .064$	.253	.26
				1.08

Lac-3, Lac-4 crosses

May 17, 1948.

1. 108 x y40 On Lac(−) and on Gna EMS'
2. W-67 X Y46 On Lac
3. W-126 X Y40. On Lac

1: gna: 4 [illegible] <10/plate; Test on glucose EMS: T1.

−R	−S	+R	+S	
22	8	1	1	32.

1: Lac.

−	+	
9	0	
20	0	
24	1	
1	0	
5	0	
4	0	
2	1	
13	1	
4	1	
3	0	
3	0	
10	2 S	
8	0	
6	0	
6	1	
4	0	
3	0	
3	0	
2	0	
130	7	137.

5.1% −

Lac+: $6^R : 1^S$ all Glu +

Lac −

R	S	
27	6	
18	6	
21	9	
16	12	
82	33	115

All Glu −

* Three tests each colony on amygenis plate was tested.

The distribution is:

−R	−S	+R	+S
.684	.275	.044	.007

Total V, ^{R} segregation:
28.2% S

(2).

+	−
3	0
1	0
1	0
2	0
1	0
1	0
1	0
3	0
3	0
1	0
4	0
4	0
1	0
3	0
2	0
1	0
32	0.

!

W-67 x Y-46.
B-M-Lac+ x T-L-B,-V,R.

R	S	
29	2	31.

(3).

+	−
1	2
0	3
7	17
6	13
1	0
10	3
2	6
2	15
1	0
4	3
1	6
16	33
2	9
1	8
54	118 / 172

	R	S	
Lac-	11	4	
	11	6	
	22	10	/ 32
Lac+	*		
	8	3	
	19	0	
	20	3	
	47	6	/ 53

−R	.47
+R	.27
−S	.22
+S.	.04

+S rather highly attenuated agrees with sep. of Lac-1.

31%+ (Maybe excessive)

On three plates, − colonies were much smaller than + possibly distorting ratio.

W-108 X Y-40.

p/187: 17+ : 254- on lactose. ie 6.7% Lac_3+

Among +, 6 V_1^r : 2 V_1^s.

- 99 : 25 (80% r.)

/191: 56+ : 463- i.e. 13% Lac_3+

/198: 105+ : 512- 17% Lac+

Among +, 52R:8S 13% S.

Among - 56R:24S (70% R) among Lac-. → of 187.

$\chi^2_1 = 2.83$

$p = .09$

for fit of V_1^R.

For agreement of Lac - segregations,

$\chi^2_2 = 22.2$

$p = < .001$

199. ~~130+~~ : 130 - : 7 + 5.1% Lac_3+

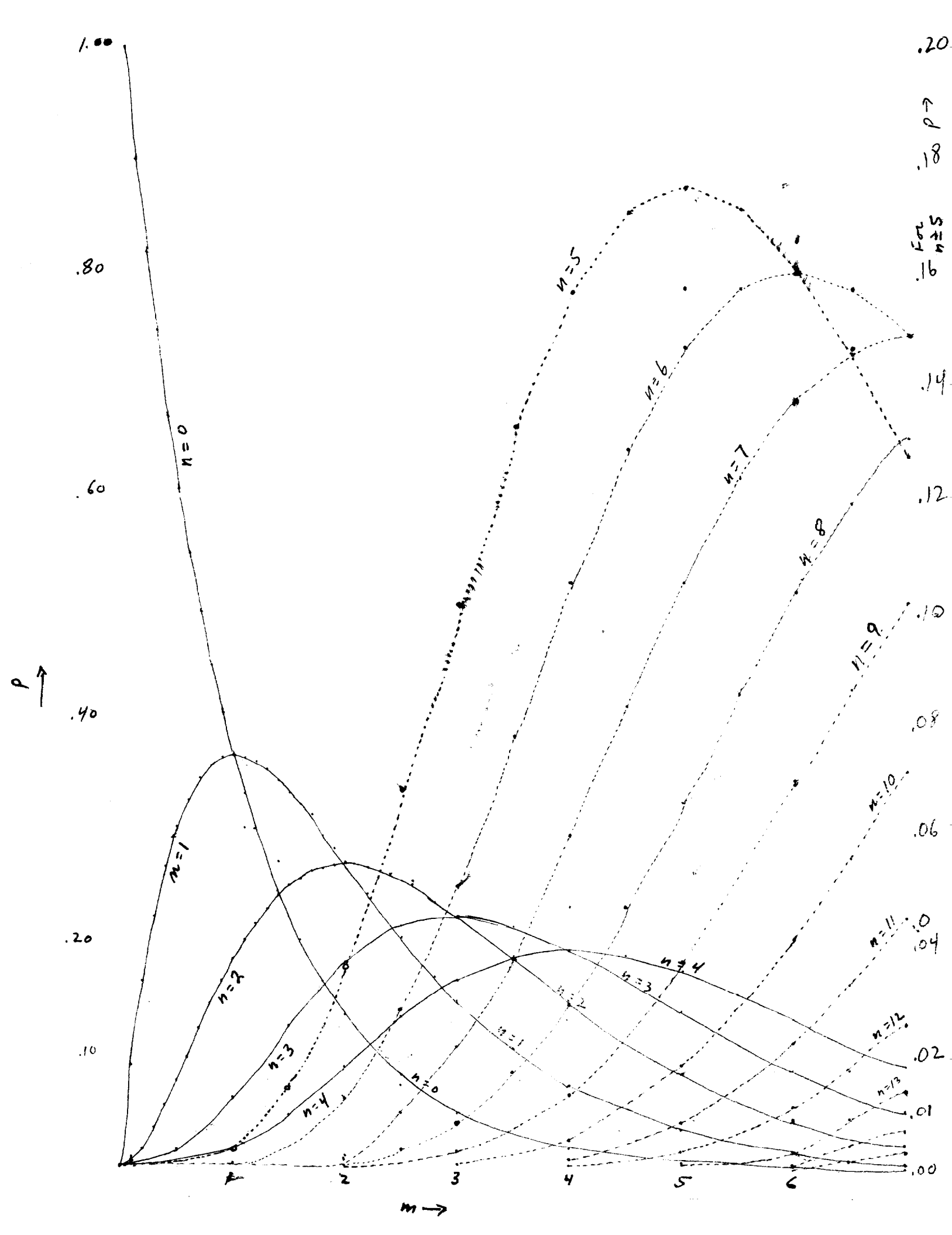
Among + $6_R : 1_S$

Among - $82_R : 33_S$ (71% R.)

K99 (transf. from gluconate EMS).

		R	S	
-	30	22	8	= 73% R.
+	2.	1	1	

All agree on Lac - = Strep.
Lac + = Strep +
on totality tests for R.
= 3+/ 5/21/48.


1.00
.80
.60
.40
.20
.10
p
n=0
n=1
n=2
n=3
n=4
n=5
n=6
n=7
n=8
n=9
n=10
n=11
n=12
n=13
1
2
3
4
5
6
m
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
.01
.00
p
For n=5

Oligosaccharides + Polysaccharides

	Coli	Coli	Coli	Aerobacter	Salm	Typhi
Maltose	+			+	±	+
Lactose	+			+	−	−
Melibiose	+					
Gentiobiose						
Cellobiose	+	some −				
Sucrose	±			+	−	
Trehalose	+					
Raffinose	±					
Salicin	±			+	+	
Amygdalin	+			+	−	

$C_2 + C_3$.

Compound.	E. coli	E. coli	E. coli	Aerobacter	Salmonella	E. typhi
Glyceraldehyde	+			+	+	+
Dihydroxyacetone	+			+		
Glycerol	+	−		+	−	+ᵛ
$CH_3-CH(O)-CH_2$	−			−		
$CH_3-CHOH-CH_2OH$	+			+		
$H_2C(O)-CH_2$	−			−		
HOH_2C-CH_2OH	−			+		

	E coli	coli	coli	Aerobacter	Salmonella Typhi
erythritol	–		–	–	–
Adonitol	–		+		

	Coli	Coli K-12	Coli	Aerobac	Salm	Typhi
d-Arabinose	+			+	+	—
L-Arabinose	+	+		+	+	—
d-Ribose	+			+	+	+
L-Ribose	+			+	+	—
D-Lyxose	+			+	+	—
D-Xylose	+	+		+	+	—
L-Rhamnose	+	K-12: —		+	+	—
arabonic ac.	— +			— +	— +	—
xylonic ac.	+			+	+	
d-methyl arabinoside	—			+ —	—	
β-methyl xyloside	—			—	—	
α-methyl mannoside	—			—		
d-arabitol	—			+	—	

C6 + dervis.

	Coli	Coli	Coli	Aerobacter	Salmonella	typhi
	~~K-12~~		K-12		(not identical typhi)	
glucose	+		+	+	+	+
mannose	+		+	+	+	+
galactose	+		+	+	+	−
sorbitol	+		+	+	+	+
dulcitol	±		−	−	±	±
inositol	−	+		+	−	−
mannitol	+		+	+	+	+
d-glucosic	+			+	+	+
l-galactanic	+			−	±	−
mucic	±			±	±	−
d-saccharic	+			+	±	−
glucosaminic	+			+	+	−
d-mannonic	+			+	−	−
glycuronic	+			+	+	
d-methyl glucoside	−	+	cloacae +	+	−	−
		occ. for ~~K~~.				
See over.						
β-methyl glucoside	+ ✓	+		+ −	+	
d-methylgalactoside	+ −	+	−	+ −	?	
β-methylgalact.	+ ✓	+	−	+ ✓		
tetramethyl glucoside	−			−		
3 methyl glucose	−			−	−	
d-methyl mannoside	−			−	−	
β-methyl fructoside	−			−	−	

	coli
α ϕ glucoside	—
β ϕ glucoside	—
α ϕ galactoside	—
β ϕ galactoside	+ (lactose adap)