

Irradiation of H-168

651

December 15, 1949.

A. 10^{-7}

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

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

H-168 Reisolated from EMS lac. Suspended in saline

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

on EMB Xyl and Lac.

A	156-	1v.		587	10
	153-	2v	1?+	1294	0
	126-	3v			
	152-	4v			
	<hr/>				
	587	10v.			

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, vac plates gave parallel results:
majority of Aureo lact, smetac -, Lacu. Ballwue Lact or -

December 15, 1949.

As above. Celloform EMS Xyl.

probably overestimated!

A. EMB Xyl.

	-	+	v
1	16	1	21
2	10	0	19
3	<u>12</u>	<u>1</u>	<u>18</u>
	48	2	58
	11	4	40
4	7	0	41
5	2	1	58
6	3	5	31
7	8	5	49
8			
	<u>31</u>	<u>15</u>	<u>199</u>

EMB lac

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 unmutated!

12/17/49.

Recount 6519 after 12 hours further incubation.

Xgl	A	-	+	v
1		11	1	31
2		10	1	30
3		12	0	26
		11	3	23
		33	4	79
				116

Xgl 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

	<u>Pure</u>	<u>Var.</u>
	32	68
	80	20

~~lac A 4 11 8~~

Scoring of lac^v vs. lac⁻ very difficult. Record only -, +.

		-	+ or v.
A	4	11	57
	5	4	50
	6	2	57
	8	7	55
		24	219

B. 14

Many more
clamb
lac⁺!

A. MHL.

9	9 9a	8	10	2	3	49	55
	u	-		+		v	

Picks + to confirm balanced diploids

6 A. 8 sec. Xyl → Lac 12L-; 3v; 30+

7 B. 3 sec MHL → Lac 4+ 4-

8 C. 3 sec Xyl → Lac 2+; 3-

D. 3 sec ~~MHL~~ Lac → Xyl 10+; 136- Xyl+ Rechecked.

E. 0 Xyl → Lac, Xyl (4)

F. 0 MHL → Lac, Xyl, MHL (4)

G. 0 Lac → Lac, Xyl bunch (8+)

Tests on possible diploids
from UV-irradiated 4168.

651bc

Dec 21, 1949.

A. 70 lac+ streaked out on lactose and xylose.

1-16 essentially pure lac+ xyl+ (+, - A₃:1-v)

A₄ (1,3 mixed lac+, -; mostly xyl+).

A₅ (6). mixed lac+, -; xyl+.

A₆ (lac v?) — hold 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~~ 11

E		xyl	lac
	1	-	+
	2	-	+
	3	+	+
	4	-	+

Exceptional recombinant?? B6

F		xyl	lac	MFL
	1	+	-	+
	2	-	-	+
	3	-	+	+
	4	+	-	+

G. Bushes. (v. dilute) #1,3 - + other 6 - (xyl)
+ - " + (lac)

Tests on presumptively
balanced diploids

651bd

December 23, 1949.

A (from	uv 8sec., Xyl + Lact):			Test on MHR TI.		
	MHR	TI	Nutr. (TO)	MHR	TI	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	+
10	-	R		+	P	

651bd 1

	MHR	TI	Nutr. (TO)	MHR	TI	TO
B-D	01	+	P	-	4	-
3sec uv	2	-	R	+	5	+
Lact + Xyl.	3	+	P	-	6	+
	4	+	P	-	7	+
	5	+	P	-	8	+
D	1	+	R	-	9	+
	2	+	P	-	10	+
	3	+	R	-	11	-
No UV.	B6	+	P	+		

651bd 2

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

12/14/49. W67 x W677

20 plates. ca 60-100/plate.
EMSlac

1500 cols.

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⁺.

1/23/50.

W67 x W1272.

ca ⁶ 500 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+ . Strains out+. all+

H-226: segregating for lac, Mal, Mtl, and probably for Stt, Ar, Gal. should also be segregating for V₁; not for V₆.

1/25/50.
67 x 1272

ca 1000.

3+ colonies.

Strains out on EMS, EMSlac; Mal.

"1"
"2"
"3"

Lac
L⁺

Mal

+, - (V)

+, - no y⁺ seen.

see 652 a.

1/30/50.

67 x 1272

January 30, 1950, et al...

3 lact+ from 1/25/50 W67xW1272.

Early gave both types

11. Mostly lac-, several lac^v ^{some} ~~some~~ ^{others} ~~others~~. ^{restreaks for type!}
 Mostly ~~lac+~~ Malt+; Mal-. ^{anchar} Mal^v ^{restreaks.}

12. lac^v (?) primarily lac+. Predominantly Mal-. Few + (contam.)

13. distinctively lac^v. Mostly ~~lac+~~ Malt+, some Mal-. Restreaks cool. lac^v.

11. Mal^v? : only Malt+, - on restreaks! Some - on Mal EMS.

12. Definitely lac^v-like H226. On Mal EMS, all Mal-.

13. 8 lac^v streaked: all Mal+. (Mal- probably contam. or admix.)

"W67xW1272" ca 1/30 lact+! (probably same user). 140 lact
 picked and streaked on EM3 lac. All pure+. (58-161 x
 Thrown out! 1272?)

See 673

Conclusions:

- 11: lac^v Mal^v H228
- 12: lac^v Mal- H229
- 13: lac^v, probably Malt+ H230

Recheck some Zelle 1-cell isolates
from H206.

653

Jan 5, 1950

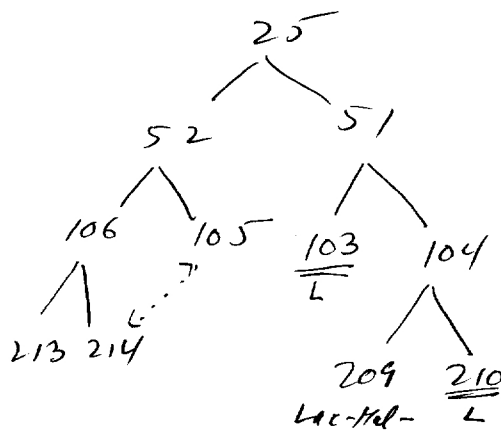
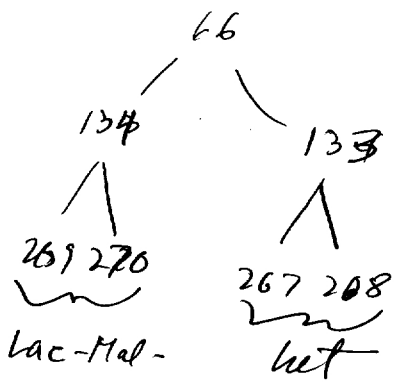
Review for pedigrees and summary of H206

		Mel	Bal	lac
1	12-267	+ ^{u?}	- ^{u?}	+
2	12-268	+	-	+
3	12-269	-	-	- ^{reverting}
4	12-270	-	-	- ^{reverting}
5	12-209	-	-	-
6	12-214	+ - v?	-	+ - v
7	12-198	all +	-	+
8	12-198 H206 stocks.	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 +. Restreaks!



$H206 = 544D4.$

W477 x W980.
 TLB, Lac-0, K x 478 Gal-Mal-. Try T5 also.

Zytle's single cell
Series 15:

Jan 5, 1950.

		lac	Mal
1	5-83	-+	-+
2	84	-+	-+
3	85	-+	-+
4	86	+	+
5	122	-+	-+
6	137	-+	-+
7	181	-+ ^v	+
8	+5-223	+	+
9	223	-+	-+
10	224	- ^{tiny}	- ^{tiny} (constant?)
11	225	-+	-+
12	+5-223	+	+
13	239	-+	+ -
14	240	-+	+ -
15	241	-+	+ -
16	242	-+	+ -
17	+5-223	+	+
18	"	+	+
19	"	+	+
20	"	+	+

-+ look for v
-+ " "

lac v

supp v

-+ look for v

Sorbitol linkage - Preliminary.

1/11/50.

W1262

~~50~~ W478 x W677. MEMS lac
" STL.

Picks 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	MHC		STL	lac	Sal	Mal	Xyl	MHC
1	+	+	+	+	+	+	24	+	+	+	+	-	+
2	+	-	-	+	+	+	25	+	+	-	-	+	-
3	+	-	+	-	-	-	26	+	-	-	+	+	+
4	+	-	-	-	-	-	27	+	-	+	+	-	-
5	+	+	+	+	+	+	28	+	+	+	+	-	-
6	+	+	+	+	+	+	29	+	-	+	+	+	+
7	+	-	-	-	+	+	30	+	-	+	+	+	+
8	+	- ⁺	- ⁺	- ⁺	- ⁺	- ⁺	31	+	-	+	-	-	-
9	+	+	+	+	+	+	32	+	-	+	-	-	-
10	+	+	+	+	+	+	33	+	-	+	+	+	+
11	+	+	+	+	+	+	34	+	+	+	+	+	+
12	+(³)	-	-	-	-	-	35	+	-	+	+	+	+
13	+	+	+	+	+	+	36	+	-	+	-	misread	-
14	+	+	+	+	+	+	37	+	+	-	-	+	-
15	+	+	+	+	+	+	38	+	+	+	+	+	+
16	+	+	+	+	+	+	39	+	-	+	+	+	+
17	+	-	+	-	-	-	40	+	-	-	+	+	+
18	+	+	+	-	-	-	41	+	-	-	-	-	-
19	+	-	-	-	-	-	42	+	-	+	+	+	+
20	+	+	+	+	+	+	43	+	-	+	+	+	+
21	+	+	+	+	+	+	44	+	+	+	+	+	-
22	+	+	-	-	-	-	45	+	-	+	+	+	+
23	+	+	+	+	+	+	46	+	-	-	-	-	-
48	+	-	+	+	+	+	47	+	+	+	-	+	+
49	+	-	+	-	-	-	52	+	+	-	-	-	-
50	+	+	-	-	-	-	53	+	+	+	+	+	-
51	+	+	+	-	-	-							

Summary - Preliminary linkage tests.
StL.

655a

4/12/50. 53 tests: inbred +. Following +:

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

Distinct linkage to Bal is apparent.

Other linkages: (in this StL + prototroph sub-sample):

Lac-Bal.	MHL-Xyl.	Mal-Xyl	Mal-Bal
++	22	30	29
+-	5	1	3
-+	17	4	9
--	11	18	12

An unusually high concordance of Mal-Bal-Xyl is noted here. It is clearly profitable to study the full sequence: Lac V6 Bal StL MHL Mal Xyl...

Characterization of W1258 (Cavalli 123)

Jan 4, 1950 Ft. in D(0):

① standard addition series: Y. tests. +++
 HC +
 O ≡

② Amino acid Run-down.

HC +
 AA +
 AA+Vits ±

No vitamin Requirement?

Vits -
 HC+V +

Add vits. in succeeding tests.

Vits + single AA groups
 HC + YDA

-
 ±

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.

Threonine
 Methionine
 Isoleucine (indicated)

- A12 series
 - A3 series
 - A6 series

testimonies??

After 24 hours, Methionine, Threonine, Isoleucine were indicated;
 AA mix gave poor growth

48 hours -M- -A3±, -A5±, but -A12+M ±

January 7, 1950. (+ pur.)

W-~~1178~~ x W-1258 on EMS lac. Pick 100 + colonies.
1178 20 ± 01 - "

[W-1177 x W-1258 " Heavy background and plating noted.
See infra. Many prototrophs, but]

Ca 99% lac+ 1% lac- do not pick.

Streak out lac+ for v. None v. Test E 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??	} Re-isolate on EMS Lac
2	+ , -, v??	-	
3	+ , ±	-	
4	+ , ±	+	as 656-1
5	+ , ±	+	-2
6	+ , ±	+	
7	+ , ±	+	
8	+ , ±	+	
9	+ , ±	+	
10	+ , ±	+	
11	+ , -	+	

#1 - definitely lac_v. Attempt re-isolation on EMS lac. However, many lac- prototrophs noted, and lac_v colonies on EMS virtually preclude selective isolation.

Jan 6-7, 1950.

In cross of W677 x W1258, heavy plating of background was noted. In view of this, tests for λ sensitivity were made in subclonal 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 12, 1.

1/10/50. Inoculate Nutri Br + Y. ext. (NB4) with 4ml young culture W1258 + ca 10^8 λ . Aerate 5-6 hours. Cool overnight and filter, first streaking out the turbid supernatant. Control was much denser.

- ① titrate filtered λ stocks
- ② Test 1258/ λ for resistance; lysogenicity

Jan. 4ff. 1950.

"A" W1262 x 58-161 m EMS loc

Pick back and loc - to STL EMB.

	STL -	STL +
L+	21	1
L-	30	2

Linkage of ϕ or ψ - linkage to M is indicated. Repeat cross, plating
on EMS-STL.

Further tests, summary:

	STL -	STL +
L-	105	9
L+	75	4

Jan 10, 1950
W478 x W1262.
Lac x

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

* idea suppression H⁺

#	Lac	Mtl	StH	Xyl.	Mal
1	+ -	+ -	+ -	+ -	-
2	+ -	+ -	+ -	v	-
3	v	-	-	v	-
4	+ -	-	- +	v	-
5	v	-	-	v	+
6	v	-	-	- +	-
7	v	+ -	+ -	v +	+
8	+ -	slow*	-	+	+
9	v	v	v ^{slow}	v	≡ H225
10	v	-	-	v	-
11	v	-	-	v	-
12	v	-	-	v	-
13	v	-	-	-	-

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

January 16, 1950.

Scrape H-168 from EMStac; dispense in water, spread on EMStac
EMS lac and irradiate as indicated.

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

#	UV	lac+	lac-	lacv
1	8	57	41	104
2	8	81	30	33
3	15	38	34	95

Spec. count?
Too crowded; only a part of area

176 105 232

281 232
95 75 199
70 199

Omitting #2

-log's
Killing

UV	lac+	lac-	lacv	% lacv	
0	0	14	131	85	
1	3	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	3	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 3sec uv.	11	1 ?
	14	0
	18	0
A 8sec.	31	1 sect
	83	3
15 sec	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; EMS lac. Haploids and stable diploids will be same on both media (P_{arr}; S); segregating diploids will be distinct. Save suspensions. 20 from each streaked.
 All were T₁^P except B10, T₁^R on both media. All were also clearly mixed \pm on EMS lac. Retards a few inportable exceptions

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

H225 segregants

1/18/50

Steak out individual lac⁻ segregants on EM10 lac. Pick 1 lac⁻ and 1 lac⁺ each for further classification.

lac	Gal	Xyl	MH	TS
-	+	+	-	R S
-	+	+	-	R S
-	+	+	+	R S
-	+	+	-	R S
-	+	+	-	R S
-	+	+	-	S S
-	+	+	-	R
-	+	+	-	R
-	+	+	-	S S
-	+	+	-	R S

lac	Gal	Xyl	MH	TS
-	+	+	-	R S
-	+	+	-	R S
-	+	+	-	R S
-	+	+	-	R S
-	+	+	-	R S
-	+	+	+	R S
-	+	+	+	R S
-	+	+	-	R S
-	+	+	-	R S
-	+	+	-	R S

all apparently sorbitol⁺, arabinose⁺

Precludes use for linkage tests. Recheck segregation.

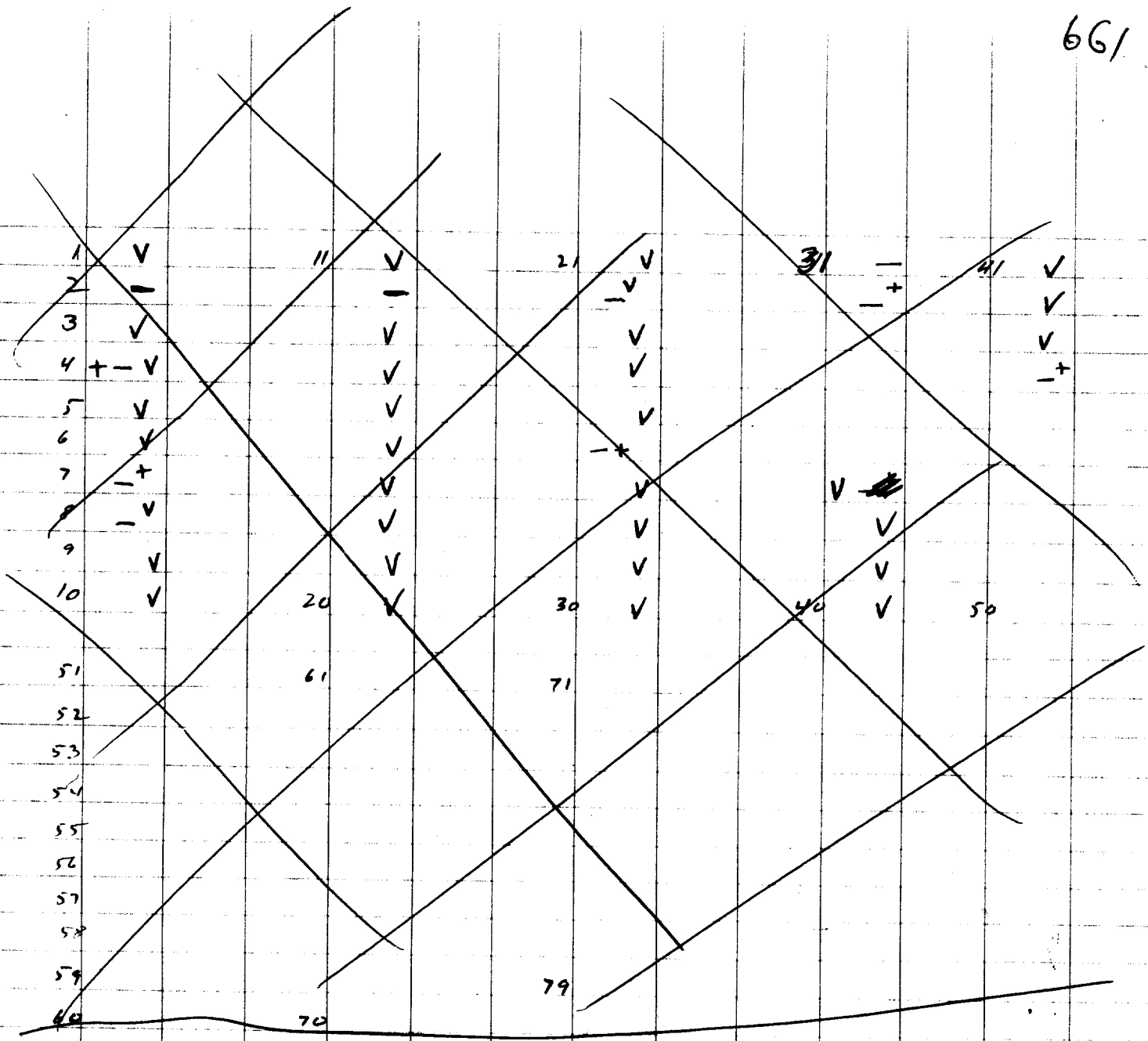
Zelle's single cell isolation

<p>A</p> <p>20</p> <p>1) - 0 - 2) ✓ - - 3) ✓ ± ± 4) + 5) ✓ + 6) ✓ + 7) + - ✓ + 8) + - ✓ + 9) + - ✓ + 10) + - ✓ +</p> <p>41) ✓ - 2) ✓ - 3) ✓ - 4) ✓ - 5) - ✓ - 6) - ✓ - 7) - ✓ - 8) - ✓ - 9) ✓ - 10) ✓ -</p>	<p>11) ✓ - ✓ - - ✓ 0 ± ↑ 2) - + ✓ ± ± ✓ - - ✓ - - ✓ - - ✓ - - ✓ - - ✓ - -</p> <p>20) ✓ -</p> <p>51) ✓ ± ✓ - ✓ - ✓ - ✓ - ✓ - ✓ - ✓ - ✓ - ✓ - ✓ - ✓ -</p> <p>60) ✓ ±</p>	<p>21) ✓ - ✓ ± ✓ - ✓ - ✓ - - ✓ - - + ✓ - ✓ - ✓ - ✓ - ✓ -</p> <p>30) ✓ -</p> <p>61) ✓ - ✓ - ✓ - ✓ - ✓ - ✓ ± ✓ - - 0 - - 0 - - 0 - - 0 - - 0 - ✓ -</p> <p>70) ✓ -</p>	<p>31) ✓ + ✓ + ✓ ± ✓ ± ✓ ± + - ✓ ± ✓ - ✓ - ✓ - - + ✓ - - 0 - - 0 -</p> <p>40) - 0 -</p> <p>71) ✓ - ✓ - - ✓ - ✓ + ✓ - - - + ✓ - - 0 - - 0 - - 0 - ✓ - ✓ -</p> <p>79) ✓ - ✓ -</p>
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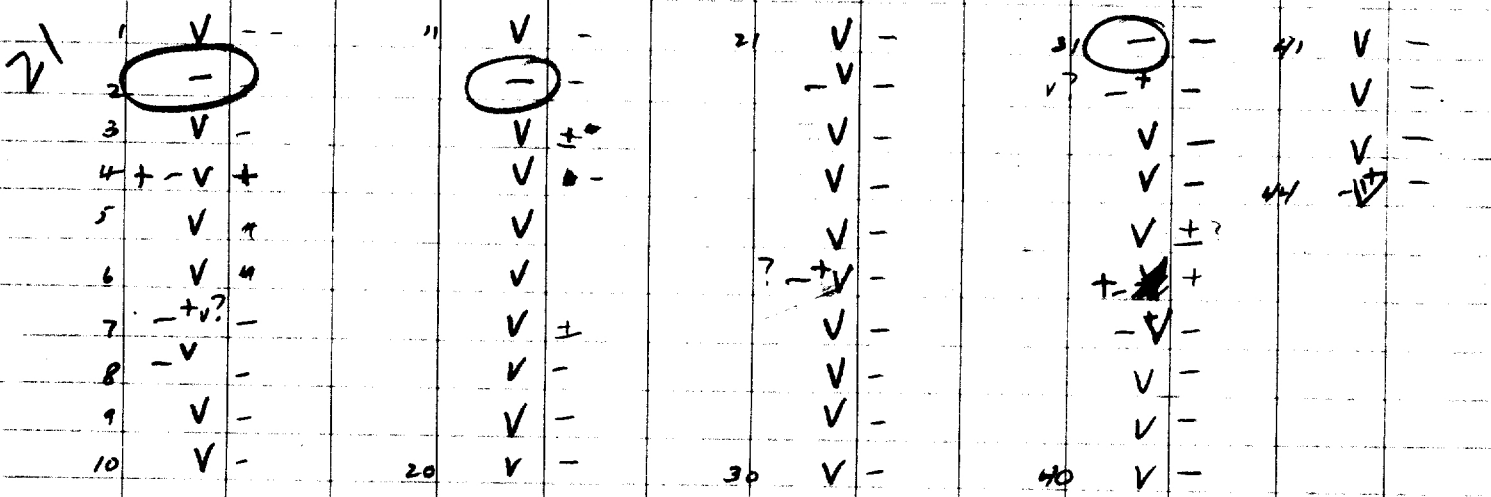
* * 4 generally very tiny colonies ° poor growth on EMS

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

A



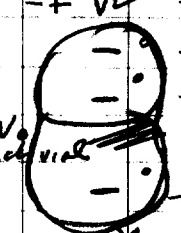
B



22
C

1	V	-
2	+ - V	+
3	V	-
4	V	-
5	V	-
6	V	-
7	V	±
8	V	-
9	V	-
10	V	-
41	V	-
42	V	-
43	-+V	-
44	V	-
45	+V	-
46	+V	-
47	V	-
48	V	-
49	-+V	→ -
50	V	±

"	V	-
	V	-
	-	-
	V	-
	+ -	+
	V	-
	V	-
	V	-
	V	-
20	V	-
51	V	±
	V	-
	V	-
	-+V	-
	V	-
	V	-
	V	-
	V	±
	V	-
60	V	-

21	V	-
	V	-
	+ - V	-
		-
	-+ (V)	-
	V	-
30	V	-
61	V	+
	V	-
	V	±
	V	-
	V	-
	V	-
67	V	-
68	V	-

31	V	-
	-V	-
	V	-
	V	-
	- (V)	-
	V	-
	V	-
	V	-
	+ - V	↑ -
40	V	±

Almost all plates predominantly - unless noted
 ° postgrowth on EMS lac

L

January 18, 1950.

A). Check possible spurious variegated (from EMS lac to EMS lac)

A17, A18, A55, -

B7, 32, 26

C15,

A

B

A from EMS
B from ^{or} take

A17	1	lacV	-V
A18	2	lacV	+,-
A55	3	lacV	lacV +-v?
B7	4	-V?	# -v?
B32	5	lacV	-V
B36	6	lacV	+,-
C15	7	lacV	+ -V

] only doubtful.

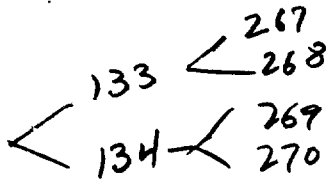
c) Segregants

✓ A10	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 lact each from 20 - [115-118] isolated and
tested:
each was lact+Mal+V₁^R 20-115: TLB, lact+Mal+V₁^R

2 from 5-223 lact+Mal+V₁^R 5-223+: TLB, lact+Mal+V₁^R

(12)



Tests on H206 agerants isolated
by M.R. Zelle

661b.

January 20, 1950.

		Lac	Mal	TS	Nutr.	
1	5-224	-	-	S	M	5-223 + colonies L+M+V ^R .
2	6-25	+	+	R	TLB ₁	
3	6-88	-	-	S	Lac v	isolated. impure? (Lac+, Mal+)
4	7-116	-	+	R	TLB ₁	
5	7-124	-	-	S	Lac v	isolated impure (Lac+)
6	12-269	-	-	S	M] slow growth
7	12-270	-	-	S	M	
8	12-209	-	-	S	M	
9	22-75	-	-	S	Lac v 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 ₁ ✓ TLB ₁	Retest!
15	-392	-	+	R	TLB ₁	
16	-393	-	+	R	TLB ₁	
17	-394	-	+	R	TLB ₁] clone 48
18	-783	-	+	R	TLB ₁	
19	-784	-	+	R	TLB ₁ ✓ TLB ₁	
20	21-98	-	+	S	B ₁	
21	-60	-	+	S	B ₁	
22	251	-	-	S	Lac v isolated	impure (Lac+, Mal+, V^R)
					from EMS Lac	Rare + seen in streak.

Retests possible impure cultures on EMS Lac.

3/21

A page must be missing here but data seem to be from the cores W178 x W1272, with lacu omitted! See 655.

662
b.

See later 662

lac+ (not v) from struck plates, picked to water and checked
another EMB.

	T6	TS	bal	AR	MHC	Xyl	STR	MAL	T6	TS	bal	AR	MHC	Xyl	STR	MAL
1		S	+	+	+	+	+	+	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	-	-	-	-	-	-	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	+	+	+	+	+	+	S		-	+	+	+	+	+
22		S	-	-	-	-	-	-	S		-	+	+	+	+	+
23		R	-	-	-	-	-	-	S		+	-	-	+	+	+
24		S	+	+	+	+	+	+	S		+	+	+	+	+	+
25		S	+	+	+	+	+	+	S		-	+	+	+	+	+
26		S	+	+	+	+	+	+	S		-	+	+	+	+	+
27	S	S	-	-	+	+	-	-	S		-	+	+	+	+	+
28		S	-	-	-	-	-	-	S		-	-	-	+	-	-
29		R	+	+	+	+	+	+	S		+	+	+	+	+	+
30		S	-	-	-	-	-	-	S		-	+	+	+	+	+
31		S	-	-	-	-	-	-	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	+	+	+	+	+	+	S		+	+	-	+	-	-

Repeat →

80

51

61

71

3

661:

MPL

42
43
12
9
41
27
60
76

+
+
+
very slow +
very slow +
very slow +
" "
" "

} score as - or +?
source of modifier?

Gal 24 intermediate + scores -
Kyl 79 pure +.

81
82
83
84
85
86
87
88
89
90

T6	T5	bal	Ar	Mtl	Xyl	Stl	Mal	T6	T5	bal	Ar	Mtl	Xyl	Stl	Mal
	S	+	+	+	+	+	+	S	+	+	+	+	+	+	+
	S	-	-	-	-	-	-	S	+	+	+	+	+	+	+
	S	-	-	-	+	-	+	S	+	+	+	+	+	+	+
	S	-	-	-	-	-	-	S	+	+	+	+	+	+	+
	S	-	-	-	-	-	-	S	+	+	+	+	+	+	+
	S	-	-	-	-	-	-	S	+	+	+	+	+	+	+
	S	-	-	-	-	-	-	S	+	+	+	+	+	+	+
	S	-	-	-	+	-	-	S	-	-	-	-	-	-	-
	S	-	-	-	-	-	-	S	-	-	-	-	-	-	-
	S	+	+	+	+	+	+	S	-	-	-	-	-	-	-
	S	+	+	-	-	-	-	S	-	-	-	-	-	-	-
	S	+	+	+	+	+	+	S	-	-	-	-	-	-	-

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

Summary of bac+ data.

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

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

V_6^R	89	All parental:	33
Xyl	57	Ar	44 (+2?)
Mtl	49	Mal	45
bal	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 = Xyl		A - BCD+
A = Mtl		
A = bal		
A = Stl		

Stl Linkage: discordances with: Ar linkage

Mal	7
bal	16
Ar	3*
Xyl	15
Mtl	13

1
2
3
4
5
6
7
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39
40

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

penicillin
penicillin

* order marker confused, especially in 21-30

- 121
- 122
- 123
- 124
- 125
- 126
- 127
- 128
- 129
- 130
- 131
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- 136
- 137
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- 155
- 156
- 157
- 158
- 159
- 160

T5T6

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

GAL

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

AR

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

NFL

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

XVL

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

STL

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

MAH

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

LAC

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

TI

U V W X Y Z A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

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

are possible tac v.
 struck out on EMPB & EMStac
 as 662-b No...

Linkage relations of STL etc.

662c

Jan. 17, 1952 ff.

~~W1262~~ W1262 x W1269 on T(0); 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 lacEMS, and check for segregation on other sugars.

	lac	Xyl	Mtl	Stl	Gal	Mal
1		<u>+</u>	-	-	<u>v</u>	-
2		+ v-	-	-	+	-
3		-	-	-	-	v?
4		-	-	-	-	v?
5		v+	- v?	-	-	v?
6		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.

"b:"

	lac	Save as
1	v	13
2	v	14
3	+	
4	+	
5	+	
6	-	
7	+	
8	+	
9	v	15
10	v	16

January 20, 1950.

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

P20. Suspend H168 from EMSlac. to ca 10^9 . Dilute in saline to 10^{-5} .

~~Spread~~ irradiate 5 ml in Petri dish ^{10 ml} under lamp and spread on EMSlac.

Suspension apparently inviable: no colonies from controls!

P22. 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	74	1	5] 79 : 10
0	10^{-7}	54				
5 s.	10^{-7}	18	15	5	5] 3 : 26
5 s.	10^{-7}	11				
10 s.	"	0		7	9	
10 s.	"	0				
20		0				

↓

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

Irradiation of H-225.

January 23, 1950.

SE UV lamp at 20 cm. Diluted coli shaken 0, 20, 30 sec.

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

Survival = ca $11/411 = \text{ca } 2\frac{1}{2}\%$.

A	+	-	Σ
71	8	13	92
78	7	12	97
88	9	17	114
95	8	5	108
<hr/>	<hr/>	<hr/>	<hr/>
332	32	47	411

C.		1	
		0	
	1	4	
	3	2	
	<hr/>	<hr/>	
0	4	7	11.

D. Blanks.

Initial T5 and T1 scores are
thoroughly satisfactory!

Recheck \bar{c} control stocks + issues
before repeating

Jan 20, 1950.

"c"

- "B" W1155 x W1258 } Backgrounds rather heavy. A few + colonies observed.
 "C" W1268 x W1262 }
 D W1268 x W1178.
 L+M+V^S L-M-V^R

B. Pick + colonies which stand out slightly over background. Spot suspensions on T(0) agar to purify. Streak these out on EMS Sucr. All Sucr - V^S, V^S, V^S but grow well on EMS Sucr with W1258!

C. Pick colonies (rather small) from T(0) directly to EMS lac. N.G.!

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

Pick ~~the~~ 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 EMS vs. T1.

95 lac+ : All V^S. 94 Mal+ 1 Mal-

5 lac- : 5 Mal+ : 3 V^S 2 V^R

∴ All lac+ are V^S; Some lac- are V^S; some V^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 (overcompensation for "dilute" suspensions).

Total counts (1/4 plate)

A	90
D	153

$\therefore 10 \text{ sec} = \text{ca } 85\% \text{ killing.}$

Relative counts

A.

v	+	-
39	2	8
41	6?	5

ca 80% v.
< 15% -

80 8 < 13

PC

6 2 6

Suspensions ca 50% -

D.

28 ~~50~~ 24 50

Duplex prototrophs

January 23, 1950.

"2" W1269 (478/6) x W1272 (1262/"6") on a variety of EMS media:
 EMSB, : Mal + observed but not clearly scoreable. Yields very high.
 Mal_s not readily detected at 48h. s = scored.

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

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

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

Mal 5 plates. Many small+. 2? ^{1,2} Mal_s. 91+ 61- [2 plates].

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

+B, 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 Mal_s
 probably are coincident colonies.

14 possible Mal_s picked and streaked on EMSB Mal.

Mal_s +, -
 compo-
 scored as.

Mal	+	-	Xyl	+	-
1	+	+	+	+	+
2	-	-	-	-	-
3	-	-	-	-	-
4	+	-	*	-	-
5	+	-	*	-	-
6	+	+	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	+	+	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	+	+	-	-	-

Very definite correlation for lac of +/- components.
 (Two exceptions *)

n.g.

Mel	Xyl	lac	Gal	MHL	stl	H ₂	T5
+ -	- -	++	- -	- -	- -	- -	S S
+ -	++	- -	- -	++	- -	- -	R R
+ -	++	- -	- -	- -	- -	- -	B B
+ -	- -	- -	- -	- -	- -	- -	S S
++	++	++	- -	- -	- -	- -	S S
+ -	++	+ -	- -	- -	+	- -	S R
+ -	+ -	++	- -	± -	- -	- -	S S
+ -	- -	- -	- -	- -	- -	- +	S S
+ -	- -	- -	+ -	- -	- +	+ -	S S
+ -	+ -	++	+ -	± -	- -	+ -	S S
+ -	- -	- -	- -	- -	- -	- -	R R
+ -	- -	- -	- -	- -	- -	- -	R R
+ -	++	++	- -	++	++	++	S S
+ -	+ -	- -	- -	- -	- -	- -	S S
+ -	- -	- -	- -	± ±	- -	- -	R R
- -	++	++	- -	- -	- -	- -	S R
+ -	+ -	+ -	+ -	± ±	- -	+ -	R R
+ -	+ -	- -	- -	- -	+	- -	S S
- -	++	- -	- -	± ±	- -	- -	S S
- +	- -	- -	- -	± ±	- -	- -	S S

no. of plaques!

January 26, 1950.

Streakout 8 Lac^v of H226, on EMBS Mal, Lac.

- a) test reduced Mal (+ or -) on lac
 b) " " Lac^v ~~on~~ on Mal. [No Lac⁺ noted].

A number of peculiar variegated colonies were noted on Mal EMBS.
 Pick and number; description. Streak out on Mal, Lac.

- c) 1.  2.  3.  4. 

- A27. a) Mal⁻. 72 tested on lac All apparently pure lac⁻.
 a ~~Mal⁺~~ Mal⁺ 84 tested. 80 definitely mixed. 4 possibly lac^v or containing lac⁺. (These may have been Mal^v misrecorded as +). Streak these out as a [1-4].
 a ~~Mal^v~~ Mal^v 24; tested 22 lac^v 2 apparently pure lac⁻; a [5-6].

~~[4-10]~~

Also, a considerable number of "pure" Mal⁺ and - colonies were pooled, and streaked out on EMBS lac. Among several hundred lac⁻ colonies there were two identifiable lac^v or +, in addition to numerous papillae in the thick streak. Attempt resolution of lac^v. a [7-8].

- b]. 52 lac^v tested on Mal: all + or contain +.

c].

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

January 27, 1950.

- J. 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. Strain 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, -
 4. Mal v, Mal+.
 5. Pure lac- } largely segregated (Mal+, -) → +, - only.
 6. Pure lac- } maybe some Mal v. [Attempt re-isolation I.]
 7. Mal-, some +, v?
 8. Mal+, some -, v?
- $\left. \begin{array}{l} \text{Mal v, -} \\ \text{lac v; lac-} \end{array} \right\}$
- $\left. \begin{array}{l} \text{lac v, -} \\ \text{for Mal purity.} \end{array} \right\}$ Reisolate lac v and test

c) 1-4 lac-, lac v. Test isolated lac v for Mal purity.

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

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

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

1-3 = a1
 4-9 = a2
 10 = a7
 11-16 = a8.

Reisolate lac v from these

These may be Mal pure. 668d. ① & ②

c) 8 each. 1 all impure (Mal+, -).
 2 " " } All were Mal v, not Mal+.

1/31/50.

EMS Lac

1. lacv; Lac-

Mal-

H227.

2. lacv; Lac- mostly Mal+, occ. Mal-, simple Malv
 keeps 4 lacv; all were Mal+; Malv.

pick H227 on EMS lacv, EMS Mal.

P31. Pick 200 lacv from 671A2. Breeds very sparsely on EMS Mal.

1-100 No Mal-. Mostly Mal+, -. 2 maybe pure Mal+ : rest of
 as 668d: 3-4. 3: No lacv (exc. papillo); 4: Mal+/-; lacv

101-200 No Mal-. Mostly Mal+ = detectable -.

201-240 All Mal+/-

Irradiation of H225 and H226

N.G. Too dilute

January 25, 1950.

Suspend H-225 from EMS lac in H₂O, 10 ml, to opt. dens. ca 120
 Dilute 1/100 for irradiation. 5 ml sample in Petri dish (10cm).

Remove 1 ml to 10 ml for each interval. 20 cm. distance.

0, 5, 10, 20 sec. This leaves suspension at 10⁻³, or an estimated 5 x 10⁵

A, B, C, D. before irradiation.

Dilutions:

- | | |
|---|-------|
| 1 | .1 ml |
| 2 | .03 |
| 3 | .01 |
| 4 | .003 |
| 5 | .001 |
| 6 | .0003 |
| 7 | .0001 |

1/10
1/10
1/10

Empty
10⁻¹⁰

H226 O.D. 090

E 0

F 20

A. 4
5
6
7

5, 4
1, 0
0, 0
0, 0

∴ initial count (OD = 120)
 was 3 x 10⁻⁶ ml = 5 colonies = ca 10⁶/ml.
 (viable)

B. 6

0, 1

C 3
4
5
6

12
7, 5
0, 1
0, 1

∴ count = 12/10⁵ = ca 10⁶/ml.
 no killing detected.

D. 01
3

0
0

E 10⁻⁶
10⁻⁷

4, 4, 3
0, 1, 1

F 10⁻⁴

0, 0, 0

Test $\text{lac}^+ [\text{Lac}, \times \text{Lac}_y]$ as diploid
mediate Seligman's lac^+ .

669

January 27, 1950.

Reupend "pure" colony of 652-2 in H_2O . Spread drops on EM13lac.
Expose to high pressure UV 6, 7, 8, 9 sec.

ca 1000 survivors in total. No Lac^- or Lac_y noted

conclude that 652-2 is probably not a stable diploid, but
a typical recombination of lac_1^- with lac_y^- .

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

streaked out on EM13lac: all +. Reupend + colonies in water, spread
on EM13lac and subject to 8 sec UV (high pressure lamp).

4 plates; ca 500 colonies. All lac^+ .

Effect of medium on segregation.

670

January 27, 1950.

FMS BYTLB, apparently does not permit hac v colonies of H168.
Check effect of various supplements.

In this run, hac v was observed on BYTLB.

Irradiation of H225-226

671

January 29, 1950

- a) H226 susp. in 10ml 0.D. 275 ca 2×10^6
 b) H225 " " " 0.D. 380. ca 3×10^6
 Count in Petroff-Hausser. ca 3×10^7

Assumed dilute ~~#/10~~ to estimated 10^5 each a) .5/10 b) .33/10
 without con. record \log_{10} est. number

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

January 20, 1950.

A. W226 No uv.
"2"

+v	-	Σ	%v
457	73	530	
432	96	528	
656	84	740	
593	107	695	
<hr/>			
2138	355	2493	86
388	46	434	

counted readily under
kino.

*

B. W226 5 secs.
2 (maybe count.)

25	382	407	
27	401	428	
<hr/>			
52	783	835	06
24		← 536	

! "1 uncount."

C 2

0	222	010000
1	338	
0	8	

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

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

D	ouv	+	-	✓	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	→ 26
		27	57	34	118	
		51 94		55	200	22
		145				

No absolute increase as ^{relative} actual count is one half that recorded

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

F					
	2	7	15	2	24
		11	22	2	35
	"20"	6	7	1	14
		9	11	2	22
		33	55	7	95
	"20"	2	1	1	
		1	2	0	

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

2/3/50.

Analysis

672

a) Initial assays were
After 20 hours, *in vitro* in water

A
 6×10^7
 2×10^7

B
 6×10^7
 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 detection.
- d) 5 mins. at 60° already kills most cells. Use at high conc. and plate various dilutions.

Irradiation of H226 - H225.

672-A

February 2, 1950.

a) Pl. Assay suspens.

		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 sec. UV. 20cm. 2ml/10cm Petri dishes

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

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

4/3 3PM	A O	+	-	v	AU/plate.
	1	8	19	36	18 19 41
	2	8	13	36	8 14 29
	3	7	24	50	% .
	4	8	56	42	
	5	8	8	42	
		10	7	31	
		41	71	145	

A 2

counted
3PM
(same as before)

	+	-	✓	Σ	%sur.
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	61	
	13	23	64		100

	+	-	✓	Σ	%sur.
A2	13	27	19	59	
	12	29	7	48	
	11	14	5	30	
	13	28	20	61	
	6	36	17	59	
Σ	55	134	68	257	
Aver.	11	27	13	51	
	22	53	25		84

	+	-	✓	Σ	%sur.
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.

	+	=	✓	-
D.	0			7
				23
Sm.	0			0
10)	0.			

Note: assays should be 10 x 80 (av. 5.5)

Lac series.
B0

+ = V	-	
4	0	
2	4	
6	1	
7	0	
0	2	
2	4	
<hr/>		
22	11	33
3.67	1.82	5.5

Med:

-	+	V
0	1	3
0	3	2
0	2	1
<hr/>		
	6	6 / 12
	2	2 4.0

B2

5	7	
0	7	
0	10	
2	4	
1	4	
1	6	
<hr/>		
4	31	35
0.8	6.2	7.0

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

B5

0	5	
0	4	
0	"	
0	4	
0	3	
<hr/>		
0	27	5.4

2	3	0
0	2	1
1	3	1 0
1	0	0
<hr/>		
4	8	2 / 14
1	2	.5 3.5

This series highly unsatisfactory owing to killing!

40 sec.
50 sec

0	26
0	0

Some original was ca. $\frac{5.5}{3} \times 10^5 = 2 \times 10^5 / .142$
The 40 sec survival was ca 10^{-4} .

Spontaneous heterozygotes
continued from 19652.

February 2, 1950

A)

W67 x W1272.

[Strains out mixture on ^{OX}EMB lac]

EMB lac

ca 2500 prototrophs

2 lac+ strains on EMB, EMB lac; EMB Mal.

2 additional A5.

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

maybe lac⁺ or very stable lac⁺. = 673-1

clear Lac⁺.
very weak +!

= 673-2

B

2/5/50.

W67 x W1177 EMB lac 21 x 50/ = ca 1200 prototrophs.

8 lac+ colonies, strains out on EMB lac; EMB lac; EMB Mal.

PROVISIONAL

1. No. to (-)	Lac	Mal
1	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 bushes.
Compare with material from 675.

39 +, apparently pure; 1-

B

Do. from c40 28 colonies brushed. 22 were pure Mal+
4 " " Mal-

2 were pure Mal-, i Mal+. Pick and restreak
→ MalEMB was badly
contaminated.

Save 677 plates!

From 677 A_x and B_x, pick isolated ~~lac~~ lac- colonies and brush
on MalEMB.

41 pure Mal-

18 "pure" Mal+

17 mixed Mal+/- : ? Restreak on MalEMB; lacEMS to resolve.

76 total.

Restreaked cultures:

	Mal	lacEMS	EMB
15	+ -	pl. -	-
16	+ -	pl. +	
17	+ -	pl. +	

Mal+ - check lacEMS
and transfer to lacEMB.

	Mal	lacEMS → EMB
1	++	
2	++ -	⊙
3	++	
4	+ -	○
5	+ -	○
6	++	
7	+ -	○
8	++	⊙ -
9	++	
10	++	
11	++	
12	+ -	○
13	+ -	-
14	++	

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

3 are lac⁺
2 are pure lac-

Heat and uv killing of H226.

Febr. 3, 1958.

* Make up from EMS lac to ^{optimal} density of 420_{4200} . H226 only.

Assume total 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 20cm.

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

Apparent proportion of lac - rather high in current suspension.
 Start a fresh restreaking

	+	V	-		+	V	-
C:	1	9	4		1	2	10
lac	0	7	4	Mal	5	0	0
wipings from each of ↑	4	5	4		0	0	0
Cx	0	28	5	1/53			
		0	21	1			
		20	9		4	2	1
		10	10		5	7	1
		2	8		12	3	1
		20	7		2	0	!
wp.		06	45751				
		2					

	+	v	-
D # 4	7	123	88
# 5	0	6	3

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

steals out apparent lac+.
 1-3 from C
 4-9 from D

all are clearly lac+. lac+ owing to spreading habit.