Irradiation of H-168

December 15, 1949.

H-168 Reisolated from EMS Lac. Suspend in saline

Unfortunately, these particular cells appear to be almost entirely segregated.

A. 10^{-7}

B. 10^{-6}; 3 seconds UV.

C. 10^{-1} 8 seconds UV.

On EMB Xyl and Lac.

A

156 –	1 v.	
153 –	2 v	1?+
126 –	3 v	
152 –	4 v	
587	10 v.	

587	10
1294	0

B

164 all Xyl –
135
263
149
215
190
178 / 1294

C

Rather heavy and uneven. Very low level of + or v. ca 1/1000

Note, although not counted, Lac plates gave parallel results: majority of A were Lac +, some Lac –, Lac v. B all were Lac + or –

Irradiate H-168

December 15, 1949.

As above. Cells from EMS Xyl.

A). EMB Xyl.

		—	+	V
1		16	1	21
2		10	0	19
3		12	1	18
		48	2	58

probably contaminated!

EMB Lac

4		11	4	40
5		7	0	41
6		2	1	58
7	x	3	5	31
8		8	5	49
		31	15	199

B Xyl.

11		3	0	0
12		39	2	12
13		10	0	0

Lac

14		28	23	23
15		31	22	26

C Xyl crowded but predom. Xyl −!

Survival much higher on EMB than on Xyl!

12/17/49.

Recount 651a after 12 hours further incubation.

Xyl A

	−	+	v	
1	10	1	30	
2	12	0	26	
3	11	3	~~31~~ 23	
	33	4	79	/116

Xyl B

11	4	0	0	
12	43	5	18	
13	13	0	1	
	71	5	19	/95

% comparison:

A	28	4	68
B	75	5	20

	Pure	Var.
	32	68
	80	20

~~Lac A 4 11 8~~

Scoring of Lac v vs. Lac − very difficult. Record only −, +.

		−	+, a v.
A	4	11	57
	5	4	50
	6	2	57
	8	7	55
		24	219

B. 14

Many more clear cut Lac+!

		—	+	v
A. Mtl.	9	8	2	49
	9a.	10	3	55

Picks + to confirm balanced duplicates

A. 8 sec. Xyl → Lac 12L−; 3v; 30+

B 3sec Mtl → Lac 4+ 4−

C 3sec Xyl → Lac 2+; 3−

D 3sec ~~Mtl~~ Lac → Xyl 10+; 136− Xyl+ Recherked.

E 0 Xyl → Lac, Xyl (4+)

F 0 Mtl → Lac, Xyl, Mtl (4+)

G 0 Lac → Lac, Xyl bursh (8+)

Tests on possible diploids from UV-irradiated 4168.

651bc

Dec 21, 1949.

A. 20 Lac+ streaked out on lactose and xylose.

1-16 essentially pure Lac+ Xyl+ ($A_1 - A_3$; v)

A_4 (1,3 mixed Lac+, −; mostly Xyl+).

A_5 (6). mixed Lac+, −; Xyl+.

A_6. (Lac v?) — held for development of v.

B. 2 Xyl+ Lac+. B1-2

C.

Lac+;				
1	Xyl+, − v?	Lac +	+ − v?	B3
2	"	+, −, v?	+	B4
3	Xyl+	+	+	B5
4	Xyl −	+	+, v?	

D. Lac+ Xyl+ #12 Lac+ Xyl− ~~D1-11~~ D1-11

E

	Xyl	Lac
1	+/−	+
2	−/+	+
3	+	+
4	−	+

Exceptional recombinant?? B6

F

	Xyl	Lac	Mtl
1	+	−	+
2	−	−	+
3	−	+	+
4	+	−	+

G. Bunches. (v. dilute) #1,3 −/+ Other 6 — . (Xyl)
+/− " + (Lac)

Tests on presumptive balanced diploids

651 bd

December 23, 1949.

A (from uv 8 secs., Xyl + Lac+): Test on Mtl T1.

	Mtl	T1	Nutr (TO)	Mtl	T1	Nutr (TO)
1	−	P	−	+	P	
2	−	P	−	−	P	+
3	+	P		−	R	
4	+	P		+	P	
5	+	P		+	R	
6	+	P		+	P	
7	+	P		−	R	+
8	+	P		−	R	
9	−	P		+	P	+ * 651 bd 1
10	−	R		+	P	

		Mtl	T1	Nutr (TO)		Mtl	T1	TO
B − D	01	+	P	−	4	−	P	−
3 sec uv	2	−	R	+	5	+	P	−
Lac + Xyl +	3	+	P	−	6	+	P	−
	4	+	P	−	7	+	P	−
	5	+	P	−	8	+	P	−
D	1	+	R	−	9	+	P	−
	2	+	P	−	10	+	P	−
	3	+	R	−	11	−	R	−
No uv.	B6	+	P	+ *				651 bd 2

No V_1^S as would indicate V_1^P/V_1^R, and very few prototrophs. i.e., no stable diploids. Recheck * (put on slants.

New sp. heterozygotes

652

12/14/49. W67 x W677

20 plates. Ca 60-100/plate. 1500 cols.
EMSLac

No Lac+ found.

W67 x 1272

Repeat 1/12/50. 500 cols.

No Lac+.

1/15/50 300 no Lac+

1/18/50 H-226 500 – 1? Lac+ ✓

This is proven Lac v.

1/23/50.

W67 x W1272. ca 6 10 all –, 1+. S.O. (652-2)

"A" pure Lac+.

[Save for tests as stable diploid] See 669.

W45 x W1272 "B" 5 plates. 87- 17+. Streak out+. all+

H-226: segregating for Lac, Mal, Mtl ~~[illegible]~~, and probably for Stl, Ar., Eal.
should also be segregating for V_1; not for V_6.

1/25/50.
67 x 1272 ca 1000. 3+ colonies.
Streak out on EMB, EMSLac; Mal.

	Lac	Mal
"1"	L1	+, – (v)
"2"		– +
"3"		+, – no v seen.

See 652a.

1/30/50.
67 x 1272

January 30, 1950, et al...

3 Lac+ from 1/25/50 W67xW1272. Each gave both types

11. Mostly Lac−, several Lac v some ⊡ others ● . Restreak for type!
Mostly ~~Lac+~~, Mal+; Mal−. Anechar Mal v. Restreak.

12. Lac v (?) primarily Lac+. Predominantly Mal−. Few+ (contam.)

13. distinctively Lac v. Mostly ~~Lac~~ Mal+, some Mal−. Restreak
isol. Lac v.

---

11. Mal v? : rely Mal+, − on restreak! Some − on Mal EMS.

12. Definitely Lac v − like H226. On Mal EMS, all Mal−.

1/31 13. 8 Lac v streaked: all Mal+. (Mal− probably contam. or admix.)

"W67xW1272" ca 1/3 Lac+! (probably some error). 140 Lac+
2/1 picked and streaked on EMB Lac. All pure+. (58-161 x 1272?)
Throw out!

See 673

Conclusion:

11: Lac v Mal v. H228

12. Lac v Mal− H229

13. Lac v, probably Mal+ H230

Rechuck some Zelle 1-cell isolates from H206.

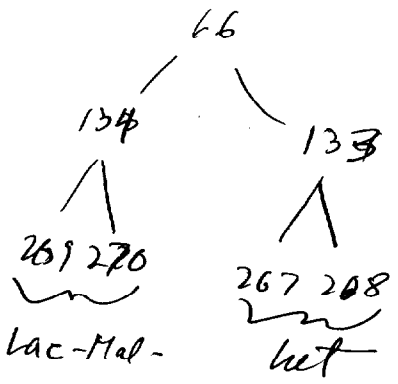
Jan 5, 1950

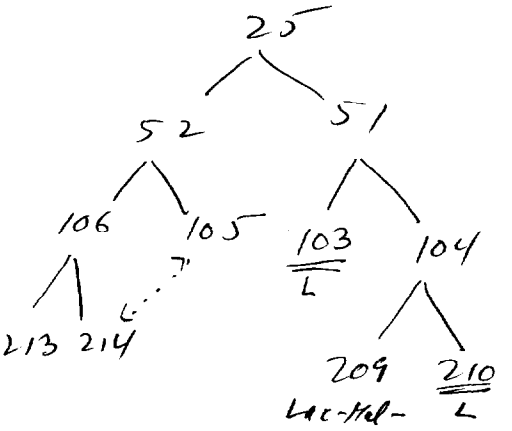
See over for pedigrees and summary of H206

		Mal	Gal	Lac
1	12-267	+, −, v?		+
2	12-268	+ −or v?		+
3	12-269	−		− verifying
4	12-270	−		− verifying
5	12-209	−		−
6	12-214	+ − v?		+ − v
7	12-198	all +		+
8	~~[illegible]~~ H206 stock.	v		v

This is in accord with Zelle's letter except that the identification of v colonies here is somewhat uncertain.

12-198 here appears to be pure +. Restreak!





H206 = 544D4.

W477 x W980.
TLB, Lac-O,K x 478 Gal-Mal-. Try T5 also.

Zylke's single cell

Series 15:

	Jan 5, 1950.	Lac	Mal	
1	5-83	−+	−+	
2	84	−+	−+	
3	85	−+	−+	
4	86	+	+	
5	122	−+	−+	−+ look for V
6	137	−+	−+	−+ " "
7	181	−+V	−+	lac V
8	+5-223	+	+	
9	223	−+	−+	
10	224	−tiny	−tiny (contam?)	
11	225	−+	−+	
12	15-123	+	+	
13	239	−+	+−	
14	240	−+	+−	
15	241	−+	+−	−+ look for V
16	242	−+	+−	segg V
17	+5-223	+	+	
18	"	+	+	
19	"	+	+	
20	"	+	+	

Sorbitol Linkage — Preliminary.

1/11/50. W1262

~~50~~ W478 × W677. on EMS Lac
" Stl.

Pick Stl+ only (ca 5%). "Purify" by spotting on EMS Lac or Sal.
Replica after 16 hours, and test directly on EMS:

	Stl	Lac	Sal	Mal	Xyl	Mtl
1	+	+	+	+	+	+
2	+	−	−	+	+	+
3	+	−	+	−	−	−
4	+	−	−	−	−	−
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	−	−	−	+	+
8	+	−	−	−	−	−
9	+	+	+	+	+	+
10	+	+	+	+	+	+
11	+	+	+	+	+	+
12	+ (−)	−	−	−	−	−
13	+	+	+	+	+	+
14	+	+	+	+	+	+
15	+	+	+	+	+	+
16	+	+	+	+	+	+
17	+	−	+	−	−	−
18	+	+	+	−	−	−
19	+	−	−	−	−	−
20	+	+	+	+	+	+
21	+	+	+	+	+	+
22	+	+	−	−	−	−
23	+	+	+	+	+	+
48	+	−	+	+	+	+
49	+	−	+	−	−	−
50	+	+	−	−	−	−
51	+	+	+	−	−	−

	Stl	Lac	Sal	Mal	Xyl	Mtl
24	+	+	+	+	−	+
25	+	+	−	−	+	−
26	+	−	−	+	+	+
27	+	−	+	+	−	−
28	+	+	+	+	−	−
29	+	−	+	+	+	+
30	+	−	+	+	+	+
31	+	−	+	−	−	−
32	+	/	+	−	−	−
33	+	/	+	+	+	+
34	+	+	+	+	+	+
35	+	−	+	+	+ mixed	+
36	+	/	+	−		−
37	+	+	−	−	+	−
38	+	+	+	+	+	+
39	+	−	+	+	+	+
40	+	−	−	+	+	+
41	+	−	−	−	−	−
42	+	−	+	+	+	+
43	+	/	+	+	+	+ −
44	+	±	+	+	+	−
45	+	−	+	+	+	+
46	+	−	−	−	−	−
47	+	+	+	−	+	+
		/	−	+	+	+
52	+	+	−	−	−	−
53	+	+	+	+	+	−

Summary – Preliminary linkage tests.
Stl.

655a

1/12/50. 53 tests: Sorbitol +. Following +:

	Lac	Gal	Mal	Xyl	Mtl
~~%~~ #	27	38	33	34	31
%	51	71	62	64	58
Standard %	ca 30		ca 15-20	ca 10	ca 10

Distinct linkage to Gal is apparent.

Other linkages: (in this Stl + prototroph subsample):

	Lac-Gal.	Mtl-Xyl.	Mal-Xyl	Mal-Gal
++	22	30	30	29
+-	5	1	3	3
-+	17	4	4	9
--	11	18	16	12

An unusually high concordance of Mal – Gal – Xyl is noted here. It is clearly profitable to study the full sequence: Lac V6 Gal Stl Mtl Mal Xyl . . .

Characterization of W1258
(Cavalli 123)

Jan 4, 1950 ft. In D(0):

(1 Standard addition series: Y. Extr. +++
HC +
O ≡

(2 Amino acid Rundown.
HC +
AA +
No vitamin Requirement ? AA + Vits ±
Vits —
Add vits. in succeeding tests. HC + V +
Vits + single AA groups —
HC + YNA ±

(3 AA mission series + Vits.
AA+ ++ (30 hours).
— A12 —
— A3 —
— A4 ++
— A5 —
— A6 ++

Characterize Requirements from A12, A3, A5. Adding vitamins and A4 + A6.

AA
AA + V.
A12 + A3 + A5
" + V.
Add A4 + A6 to + Vits.
— A12 series
— A3 series
— A6 series

Threonine
Methionine
Isoleucine indicated

histidine ??

After 24 hours, Methionine, Threonine, Isoleucine were indicated;
AA mix gave poor growth
48 hours — M —, — A3 ±, — A5 ±, but — A12 + M ±

K-12 × Cavalli 123

January 7, 1950. (+ pur.)

W-1178 × W-1258 on EMS lac. Picks 100 + colonies.
20 ± or − " .

[W-1177 × W-1258 " . Heavy background and plaguing noted. See infra. Many petites, but do not pick.]

Ca 99% lac+ 1% lac−

Streak out lac+ for x. None v. Test ē Mal, T1.

lac+.	Mal+ V_1^R	Mal+ V_1^S	Mal− V_1^R	Mal− V_1^S	Σ
	1	98	0	1	100
lac−	3	5	1	0	9
lac ±	1	8±	2 mixed.		

At least #1 of the lac ± is lac v after 30 hours.

	Lac	Mal	
1	v, +, −	+, v??	} Reisolate on EMS lac
2	+, −, v??	−	
3	+, ±	−	
4	+, ±	+	as 656-1
5	+, ±	+	-2
6	+, ±	+	
7	+, ±	+	
8	+, ±	+	
9	+, ±	+	
10	+, ±	+	
11	+, −	+	

#1 — definitely lac v. Attempt reisolation on EMS lac. However, many lac− petites noted, and lac v colonies on EMS virtually preclude selective isolation.

Jan 6-7, 1950.

In cross of W677 x W1258, heavy plaquing of background was noted. In view of this, tests for λ sensitivity were made in seeded layer plates.

	λ	W518	W677	W1258	T1	T6
W1258	L	—	L	—	L	L
W677	—	—	—	—	—	L

To obtain resistant mutants, plate W1258 with λ at various dilutions. Plates in j.

1/10/50. Inoculate Nutr Br + Y. Extr. (NBY) with 5ml young culture W1258 + ca 10^8 λ. Aerate 5-6 hours. Cool overnight and filter, first straining out the turbid supernatant. Control was much denser.

① titrate filtered λ stock

② Test 1258/λ for resistance; lysogenicity

Map Stl -

657

Jan. 4ff. 1950.

"A"

W1262 x 58-161 on EMS Lac

Pick Lac+ and Lac — to Stl EMB.

	Stl -	Stl +
L+	21	1
L-	30	2

Linkage ~~of~~ or ~~#~~-linkage to M is indicated. Repeat cross, plating on EMS-Stl.

Further tests summary:

	Stl -	Stl +
L-	105	9
L+	75	4

Stl heterozygotes.

Jan 10, 1950
W478 x W1262.
Lac v.

Pick Lac+ from EMS Lac and streak for Rastrals on EMS Lac; EMB ...

* like suppressor Mtl+

	Lac	Mtl	Stl	Xyl.	Mal
#1	+ −	+ −	+ −	+ −	−
2	+ −	+ −	+ −	v	−
3	v	−	−	v	−
4	+ −	−	−+	v	−
5	v	−	−	v	+
6	v	−	−	−+	−
7	v	+ −	+ −	v+	+
8	+ −	slow*	−	+	+
9	v	v	v see note	v	− = H225
10	v	−	−	v	−
11	v	−	−	v	−
12	v	−	−	v	−
13	v	−	−	−	−

Select for further study.
↓
Stl+ (not v)

H168 Irradiation

January 16, 1950.

Scrape H-168 from EMS Lac; disperse in water, spread on EMB or EMS Lac and irradiate as indicated.

A). Undiluted .1 ml samples. 8 and 15 secs. UV. EMB Lac

#	UV	Lac+	Lac-	Lac v
1	8	57	41	104
2	8	81	30	33
3	15	< 38	34	95
		176	105	232
		281		232
		95	75	199
		170		199

Omitting #2

?

Macc. count? Too crowded; only a part of area ctd.

-Logs Killing	UV	Lac+	Lac-	Lac v	% Lac v
0	0	14	9	131	85
1	3	56	55	38	26
5-6	8-15	176	105	232	48

B). 10^{-6} ml samples. 3 secs. UV.

4	14	23	13
5	20	16	11
6	22	16	14
	56	55	38
	111		38

C) 10^{-7} ml. No UV.

7	4	5	35
8	5	1	43
9	5	3	53
	14	9	131
	23		131

January 16, 1950.

Similar samples (659) irradiated on EMS Lac to test for occurrence of balanced lethals here.

		+	−
C: (No UV). Counts:		44	0
		21	0
B	3 secs UV.	11	1 ?
		14	0
		18	0
A	8 secs.	31	1 sect
		83	3
	15 secs.	18	1
		18	1 sect
		52	1?

Pick and streak ~~out~~ 20+ colonies in each category (and all −).
For 1st test. Compare cross streaks vs. T1 on EMS Lac; EMB Lac. Haploids and stable diploids will be same on both media (P or R; S); segregating diploids will be distinct. Save suspensions. 20 from each tested.
All were T_1^P except B10, T_1^R on both media. All were also clearly mixed ± on EMB Lac. Restreak a few unidentifiable exceptions

Recessive lethals can account for only a part of the killing.

H225 segregants

1/18/50

Streak out individual lac V segregants on EMB Lac. Pick 1 Lac- and 1 Lac+ each for further classification.

Lac	Gal	Xyl	MR	TS		Lac	Gal	Xyl	MR	TS	
−	+	+	+	−	−	−	−	R	S	−	+	+	+	−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−						+		R	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S
−	+					−	−	S	S	−	+			−	−	+	−	R	S
−						−		R		−	+			+	−	+	−	R	S
−						−		R		−	+			−	−	−	−	R	S
−	+					−	−	S	S	−	+			−	−	−	−	R	S
−	+					−	−	R	S	−	+			−	−	−	−	R	S

all apparently sorbitol+, arabinose +

Precludes use for linkage tests. Recheck segregation.

Zelle's single cell isolations

A

20

1	—	° —
2	V	—
3	V	±
4 *	—	
5	V	+
6	V	+
7	+ — V	+
8	+ — V	+
9	+ — V	+
10	+ — V	+

11	V	—
	V	—
	V	° ±
s.col. cont.	V	±
	V	—
	V	—
?	— +	—
?	+ —	±
	V	—
20	V	—

21	V	—
	V	±
	V	—
	V	—
—	V	—
—	+ V	—
	V	—
	V	—
	V	—
30	V	—

31	V	+
	V	+
	V	±
	V	±
+ —	V	+
	V	—
	V	—
	V	—
—	+ V	—
40	—	° —

41	V	—
2	V	—
3	V	—
4	V	—
5 —	V	—
6 —	V	—
7	— V	—
8	— V	—
9	V	—
50	V	—

51	V	±
	V	—
	V	—
	V	—
?	— + no V	±
	— V	—
	V	—
	V	—
	— + V	—
60	V	±

61	V	—
	V	—
	V	—
	V	—
	V	±
	V	—
	—	° —
	—	° —
	—	° —
70	V	—

71	V	—
	V	—
	— V	—
	V	±
	V	—
— —	+ V	—
	—	° —
	—	° —
79	V	—
	V	—

* #4 gave only very tiny colonies

° poor growth on EMS

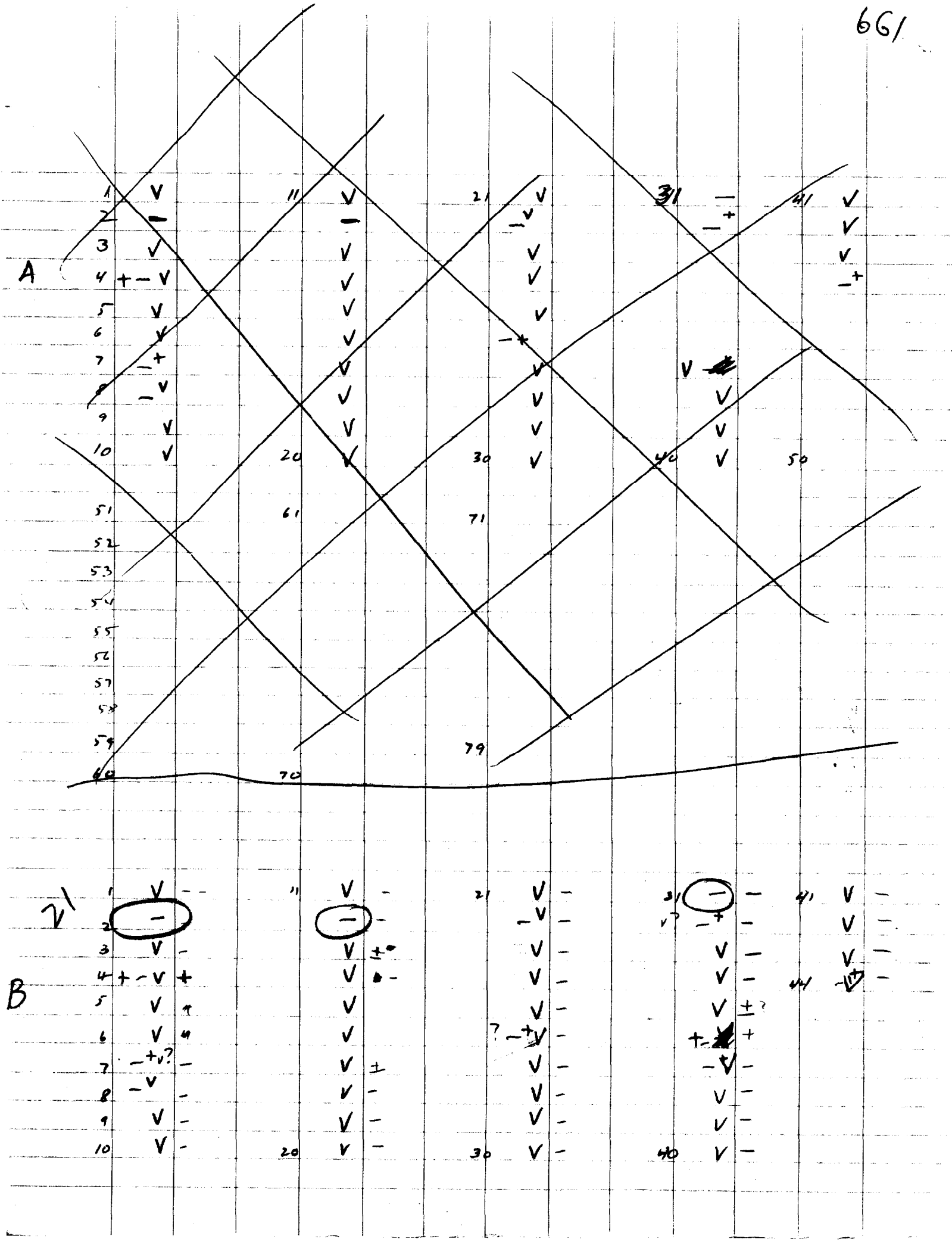
A: 1-10 show a special propensity for + colonies.

A

1	V	11	V	21	V	31	—	41	V
2	—		—		—V		—+		V
3	V		V		V				V
4	+—V		V		V				—+
5	V		V		V				
6	V		V		—+				
7	—+		V		V		V		
8	—V		V		V		V		
9	V		V		V		V		
10	V	20	V	30	V	40	V	50	
51		61		71					
52									
53									
54									
55									
56									
57									
58									
59				79					
60		70							

B

1	V	— —	11	V	—	21	V	—	31	—	—	41	V	—
2	—			—	—		—V	—	v?	—+	—		V	—
3	V	—		V	±		V	—		V	—		V	—
4	+—V	+		V	—		V	—		V	—	44	—V	—
5	V			V			V	—		V	±?			
6	V			V		?	—+V	—		+—	+			
7	—+v?	—		V	±		V	—		—V	—			
8	—V	—		V	—		V	—		V	—			
9	V	—		V	—		V	—		V	—			
10	V	—	20	V	—	30	V	—	40	V	—			



1	V	−	11	V	−	21	V	−	31	V	−
2	+− V	+		V	−		V	−		−V	−
3	V	−		−	−		−+ V	−		V	−
4	V	−		V	−		−	−		V	−
5	V	−		+−	+		−	−		−(V)	−
6	V	−		V	−		No such vial	⊖		V	−
7	V	±		V	−		−	−		V	−
8	V	−		V	−		−+ (V)	−		V	−
9	V	−		V	−		V	−		+− V	−
10	V	−	20	V	−	30	V	−	40	V	±

22

C

41	V	−	51	V	±	61	V	+
42	V	−		V	−		V	−
43	−+V	−		V	−		V	±
44	V	−		−+V	−		V	−
45	−+ V	−		V	−		V	−
46	−+ V	−		V	−		V	−
47	V	−		V	−	67	V	−
48	V	−		V	±	68	V	−
49	−+V	−		V	−			
50	V	±	60	V	−			

Almost all plates predominantly − unless noted

° poor growth on EMS Lac

Zelle isolates

January 18, 1950.

A). Check possible spurious variegated (from EMS Lac to EMB Lac)

A17, A18, A55, — B7, 32, 36

C15, A B

A from EMS
B from ori. tube

A17	1	lac v	−V
A18	2	lac v	+, −
A55	3	lac v	~~lac v~~ +−v?
B7	4	−v?	−v?] only doubtful.
B32	5	lac v	−V
B36	6	lac v	+, −
C15	7	lac v	+−V

C)

Segregants

A1	1
A40	2
A67	3
A68	4
A69	5
A77	6
A78	7
B2	8
B12	9
B31	10
C13	11
C24	12
C26	13
C27	14

1 Lac+ each from 20 - [115-118] isolated and tested:
each was Lac+Mal+ V_1^R 20-115: TLB1, Lac+Mal+ V_1^R

2 from 5-223 Lac+Mal+ V_1^R 5-223+ : TLB1, Lac+Mal+ V_1^R

---

(12)

133 < 267 / 268
134 < 269 / 270

Tests on M206 segregants isolated by M.R. Zelle

January 20, 1950.

		Lac	Mal	TS		Nutr.	
1	5-224	−	−	S		M	5-223 + colonies L+M+V_1^R.
2	6-25	+	+	R		TLB_1	
~~3~~	~~6-88~~	~~−~~	~~−~~	~~S~~		Lac ✓ isolated. impure? (Lac+, Mal+)	
4	7-116	−	+	R		TLB_1	
~~5~~	~~7-124~~	~~−~~	~~−~~	~~S~~		Lac ✓ isolated impure (Lac+).	
6	12-269	−	−	S		M	slow growth
7	12-270	−	−	S		M	
8	12-209	−	−	S		M	
~~9~~	~~22-85~~	~~−~~	~~−~~	~~S~~		Lac ✓ isolated from EMS lac impure?	Rare + seen
10	22-103	−	+	S		+	
11	22-104	−	+	S		+	
12	22-106	−	+	S		+	
13	20-113	−	−	S		M	
14	-198	−	+	R		T.. ✓ TLB_1	Retest!
15	-392	−	+	R		TLB_1	
16	-393	−	+	R	clone 48	TLB_1	
17	-394	−	+	R		TLB_1	
18	-783	−	+	R		TLB_1	
19	-784	−	+	R		TLB_1 ✓ TLB_1	Retest B_1
20	21-98	−	+	S		B_1	
21	-60	−	+	S		B_1	
~~22~~	~~25~~	~~−~~	~~−~~	~~S~~		Lac ✓ isolated from EMS lac	impure (Lac+, Mal+, V_1^R) Rare + seen in streaks.

Restreak possible impure cultures on EMS Lac.

3/21

A page must be missing here but data seem to be from the cross W478 x W1272, with Lac+ omitted! See 655.

662
b.

See later 662

Lac+ (not ±) from streak plates, picked to water and checked on other EMB.

	T6	T5	Sal	AR	Mtl	Xyl	Ste	Mal		T6	T5	Sal	AR	Mtl	Xyl	Ste	Mal	
1		S	+	+	+	+	+	+	41		S	−	−	±	+	+	−	+
2		S	−	+	+	+	+	−			S	+	+	±	+	+	+	+
3		S	+	−	−	+	−	−			S	+	+	±	+	+	+	+
4		R	−	−	−	−	−	−			S	+	+	+	+	+	+	+
5		R	+	+	+	+	+	+			S	−	−	+	−	−	−	−
6	S	S	−	−	−	−	−	−		S	S	+	+	−	+	+	+	+
7		R	−	−	−	−	−	−			S	+	+	+	+	+	+	+
8		S	+	+	+	+	+	+			S	+	+	−	+	+	+	+
9		S	−	−	±	+	−	+			R	−	−	−	−	−	−	−
10		S	+	+	+	+	+	+			S	−	−	−	−	−	−	−
11		S	−	−	−	+	−	−	51		S	−	−	−	−	−	−	−
12		S	+	+	± ⊕	+	+	+			S	−	−	−	−	−	−	−
13		S	−	−	−	−	−	−			S	−	−	+	−	−	−	−
14		S	−	−	−	−	−	−			S	+	+	+	+	+	+	+
15		S	−	−	−	−	−	−			S	−	−	−	−	−	−	−
16		S	−	−	−	−	−	−			S	+	+	−	+	+	+	+
17		S	−	−	−	−	−	−			S	+	+	+	+	+	+	+
18		S	+	+	+	+	+	+			S	−	−	+	+	−	−	−
19		S	−	−	−	−	−	−			S	−	−	+	+	−	+	−
20		S	−	−	−	−	−	−		S	S	−	−	±	+	−	−	−
21		S	+	+	+	+	+	+	61		S	−	+	+	+	+	+	+
22		S	−	−	−	−	−	−			S	−	+	+	+	+	+	+
23		R	−	−	−	−	−	−			S	+	−	−	+	+	−	−
24		S	⊖ ±	−	−	+	−	−			S	+	+	+	+	+	+	+
25		S	+	+	+	+	+	+			S	−	+	+	+	+	+	+
26		S	+	+	+	+	+	+			S	−	+	+	+	+	+	+
27	S	S	−	−	± ⊕	+	−	−				−		+	+	+		
28		S	−	−	−	−	−	−			S	−	−	−	−	−	−	−
29		R	+	+	+	+	+	+			S	+	+	+	+	+	+	+
30		S	−	−	−	−	−	−			S	−	+	+	+	+	+	+
31		S	−	−	−	−	−	−	71		S	+	−	−	−	−	−	−
32		S	−	−	−	−	−	−			S	+	+	+	+	+	+	+
33		S	−	−	−	−	+	−			S	−	−	−	−	−	−	−
34		S	+	+	+	+	+	+			S	+	−	−	−	−	−	−
35		S	+	+	+	+	+	+			S	+	−	−	+	+	−	−
36		S	+	+	+	+	+	+			S	+	−	±	+	+	−	−
37		S	+	+	+	+	+	+			S	+	+	±	+	+	+	+
38		S	+	+	+	+	+	+			S	−	+	−	−	−	−	−
39	S	S	−	−	−	−	−	−			S	+	−		±		−	−
40		S	+	+	+	+	+	+	80		S	+		−	−		−	−

Repeat ?

661:

		MHL
42	+	
43	+	
12	+	
9	very slow +	score as − or +?
41	very slow +	score as − or +?
27	very slow +	score as − or +?
60	" "	source of modifier?
76	" "	source of modifier?

Gal 24 intermediate + score as −

Kyl 79 Pure +.

	T6	T5	Gal	Ar	Mtl		Xyl	Stl	Mal
81		S	+	+	+	+	+	+	+
82	S	R	−	−	−	−	−	−	−
83	S	S	−	−	−	+	−	−	+
84		S	−	−	−	−	−	−	−
85		S	−	−	−	−	−	−	−
86		S	−	−	−	−	−	−	−
87		S	−	−	−	−	+	−	−
88		S	+	+	+	+	+	+	+
89		S	+	+	−	−	−	−	−
90		S	+	+	+	+	+	+	+

	T6	T5	Gal	Ar	Mtl	Xyl	Stl	Mal
91		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		S	+	+	+	+	+	+
		R	−	−	−	−	−	−
96		S	−	−	−	−	−	−

All are V_6^R unless S is indicated. Scoring with T5 was unsatisfactory

Summaries of lac+ data.

Parental combinations were +++++ $V_6^R V_1^R$ V_6 is known to be linked to lac.

Of 96 lac+ the following parental couplings (single) were observed:

V_6^R	89	All parental:	33
Xyl	57	Ar	44 (+2 ?)
Mtl	49	Mal	45
Gal	46	V_1	88
Stl	44		

Mal, V_1 should also be scored

NOTE: All 8 exceptions were V_6^R and either all − or all +.

	A+ BCD+	A− BCD+
A = Xyl	||	
A = Mtl	||	|||
A = Gal	||||	++++ ||
A = Stl	|	

Stl Linkage: discordances with:

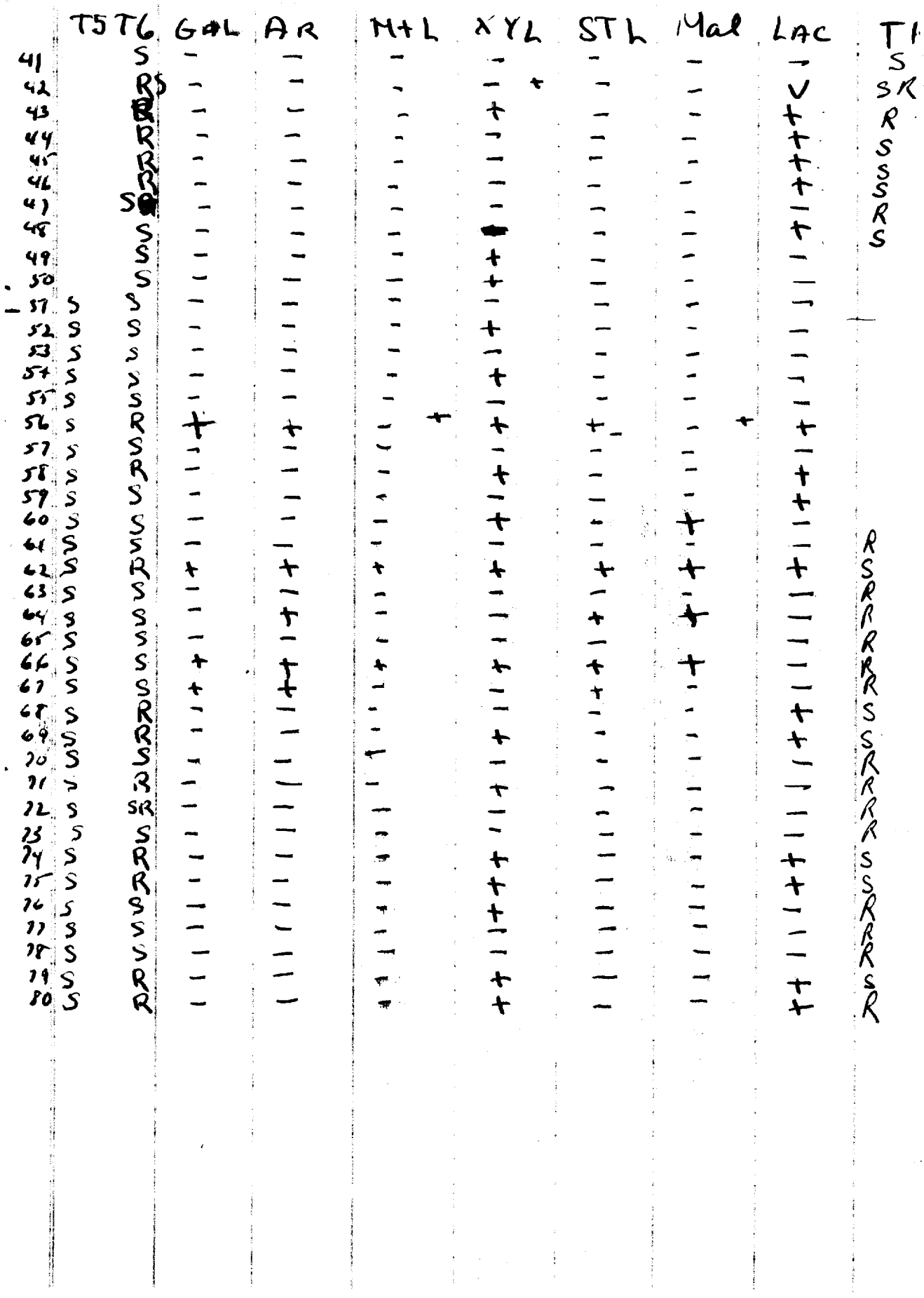
		Ar Linkage
Mal	6	
Gal	16	
Ar	3 *	
Xyl	15	
Mtl	13	

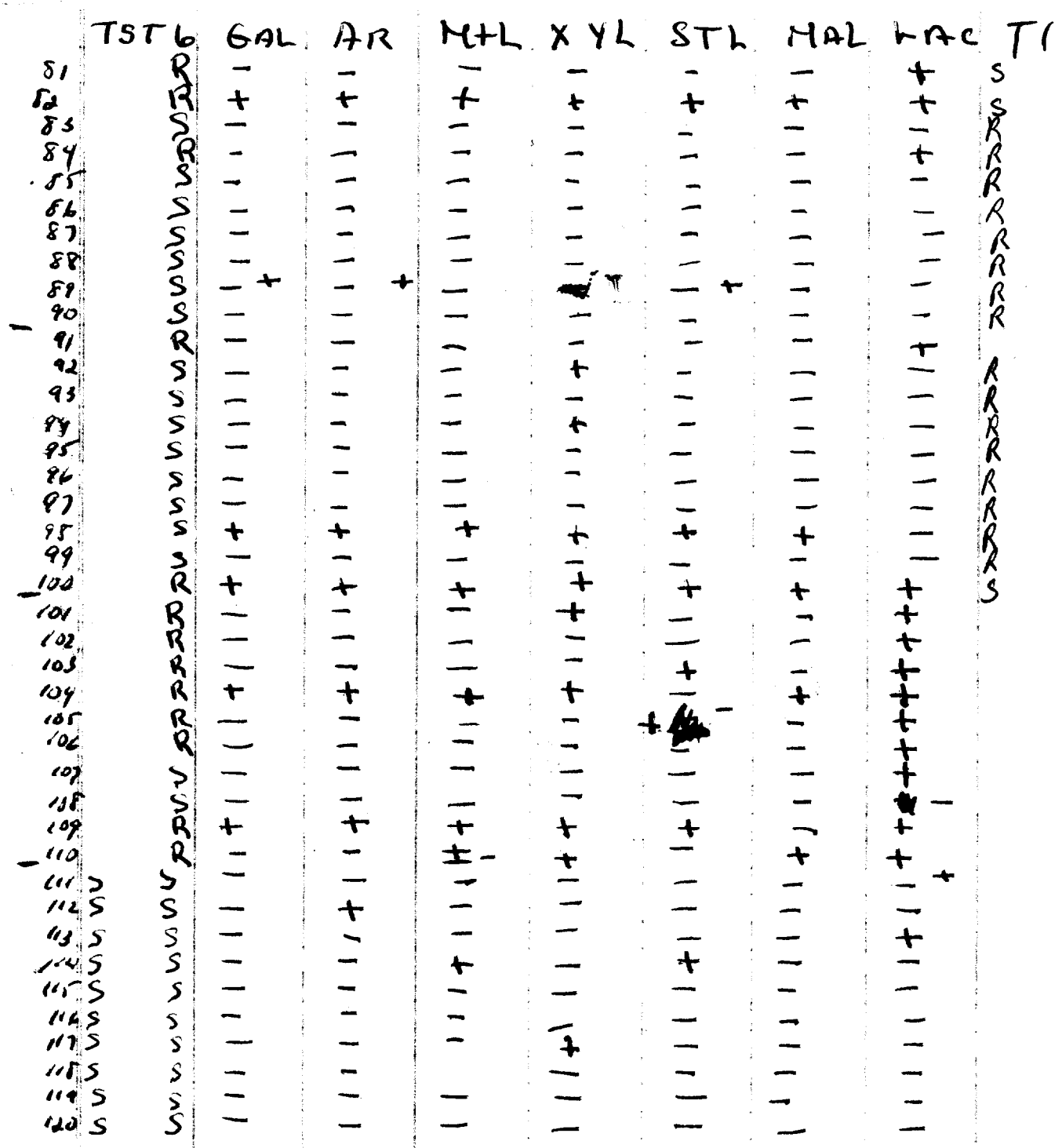
662
D

	T5	T6	Gal	Ar	Mtl	Xyl	Stl	Mal	Lac	T1
1	R	S	−	−	−	−	−	−	−	R
2	S	S	−	+	−	−	+	−	−	R
3	R	S	−	−	±	+	−	+	−	R
4	S	S	−	−	−	−	−	−	+	S
5	R	S	−	−	−	−	−	−	−	R
6	S	R	−	−	±	+	−	−	+	S
7	S	R	+	+	+	+	+	+	+	S
8	S	R	−	−	−	−	−	−	+	S
9	S	S	−	−	−	−	−	−	+	S
10	S	S	−	−	−	−	−	−	+	S
11		RS	−	−	−	+	−	−	+ −	RS
12		S	−	−	−	−	−	−	−	R
13		S	−	−	−	−	−	−	−	R
14		S	−	−	−	−	+ −	−	−	S
15		S	+	−	−	−	+	+	−	S
16		R	−	+	+	+	−	−	+	S
17		R	−	−	−	− +	−	−	+	S
18		S	−	−	−	+	−	−	−	R
19		S	−	−	−	−	−	−	−	R
20		S	0	−	−	−	0	−	−	S
21		R	−	−	−	−	0	−	+	S
22		S	−	−	−	− +	−	−	−	R
23		S	−	−	−	+	−	−	−	R
24		S	−	−	−	−	−	−	+	S
25		S	−	−	−	−	−	−	+	S
26		S	+	−	−	−	+	−	−	R
27		S	+	−	−	−	+	−	−	S
28		S	−	+	−	+	0	−	−	R
29		S	0	+	−	−	−	0	−	S
30		S	0	−	0	−	−	−	−	R
31		S	−	−	−	−	−	−	−	S
32		S	−	−	−	−	−	−	−	R
33		S	−	−	−	−	−	−	−	S
34		S	−	−	−	−	−	−	−	S
35		S	−	−	−	−	−	−	−	S
36		S	−	−	±	+	−	+	+	S
37		R	−	−	+	−	+ −	−	+	RS
38		RS	−	−	−	+ −	−	+	V	RS
39		RS	−	−	−	−	+	−	+	S
40		S	−	−	0	−	−	−	−	R
			*		*		*	*		

* order maybe confused, especially in 21-30

662
D




TST
GAL
AR
MTL
XYL
STL
MAL
HAC
TI

662b

	T5T6	GAL	AR	M+L	XYL	STL	MAL	LAC	T1
121	R	−	−	−	−	−	−	+	S
122	R S	−	−	−	+	−	−	−	R
123	S	−	−	−	−	−	+	− +	R
124	R	−	−	−	−	−	−	+	S
125	R	+	+	+	+	+	−	+	S
126	O	O	O	O	O	O	+	O	O
127	S	−	−	−	−	−	−	−	R
128	S	−	−	−	−	−	−	−	R
129	S	−	−	−	+	−	−	−	R
130	R	+	+	−	+	+	−	+	S
131	S	−	−	±	+	−	+	−	R
132	R	−	−	−	−	−	−	+	S
133	S	−	−	−	−	−	−	−	R
134	R	+	+	+	+	+	+	+	S
135	R	−	−	−	−	−	−	+	S
136	R	+	+	+	+	+	+	+	S
137	S	−	−	+	+	−	+	−	R
138	R	−	−	−	− +	−	−	+	S
139	S	−	−	−	−	−	−	−	R
140	R	−	−	−	−	−	−	+	S
141	S	−	−	−	−	−	−	−	
142	S	−	−	−	−	−	−	−	
143	S	−	−	−	−	−	−	−	
144	S	−	−	−	−	−	−	−	
145	R	−	−	−	−	−	−	+ −	
146	S	−	−	+	+	−	+	−	
147	S	−	−	−	−	−	−	−	
148	S	−	−	−	−	−	−	−	
149	S	−	−	−	−	−	−	−	
150	S	−	−	−	−	−	−	−	
151	S	−	−	−	−	−	−	−	
152	S	−	−	−	−	−	−	−	
153	S	−	−	−	−	−	−	−	
154	S	+	+	−	−	−	−	−	
155	S	−	−	−	−	−	−	−	
156	S	−	−	+	+	−	−	−	
157	S	−	−	?	−	−	−	−	
158	R	−	−	−	+	−	−	+	
159	S	−	−	−	−	−	−	−	
160	S	−	−	?	−	+	−	−	

	No.
# 11	1
38	2
39	3
42	4
84	5
122	6
145	7
162	8
171	9
179	10

are possible lac v.

struck out on EMB & EMS lac

as 662-b No...

	TJTG	GAL	AR	MAL	XYL	STH	MAL	LAC	TI
161	S	−	−	−	−	−	−	−	−
162	R	+	+	+	+	+	+	+	K
163	S	−	−	−	−	−	−	+	S
164	S	−	−	−	−	+	−	+	S
165	S	−	−	−	−	−	−	−	
166	S	−	−	−	+	−	−	−	
167	S	−	−	−	+	−	+	−	
168	S	−	−	−	−	−	−	−	
169	S	−	−	−	−	−	−	+	
170	S	−	−	−	−	−	−	−	
171	R	−	−	+	+	−	+	V	
172	S	−	−	−	−	−	−	+	
173	R	−	−	−	+	−	−	−	
174	S	−	−	−	−	−	−	−	
175	S	−	−	−	+	−	−	+	
176	S	−	−	−	−	−	−	−	
177	S	−	−	−	−	−	−	−	
178	S	−	−	−	−	−	−	−	
179	R	−	−	−	+	−	−	V	
180	S	−	−	−	−	−	−	−	
181	V	+	−	+	+	+	+	−	+
182	R	+	−	−	−	−	−	+	+
183	S	−	−	−	−	−	−	−	+
184	S	−	−	−	+	−	−	−	+
185	V	+	−	+	+	+	+	−	+
186	S	+	−	+	+	+	+	−	+
187	S	−	−	−	−	−	−	−	
188	S	−	−	−	−	−	−	−	
189	S	−	−	−	−	−	−	−	
190	S	−	−	+	−	+	−	−	
191	R	−	−	−	+	−	−	+	
192	S	−	−	−	−	−	+	−	
193	R	+	−	−	+	+	−	+	
194	S	+	−	−	−	−	−	−	
195	S	−	−	−	−	−	−	+	
196	S	−	−	−	−	−	−	−	
197	R	−	−	−	−	−	−	+	
198	S	+	+	−	−	−	−	+	
199	S	−	−	−	−	−	−	−	
200	R	−	−	−	−	−	−	+	

Linkage relations of Stl etc. 662c

Jan. 17, 1950 ff.

~~W1278~~ W1262 × W1269 on T(o); EMS Lac; EMS Stl.

1) From EMS lac, test 100 lac+ for lac v. (struck out on EMS Lac).

8 lac v found. Reisolate to lac EMS, and check for segregation on other sugars.

	Lac	Xyl	Mtl	Stl	Gal	Mal
1		—	—	—	V	—
2		~~m~~ V⁻	—	—	+	—
3		—	—	—	— v?	—
4		—	—	—	— v?	—
5		V+	~~—~~ v?	—	— v?	—
6		V⁻	v?	—	V⁻	—
7		—	—	—	V	—
8		—	—	—	V	+

1/23 Additional 100 Lac+ picked and tested:

8 possible lac v: Retest 4 confirmed (not certainly).

Check on all sugars as 9–12 662c.

		Lac	Save as
"b"	1	V	13
	2	V	14
	3	+	
	4	+	
	5	+	
	6	—	
	7	+	
	8	+	
	9	V	15
	10	V	16

Irradiation of H-168.

January 20, 1950.

All previous irradiations have been done with Hanovia high pressure Hg lamp. To prepare for further experiments, calibrate killing with G.E. sunlamp.

P20. Suspend H168 from EMS bac. to ca 10^9. Dilute in saline to 10^{-5}.
~~Spread~~ Irradiate 5 ml in Petri dish (10 ml) under lamp and spread on EMS bac.
Suspension apparently viable: no colonies from controls!

P23. Repeat with H225.
By mistake, plated 10^{-6} and 10^{-7} ml with 0 irradiation and 10^{-7} only with various U.V. doses.

UV	Dil.	Count		V	+	−	
0	10^{-6}	~~~					
0	10^{-6}	~~~					
0	10^{-7}	35	45	✻	1	5	79 : 10
0	10^{-7}	54			1	3	
5 s.	10^{-7}	18	15		5	5	3 : 26
5 s.	10^{-7}	11			7	9	
10 s	"	0					
10 s.	"	0					
20		0					

↓

For future work, increase distance of lamp to 20 cm and multiply dosages by 4×.

Irradiation of H-225.

January 23, 1950.

BE UV lamp at 20 cms. Diluted coli shaken 0, 20, 30 secs.

	UV	Dil.
A.	0	10^{-7}
B.	0	10^{-6}
C.	20	10^{-6}
D.	30	10^{-6}

Survival = ca 11/411 = ca 2½ %.

	V	+	−	Σ
A	71	8	13	92
	78	7	12	97
	88	9	17	114
	95	8	5	108
	332	32	47	411
C.			1	
			0	
		1	4	
		3	2	
	0	4	7	11.

D. Blank.

Initial T5 and T1 scores are thoroughly unsatisfactory!

Recheck c̄ control stocks + unises before repeating

Jan 20, 1950.

"C"

"B" W1155 × W1258 } Backgrounds rather heavy. A few + colonies observed.
"C" W1268 × W1262 }

D W1268 × W1178.
L+M+V_1^S L-M-V_1^R

B. Picks + colonies which stand out slightly over background. Spot suspensions on T(0) agar to purify. Streak these out on EMB Sucr. All Sucr – V_1^S V_6^S but grow well on EMB Sucr with W1258!

C. Picks colonies (rather small) from T(0) directly to EMS Lac. V.G.!

D. (Colonies rather larger than C). Background very noticeable on EMS; OK on T(0).

Picks colonies from T(0) and streak out on EMS

100 tests: 5 lac – [may contain Lac+] 95 lac+.

Pick purified colonies and test on Mal EMB vs. T1.

95 Lac+: All V_1^S. 94 Mal+ 1 Mal –

5 lac – 5 Mal+ : $3V_1^S$ $2V_1^R$

∴ All lac+ are V_1^S; Some lac– are V_1^S; some V_1^R.

January 24, 1950.

Prepare suspensions from EMS Lac. Prepare rather dilute susp.

	UV	Dil.
A.	0	10^{-7}
B.	0	10^{-6}
C.	5	10^{-6}
D.	10	10^{-6}

All plates too dense for accurate count (over compensation for "dilute" suspensions.

Total counts (¼ plate)

A 90

D 153

∴ 10 sec ≐ ca 85% killing.

Relative counts

	v	+	—	
A.	39	2	8	ca 80% v.
	41	6?	5	< 15% —
	80	8 <	13	
C	6	2	6	

Impression ca 50% —.

D.	28 ~~30~~	24	50

Duplex prototrophs

January 23, 1950.

"C". W1269 (478/6) x W1272 (1262/"6") on a variety of EMS media:

EMSB$_1$: Mal + observed but not clearly scoreable. Yields very high.
Mals not readily detected at 48h. s = sectored.

P25. : Xyl clear, small colonies. A few possible Xyls noted and marked.
Reincubate for further development.

P26: Lac 6 plates. No s colonies. 92+ 128−

−B$_1$ Xyl 5 plates ca 100/plate. No s seen, but colonies too small for accurate + − scoring.

Mal 5 plates. Many small +. 2? 1,2 Mals. 91+ 61 − [2 plates].

Lac 6 plates. heavy background. No Lacs seen. ca 400+.

+B$_1$ Xyl 4 plates. 6 fairly clear Xyl v. [3 − 8].
ca 136 scoreable +. 42+ : 50 − (1 plate)

Mal Too crowded to estimate precisely 7 plates. Some Mals probably are coincident colonies.

14 possible Mals picked and streaked on EMB Mal.

Mals, +, − comps. scored on Lac.

Lact Mal →	+	−	
1	+	+	
2	−	−	
3	−	−	
4	+	−	*
5	+	−	*
6	+	+	
7	−	−	
8	−	−	
9	+	+	
10	−	−	
11	−	−	
12	+	+	

Xyl →	+	−
1	+	+
2	−	−
3	−	−
4	−	−
5	−	−

Very definite correlation for Lac of +/− components.
(Two exceptions *)

667a

	Mal		Xyl		Lac		Gal		Mtl		Sbl		Rz		T5	
	+	−	−	−	+	+	−	−	−	−	−	−	−	−	S	S
	+	−	+	+	−	−	−	−	+	+	−	−	−	−	R	R
	+	−	+	+	−	−	−	−	−	−	−	−	−	−	B	B
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	S	S
n.g.	+	+	+	+	+	+	−	−	−	−	−	−	−	−	S	S
	+	−	+	+	+	−	−	−	−	+	−	+	−	−	S	R
	+	−	+	−	+	+	−	−	"±"	−	−	−	−	−	S	S
	+	−	−	−	−	−	−	−	−	−	−	−	−	+	S	S
	+	−	−	−	−	−	−	+	−	−	−	+	+	−	S	S
	+	−	+	−	+	+	+	−	±	−	−	−	+	−	S	S
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	R	R
	+	−	−	−	−	−	−	−	−	−	−	−	−	−	M	R
	+	−	+	+	+	+	−	−	+	+	+	+	+	+	S	S
	+	−	+	−	−	−	−	−	−	−	−	−	−	−	S	S
	+	−	−	−	−	−	−	−	±	±	−	−	−	−	R	R
	−	−	+	+	+	+	−	−	−	−	−	−	−	−	S	R
	+	−	+	−	+	−	+	−	±	−	−	−	+	−	R	R
	+	−	+	−	−	−	−	+	−	−	−	+	−	+	S	S
	−	−	+	+	−	−	−	−	±	±	−	−	−	−	S	S
	−	+	−	−	−	−	−	−	−	−	−	−	−	−	S	S

Need plaques!

Segregation of H226

January 26, 1950.

Streaked out 8 Lac v of H226, on EMB Mal, Lac.

a) test reduced Mal (+ or −) on Lac

b) " " Lac v on Mal. [No Lac+ noted].

A number of peculiar variegated colonies were noted on Mal EMB. Picks and number & description. Streaks out on Mal, Lac.

c) 1. 2. 3. 4.

A27. a) Mal−. 72 tested on Lac All apparently pure Lac−.

a Mal+ 84 tested. 80 definitely mixed. 4 possibly Lac v or containing Lac+. (These may have been Mal v miscounted as +). Streak these out as a [1-4].

a Mal v 24; tested 22 Lac v 2 apparently pure Lac−; a [5-6].

Also, a considerable number of "pure" Mal+ and − colonies unpooled, and streaked out on EMB Lac. Among several hundred Lac− colonies there were two identifiable Lac v or+, in addition to numerous papillae in the third streak. Attempt recisolation of Lac v. a [7-8].

b]. 52 Lac v tested on Mal: all + or contain +.

c].

All these tests were made by picking media directly from colony to agar ∴ + cannot be distinguished from v.

January 27, 1950.

C]. 1. Lac — virtually all −, rare ∀, papillate.
Mal — only + and − seen.

2. Lac — rare-occ. ∀, heavily papillate
Mal — Mostly or all pure +, some −.

3. Lac — all − colonies, but papillate
Mal — only +, −

4. Lac — occ. ∀ colonies.
Mal — only +, − noted.

Pick Lac ∀ and pool as far as possible. Streak out on lactose for resolution of Lac ±

January 30, 1950

a) 1. Mal+ with occ. Mal−, and simple sectors. lac− with occasional lac v.

2. Do.

3. Mixed Mal+, v, − } ~~Mal v, M~~

4. Mal v, Mal+. } lac v; lac−.

5 Pure lac− } largely segregated (Mal+, −) → +, − only.

6 Pure Lac− } maybe some Mal v. [Attempt reisolation].

7 Mal−, some +, v?

8. Mal+, some −; v? } lac v, −. Reisolate lac v and test for Mal purity.

c) 1–4 Lac−, Lac v. Test isolated lac v for Mal. purity.

---

a) Pick lac v from 1, 2 and 7, 8 above. Streak on Mal.

1–3 = a1
4–9 = a2
10 = a7
11–16 = a8.

	Mal
1	+, −, v
2	+ −
3	+ −
4	+ −
5	+ −
6	+ −
7	+ −
8	−

	Mal
9	+ −
10	+ −
11	+ −
12	+ −
13	+ −
14	+
15	+ −
16	+ −

Reisolate lac v from these

These may be Mal pure. 668d. (1) & (2)

---

c: 8 each.

1 all impure (Mal+, −).
2 " "
3
4

} All were Mal v, not Mal+.

Segregation of H226

1/31/50.

EMS Lac

1. Lac$\underline{v}$; Lac− (Mal−) H227.

2. Lac$\underline{v}$; Lac− mostly Mal+, occ. Mal−, simple Mal$\underline{v}$
Repeats 4 Lac$\underline{v}$; all some Mal+; Mal$\underline{v}$.

---

push H227 on EMS Lac, EMS Mal.

---

131. Picks 200 Lac$\underline{v}$ from 671 A2 Breeds very sparsely on EMS Mal.
1-100 No Mal−. Mostly Mal+, −. 2 maybe pure Mal+ : retests as 668d: 3-4. 3: No Lac$\underline{v}$ (exc. papillae); 4: Mal+/− ; Lac$\underline{v}$
101-200 No Mal−. Mostly Mal+ & detectable −.
201-240 All Mal+/−

Irradiation of H225 and H226

668+

N.G. Too dilute

January 25, 1950.

Resuspend H-225 from EMS lac in H_2O, 10 ml, to opt. dens. ca 120
Dilute 1/100 for irradiation. 5 ml sample in Petri dish (10 cm).
Remove 1 ml to 10 ml for each interval. 20 cm. distance.
0, 5, 10, 20 sec. This leaves suspension at 10^{-3}, or as estimated 5×10^5
A, B, C, D. before irradiation.

Dilutions:

	1	.1 ml
	2	.03
	3	.01
	4	.003
E only	5	.001
F 10^{-6}	6	.0003
	7	.0001

H226 O.D. 090

E 0

F 20

A.	4	5, 4	∴ Initial count (00 = 120)
	5	1, 0	
	6	0, 0	was 3×10^{-6} ml = 5 colonies = ca 10^6/ml. (viable)
	7	0, 0	
B.	6	0, 1	
C	3	12	∴ count = 12/10^5 = ca 10^6/ml.
	4	7, 5	
	5	0, 1	no killing detected.
	6	0	
	01	0	
D.	3	0	

E	10^{-6}	4, 4, 3
	10^{-7}	0, 1, 1
F	10^{-4}	0, 0, 0

Test lac+ [Lac$_1$ x Lac$_4$] as diploid
Irradiate Seligmann's lac+.

January 27, 1950.

Suspend "pure" colony of 652-2 in H_2O. Spread drops on EMBlac.
Expose to high pressure UV 6, 7, 8, 9 secs.
Ca 1000 survivors in total. No Lac- or Lac$^{\pm}$ noted

Conclude that 652-2 is probably not a stable diploid; but a typical recombination of lac$_1$- with lac$_4$-.

2/4/50. #2922A received from E. Seligmann, Beth Israel Hospital, New York, N.Y.

streaked out on EMBlac: all +. Suspend + colonies in water, spread on EMBlac and subject to 8 secs UV (high pressure lamps).
4 plates; ca 500 colonies. All lac+.

Effect of medium on segregation.

January 27, 1950.

EMS BMTLB, apparently does not permit lac v colonies of H16B. Check effect of various supplements.

In this case, lac v was observed on BMTLB.

Irradiation of H225-226

January 29, 1950

a) H226 susp. in 10 ml O.D. 275 ca 2×10^6

b) H225 " " " O.D. 380. ca 3×10^6

Count in Petroff-Hausser. ca 3×10^7

Approved dilutions. dilute ~~1/100~~ to estimated 10^5 each a) .5/10 b) .33/10

record $\log_{10}$ est. number

			uv
1x	A=	226	0
2x	B=		5
50x	C		20
1x	D	225	0
2x	E		5
50x	F		20

January 30, 1950.

			+ = V	−	Σ	% V	
A.	W226	No UV.					
		"2".	457	73	530		Counted readily under lens.
	*		432	96	528		
			656	84	740		
			593	102	695		
			2138	355	2493	86	
		"1"	388	46	434		
B.	W226	5 secs.					
	2	(+ maybe count.)	25	382	407		
			27	401	428		
			52	783	835	06	
	!	"1 uncon".	24	←	536		
C	2					0	
			0	2		2	
			1	2		3	
			0	3		3	
			0	8		8	

No full or stable + noted. Reincubate to search for same.

Note re counts. Two sets of "2" were made.
The first set was uncountable, probably ca 10 × 434
The second is given. Platings appear very erratic!

D	ouv	+	−	V	Σ	%v
	1	10	21	110	141	
		22	21	128	171	
		32	42	238	312	76
			74			
E	ss. uv.					
	1	24	37	21	82	≈ 36
		27	57	34	118	
		51	94	55	200	22
			145			
F	2	7	15	2	24	
		11	22	2	35	
		6	7	1	14	
	"7"	9	11	2	22	
		33	55	7	95	
	"20"	2	1	1		
		1	2	0		

No absolute increase, as relative; actual count is one half that recorded

i.e., survival was $\frac{100}{312}$ = ca 32%

These counts seem more consistent than those of A–C: even shorter doses should be used (ca 3 seconds)

lysis

2/3/50.

	A	B
a) Initial assays were	6×10^7	0×10^7
After 20 hours, refr. in water	2×10^7	5×10^6

Note drop in titer, especially with H226!

b) H225 data show uncomfortably high variance, but clearly indicate absolute increase of segregant types with 2 sec. irradiation.

c) H226 data not very useful owing to too low deletion

d.) 5 mems. at 60° already kills most cells. Use at high conc. and plate various deletions.

Irradiation of H226 - H225. 672 A

February 2, 1950.

a) P1. Assay suspns.

		O.D.^{4200}	Titer
A	H225	340	6×10^7
B	H226	290	6×10^7

Note error in calculation!!!

For critical, low dose irradiations, dilute to 3×10^{-5} (estimated 1800/ml), irradiate, and plate .1 ml samples. Use 2 and 5 secs. UV. 20cm. 2 ml/10cm Petri dish

C. H226 Take 3 ml sample, undiluted, and give ~~xx~~ doses at 10 sec intervals. Plate .1 ml for each. Start at 40 sec. 27 survivors at 40 sec.

D. 60° exposure. (in oven) Use 3×10^{-4} dilution H226, 3 ml in small test tube. Sample at 5 min intervals (.1 ml).

2/3 3PM

A O	+	−	V
1	8	19	36
2	8	13	36
3	7	24	50
~~4~~	~~23~~	~~56~~	~~42~~
4	8	8	42
5	10	7	31
~~22~~ 22	41	71	145

% .

Av/plate.

~~28~~	~~14~~	~~41~~
8	14	29

A 2.

survival
3PM
(somewhat early)

		+	−	V	Σ	% surv.
A0		8	19	36	63	
		8	13	36	57	
		7	24	50	81	
		8	8	42	58	
		10	7	31	48	
	Σ	41	71	195	~~307~~	
	Av.	8	14	39	~~31~~ 61	
		13	23	64		100
A2	1	13	27	19	59	
	2	12	29	7	48	
	3	11	14	5	30	
	4	13	28	20	61	
	5	6	36	17	59	
	Σ	55	134	68	257	
	Aver.	11	27	13	51	
		22	53	25		84
A5		13	16	11	40	
		17	25	15	57	
		5	8	3	16	
	Σ	35	49	29	123	
	Av	12	16	18	41	
		29	39	32		67

These results indicate an increase in the absolute number of segregant types with very low doses, with appreciable killing.

		+ = V	−	
D.	0	16	7	23
	5m.	0	0	
10 ↓	0.			

Note: assay should be 10 × B0 (av. 5.5)

Lac series.

	+ = V	−	
B 0	4	0	
	2	4	
	6	1	
	7	0	
	0	2	
	2	4	
	22	11	33
	3.67	1.82	5.5

Mel.

	−	+	V	
	0	1	3	
	0	3	2	
	0	2	1	
		6	6	/ 12
		2	2	4.0

B2	~~5~~	~~B~~	
	0	7	
	0	10	
	2	4	
	1	4	
	1	6	
	4	31	35
	0.8	6.2	7.0

		2	3	
	1	3	0	
	2	3	3	
	0	9	1	
	3	17	7	/ 27
	.75	4.25	1.75	6.75

B5	0	5	
	0	4	
	0	11	
	0	4	
	0	3	
	0	27	5.4

	2	3	0	
	0	2	1	
	1	3	1	
	1	0	0	
	4	8	2	/ 14
	1	2	.5	3.5

This series highly unsatisfactory owing to killing!

40 sec.	0	26
50 sec	0	0

Same original was ca. $\frac{5.5}{3} \times 10^5 = 2 \times 10^5$ / .1 cc

The 40 sec survival was ca 10^{-4}.

Spontaneous heterozygotes
continued from ~~1~~ 652.

February 2, 1950

A) W67 x W1272. [Streaks out mixture on EMB Lac OK]

EMSlac ca 2500 prototrophs

2 lac+. Streaks on EMB, EMSlac; EMBMal. 2 additional A5.

1	Lac?	Mal−	maybe lac+ or very stable lac v. = 673−1
2	lac−	Mal−	
3	Lac−	Mal−	
4	Lac v	Mal−	clear Lac v. = 673−2

very weak + !

B 2/5/50.

W67 x W1177 EMSlac 21 x 60/ = ca 1200 prototrophs.

8 lac+ colonies streaks out on EMSlac; EMBlac; EMBMal.

		Lac	Mal
PROVISIONAL	1. Adj. to (−)	v	− +
	2	+	−
	3	v	!
	4	?	+
	5	?	−
	6	v	v?? mod.
	7	v	−
	8	v	+

Keep on D(Lac) agar.

Evidence concerning dikaryon intermediate in segregation of H226.

2/3/50.

A Pick well isolated Lac - colonies from pre-assay plates B5 - etc. of 672. Some should be Mal+/-. 40 tested by sparse brushes.

Compare with material from 675.

39 +, apparently pure; 1 -.

B. Do. from C40 28 colonies brushed. 22 were pure Mal+
4 " " Mal-

2 were predom. Mal -, c Mal+. Pick and restrak → Mal EMB was badly contaminated.

Save 677 plates!

From 677 Ax and Bx, pick isolated ~~Mal~~ Lac - colonies and brush on Mal EMB.

41 pure Mal-

18 "pure" Mal+

17 mixed Mal+/-: ? Restrak on Mal EMB; Lac EMS to reisolate.

76 total.

Restraked cultures:

	Mal	Lac EMS	EMB
15	+ -	gr. - +	-
16	+ -	gr. +	
17	+ -	gr. +	

Mal + - check Lac EMS and transfer to Lac EMB.

3 are Lac x
2 are pure Lac -

	Mal	Lac EMS → EMB	
1	++		
2	++ -	O	1
3	++		
4	+, -	O	2
5	+ -	O	3
6	++		
7	+ -	O	4
8	++	-	7
9	++		
10	++		
11	++		
12	+ -	O	5
13	+ -	-	6
14	++		

∴ 7 of these colonies are apparently Lac - pure, but Mal+/- !

Heat and uv killing of H226.

Febr. 3, 1950.

Make up from EMS Lac to optical density of 420_{420}. H226 only.

Assume titre ca. 10^8 (but also work on basis of 10^9; 10^7)

A. (assume 10^8). Dilute to 2×10^{-5}. Use. 1 ml samples/plate before and after 2 secs. irradiation. A and AX

B (assume 10^9) Dilute to 2×10^{-6}, etc.

C. (assume 10^7) Dilute to 2×10^{-4}.

D. Heat 2ml original culture 5 mins., 60° and plate out at various dilutions

irradiate A, B, C 2 secs. UV 20 cm.

P4: Viable count too low! Ca 10/plate at 2×10^{-4} $\frac{a}{=} 5 \times 10^5$.
D5 gave 9 colonies, indicating no killing by the heat treatment!

Apparent proportion of Lac – rather high in current suspensions.
Start a fresh restreaking

	+	V	−		+	V	−
C:	1	1	4		1	2	0
	2	9	4		5	1	1
Lac	0	7	4	Mal	0	0	0
	0	5	4				
	1	6	5				
	4	28	21 /53				
wipings from each of ↑	0	0	1				
		2	9		4	2	1
Cx		0	10		5	7	1
		2	8		12	3	1
		0	7				
		2	11		2	0	1
wp.		06	2	45751			

		+	v	−
D	4	7	123	88
	5	0	6	3

These ratios are comparable to C, which is consistent with the lack of killing.

---

streaks out apparent Lac +.
1-3 from C
4-9 from D

all are clearly Lac v. Lac + owing to spreading habit.