March 10-12, 1942.
Y10 x Y45.

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

	+	−	
	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

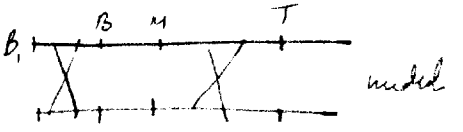
5.7%.

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 told.

W72 3 Lac - colonies all told.

Y87 9 Lac - colonies.

Crosses should be repeated.

Glucose - 1 - phosphate.

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

		Y10	W-108	W-327
1. K. glucose-1-phosphate (Lardy)	P11	–	–	–
	A12	–	–	–
	~~A~~12			
	A 13.	–	–	–
2. Glucose.		++	–	–
		✓	–	–
		✓	–	–

Glutamate + Riboflavin on Recombination.

March 9, 1948.

T (B_1) Y10 + Y87. Measured digs dilute suspension:

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

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

B. 34 / 4, 11 (2 dups), 6, 13 m = 8½.

No pronounced effect of B_2 + glutamate.

(More colonies may appear later). 12 appeared altogether.

See 155.

Map Lac-1

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 (−B₁) plates.

Sectors counted twice.

	+	−		−R	−S	+R	+S.
a) Readings from plates.	13	5	b.				
	13	5	S.O.				
	10	4	T₁-Lac S'				
	~~22~~	~~0~~					
X W45 according to label!	36	14					
	~~63~~	~~14~~	77.				
a' Repeat A12. :	~~64~~	~~18~~	~~82~~ 52.	14	3	27	8
	36	18					

1B. On EMS(B₁) Lac plates

a. direct counts.

a' repeat A12

	17	4	
	16	8	
	16	3	
1S.	30	15	
	30	5	
1S.	16	3	
	22	11	
	28	11	
	19	3	
	194	63	257.
	227	101	338

Total: 6 sectors.

1C. From T(B₁) 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:

[illegible]	13	247	[illegible]

1a. Scored originally as Lac +.

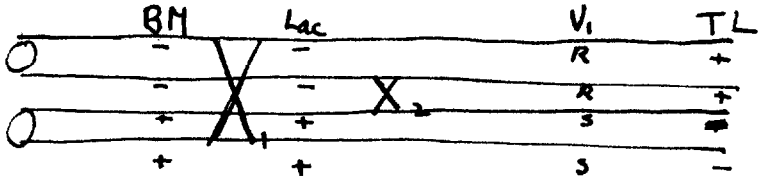
	−R	−S	+R	+S.	
	0	0	14	3	
	0	0	13	5	
As Lac−	14	3			
	14	3	27	8	52:
	[.269	.058	.519	.154]	

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	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 Y/O on pyrimidine + thiazole:

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

Specific Reversions.

#64

April 8.

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

Apr 17. No papillae seen on these 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	Lac1 Y53	-	-	-	-	+
4	Lac2 W45	-	-	-	-	-
5	Lac3 W-108	-	- P	-	-	+
6	Lac4 W-126	-	-	-	-	-
7	Lac5 W-145	(+)	-	-	-	+
8	Lac6 W-125	(+) !	-	±	-	-
9	Lac7 W-133	- P	-	-	-	-
10	Sl1 W-117	- P.	- P.	-	-	-
11	Sl W-252	+	±	+	-	+
12	Sl W-328	+	-	+	-	+
13	Gal- W-254	+	-	+	-	-
14						

[5/16/50 - Note that W252 is recorded as neolactose pos!]

* Papillates to form showing 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 168

Inoculate 58-161[R] into 25 ml T(m) + Nidactose .05%.
+ galactosan

Delayed growth on nidactose.

Streak out and test on nidactose EMB. 11- 0+.

Repeat streaking.

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 24h. aer., no antifoam, unaided, with cerelose. Collect 44g. cells Divide & incubate each portion for 3½ hours in 100 ml 1% peptone + 5% lactose or glucose. for adaptation Sediment after 3½h. + resuspend each in 50 ml M/100 Na Citrate under toluene + autolyse. P8 – P10.

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

∴ ~~Assay~~ To 10 ml samples add 3.5 g AS + sediment. Assay ppt redissolved in M/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 [Blank].
Glucose + galactose 10 mg.	19.06
" " 1 mg.	1.97
163 B2 + Lac.	5.42
" (blank)	< 1 drop

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

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

W-125, W-145

April 9, 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 Gra −, 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 o.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 Neol+ — ± ~~slow~~

58161 Neol+ —

See p. 170 for selection of Lol+ mutants.

Outcross Lactose mutants.

W125, W145. Predominantly lac + on streaking.

W126 x ~~Y10~~ 58-161.
Lac – v. small colonies on EMS

+	–
26	16

W133 x 58-161
not so small

+	–
33	53
16	45
49	128

W45 x Y10 >10+ : 1 –

W108 x 58-161. 3 types noted.
original streaks shows not but some variation.

++	±	–	
8	25	31.	76.

see W-342 ff.

Pentose mutants.

April. 9/9/8.

410 5 mins UV Hanovia.

l-Arabinose EMB. Ca 2000/plate unevenly spread + difficult to score. 36 plates = ca. 70,000 colonies.

11 - 30. 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	Gna	Gal	T1
351	1	–		+	+	+	+	+	
352	2	–							
353	3	–							
354	4	–							
–	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	+	–						
	27	+	–						S
	28	+	–						S
? ?	29	–	–	–	–	–	–	–	S
380	30	–	–	–	–	–	–	–	R
									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.