V mapping. 251.

July 12, 1948.

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

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

(3) Y87 x W401.

(4) W415 x Y10.

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

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

small: 8-: 40+.

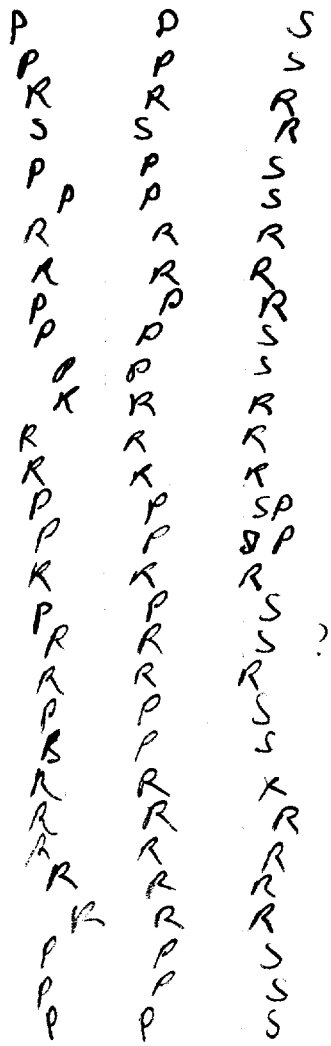
	T1h	T5	T1
-:			
+:			

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

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

R, S, + P

—:



?

?

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

Restreak from broad streak of 1st plate:

251-2.

EMS' isolates to EMS'. mostly Lac−

T1	T5		T1	T5		T1	T5
R	S		R	R		P	B
P	S		P	S	Lac+ →	R P	S
P	S		R	S	Lac+ →	R P	S
P	S		R	R		P	S
P	S		R	R		R	S
R	S		R	S		R	S
P	S		R	S		R	S
P	S		P	S		R	R
R	S		R	R		R	P
P	S		R	R		R	R
R	S		P	S	Lac+ →	R	R P
R	S		R	R	Lac+ →	R P	R
R	S		R	S		R	P
B	S		R	R		R	R
B	S		R	S		R	R
R	R		R	R		R	R
R	S		R	R		R	S
R	R		R	R		R	R
R	R		P	S	Lac+ →	R	S
			R	R			
R	R	Lac+ (struck out)	R	S P			
R	S		R	R			
P	S		R	R			
R	P		R	S			
R	S		R	S	Lac+		
R	S		R	R			
S	S		P R	S	+		
R	R		R	R			
R	R		R	R			
R	S		R	R			
			R	R			
P	S						
R	R	Lac+	R	S			
R	S		R	R			
R	R	Lac+	R	R			
R	S		R	R			
R	R		R	S			
R	R	Lac+	P	S			
R	S		P	S			
P?	S		R	R			
R	R		R	S			
			R	R			

251-1 definite ! (S, T1 column) purify + recheck.

Possibly diauxic?

251aa

July 21, 1948.

See 251b-c.

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

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

I +S −R

Note: parents were W416 and Y64.

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

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

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

July 13, 1948

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

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

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

July 15, 1948. T2 Glen cum.

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

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

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

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

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

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

SW10. do. B10:1

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

SW7 1:100 some inhibition B7:1

1:50 appreciable "

SW10. 1:50 " " B10:1

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

Wash cells for all transfers.

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

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

A10-5.

10 tested 9 camphage.

1? Repeat test.

A7-5.

16 cultures tested on S436. + SW-10

all still camphage.

> [illegible] phage.

Check new phage ~~strains~~ resistants

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

Salmonella:

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

July 17. Recheck:

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

July 16, 1948.

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

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

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

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

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

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

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

July 19, 1948.

Irradiate W252, purified, 6 secs. on a) ~~EMB Lac 45 plates~~
b) T2 Lac 45 plates.
ca 200 pu = 9000.

D.G. fecit) W327 " 6 secs. on a) EMB Mal
b) E2 Mal } 45 plates. 200 ea. = 18,000.

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

1 plate {
1. ○ slow
2. ○ slow
3. ○ slow
4. ○ slow
5. ○ + and − W−438
6. ○ slow. ~~EMB~~
}

13. ○ + and − 448.
14. ○ (12) all − 449
15. ○○ all +
16. ● (13) all − 450.
17. ○ + and slow +
18. ● + and − 451
19. ○ all + S.O. on (T2).
20. ○ + and − 452.
21. ○. slow + small
22. ○ " "
23. ○ mostly −; some +
24. ○. slow + small
25. ○ (temperature?) all +
26. ○ − (slow ±?) 453.
27. ○ all − 454
28. ○ − occ. + 455
29. ○ (14) + and − 456.
30. ○ + and − 457

31. (21) ○ + & − 458
32. (22) ○ + & − 459
33. (23) ○ mostly − 460
34. (24) ○ mostly − 461

W327). b). 1. ● − or slow. W439.
2. ○ + and slow
3. ○ + and − occ. s. W440
4. ○ mostly −. W441
5. ○ all +
6. ○ + and slow
7. ○ + and slow 442
8. ○ all +.
9 ~~8a~~ ○ +, −, and slow 443 − 444 s 445 +
10. ~~9~~ ○ + and slow
11. ○ + and − 446
12. ○ + and slow 447

EMB. →

All cultures tested on Lac V1 5

July 16, 1948.

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

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

No plates showed colored mycelia.

Next day: growth similar + advanced Neocolor.

July 20 ff.

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

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

19 R
1 S.

1R inoculum for cross

July 22, 1948. SW7/6 × SW10

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

also S.O. parental suspensions

July 25, 1948.

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

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

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

all sensitive!

Contn. 251.

Test five "±" colonies from 251a for mutation

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

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

MTH-W472 to solar transfer.

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

T5 S!

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

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

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

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

-S +R.

July 23, 1948.

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

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

July 23, 1948.

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

P25.

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

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

Streak out on EMB: M colonies.

Test for sensitivity.

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

July 25, 1948.

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

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

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

EMB. +, -, and M colonies predominating.

A27. S.O. 4 Colonies from A1.

EMS 4 cols from B1. ⇒

B.

EMB. +, - and M predominant.

C. EMS. All four are +

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

Take 1 col. each from C for D +.

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

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

② all +.

③ all +

④ 1% - ; others +.

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

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

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

F↓

Aug. 12, 1948.

J. EMS: 1-4 All +

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

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

EMS. All +. EMB. All Var.

K. EMB

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

L. EMB All V.

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

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

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

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

persistence of heterogenic.

August 3+, 1948.

F. EMS. All 4: all +. 4 cols. from ①

EMB. all predominantly variegated.

A7.

G. EMS All +.

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

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

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

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

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

EMB: 1-8 all prominently variegated.

EMS (A9) 1: appreciable — (1-2)

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

A9. EMS
EMB.

EMB.

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

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

P10.

J

July 26, 1948.

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

~~#~~ 259-6. MTL

~~K~~

~~B~~.

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

263: Test - segregants.

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

T - 2 M-1 ML 2 MT 1.

S. M 6.

O 2.

+ :

R. TLB, (M?) 1.

S. M 8.

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

Save as (HFS)

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

Retest single colon

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

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

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

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

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

Lac− (4 colonies) 4 S 0 R.

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

Rechecked of 4 S's. all were S.

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

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

Search for Syncaryon:
W-1 × Y40.

July 27, 1948.

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

Replate,

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

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

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

July 26, 1948.

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

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

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

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

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

O = +

S.

O = #− Test on EMBLac / TS.

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

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

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

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

Lac+: ~~9R · 1S~~.
9S : 1R

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

July 24, 1948.

1A: 1-9 Lac− 10 Lac+
1B: 11-7 Lac− 8-10 Lac+
1C: 21-25 Lac− 26-30 +
1D. 31-35 − 36-40 +
2A: 41- -50
2B 51- -60
2C 61- -70
2D 71- -80

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

parents were M− Lac+ V_5^S
\# T−L− Lac− V_5^R.

Test sensitivity to T5:

	1A 0	1B 10	1C 20	1D 30	2A 40	2B 50	2C 60	2D 70.
1	R	R	R	S	R	R	S	Lac− R ~~+++~~
2	R	R	R	S	R	R	S	R
3	R	R	R	S	R	R	S	R
4	R	R	R	S	R	R	S	R
5	R	R		S	R	R	S	(S)
6	R	R	S	S	S	S	S	R
7	R	− R	S	S	S	S	R	S
8	R	+ ~~R~~ S	S	S	S	S	R	R
9	− R	(R)	S	S	S	S	RS	R
10	+ S	(R)	S	S	S	S	S	(Lac+ R. ~~TL~~)
Nutrition: 1	MTLB_1	(M.)	TL	(M)	MTL	TL(B_1)	L	+++
10.	M	+++	M (T?)	M	TL.	T(L)	TL.	~~TL~~

[illegible] completely [illegible], 21, 51, 10, 50, 60 − 5 were [illegible] (i.e., had [illegible] TL,

W − 471.

July 30, 1948.

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

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

heterozygotic

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

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

See 271

These colonies obviously have more than 4 kinds of recombinants

July 28, 1948.

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

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

13: 3 plates No cols.

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

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

1: Gal- Ar+

7. Gal+ Ar-

A29. Pick 9 further cols + test:

9 tests: all Gal+ Ar-

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

segregation from 1046S.

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

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

1-20 are from earlier streakings.

M+ } 5
M− } 14.

R+ } 7
S− } 11

T+ } [illegible] 14
T− } 5

L+ } 13.
L− } 6

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

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

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

Preserve (2a).

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

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

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

phage ?↑

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

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

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

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

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

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

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

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

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

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

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

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

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

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

68, 110, (b).

Nutritional Tests.

On liquid:

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

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

See 274.

~~Today~~ August 1, 1998.

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

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

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

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

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

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

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

D.'s tests on W-470.

August 2-3, 1948.

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

Tests 16h. fermentation tubes.

M3/1 " "

August 3, 1948.

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

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

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

Chemical control of segregation
Phosphate and nucleate

August 3, 1948.

Use same inoculum as in 269. (Washed)
Inoc. .5 ml into each of following: (additions / 10 ml tubes) All media

		Turbidity	8P4:	ctrs. TLB, BM.
1. Basal (see infra) — phosphate		18	22	
2. "	+ .05 ml "	29	42	
3. "	0.1 " "	35	45+	
4. "	.5 " "	48	75	
5. "	1.0 " "	43	96	
6. "	+ .5 ml P. + 5% Na nucleate	3 (deposit on bottom)	9	unsolved
7. "	" 2%	11 (colored.)	15	
8. "	" 1%	21	57	
9. "	" .5%	27	63	
10. T(0)		60	87 (colored).	
11. Penassay broth.				
12.				
	H_2O	4.	2-	
	Inoc.	14	14	
	Standard A. = 100.			

Basal = 1 l. See Columbia, p. 109 ff.

KNO_3	1
Na AcO (Na acetate)	.5
Na citrate	.2
Am Sulf.	2
$MgSO_4$	.1
~~$CaCl_2$~~	~~.1~~
Glucose	5

phosphate solution: class:
30 g K_2HPO_4 / l. ≐ 10 mg P/cc.
10 g KH_2PO_4

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

	1	3	5	9	10	11
v	26	21	5	mostly v or +	1	could not be read!
+	4	6	11		6	
~~+~~	5	4	15		4	

Aug. 1-3, 1948.

Ref. 265c.

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

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

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

EMS: none grew.

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

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

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

Aug. 6, 1948.

① 477 × 478 — Lac EMS.

② 477 × W-21

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

④ W21 × W-1/1. (on Mal EMS)

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

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

③ 8 Lac S plates.

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

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

---

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

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

W482 - 483. 212b.

Aug. 11, 1948.

See 272 last IP.

W482 } colonies on MalEMB: all —
W483 }

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

482: 1
2
3
4.

483 - showed more frequent variants.

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

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

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

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

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

A10.

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

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

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

S.O. as 272a 9-11.

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

On Lac EMB.

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

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

485 – Pure Mal+ Lac +, – and Var. " "

486 – Mal+ or ± Var, + and Lac – "

Aug. 13-14.

Isolate + check W482- W486.

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

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

3 + V } all +!

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

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

486. (1 V. 2. V, +, − 3. V, V.

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

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

Phage tests on T5 Lac S

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

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

Chemical control of segregation.

August 7, 1948.

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

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

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

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

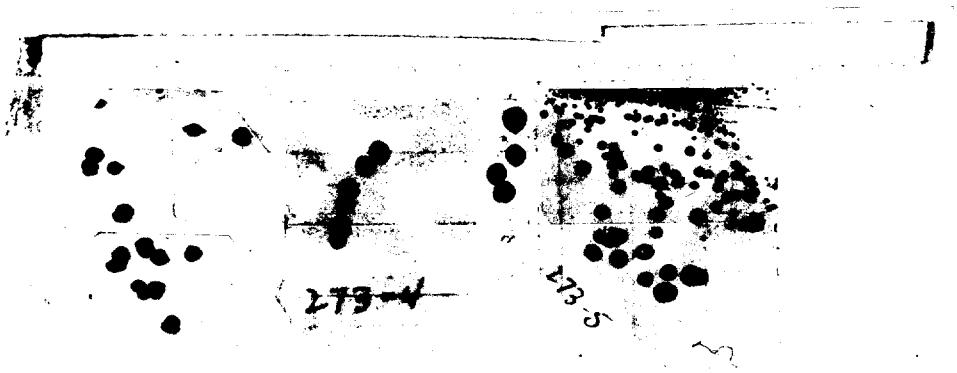
A9. well! Pick 10 colonies each from 2, 4, & 5 + S.O. on Loc EMB.

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

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

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

(5).



273-1

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

Segregates from W-465 continued.

August 8, 1948.

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

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

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

8/11-12-24

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

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

\# 75 Vs-R Recheck: S.

104 is of special interest.

Test W465 segregation monoplex 275

Aug. 9.

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

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

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

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

Selective media for ferm. controls.

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

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

No Buffer:
Sod. sulfite ½% +++ ++

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

Na Salicylate 1%
.1% ± ±

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

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

B = phosphate buffer M/50 7.0

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

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

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