Preparation of diploid inocula

February 4, 1950

D(@) liquid + 0.1% lactose

1-4 single colonies from old EMS stac

5,6 from broad streaks. Incubate 5 aeration or shaking.

Streak out initially and after growth. Estimate % lac⁻:

	Initial	20 h.
1	25	85
2	<10	90
3	40	90
4	80	>90
5	50	>90
6	40	>90

This appears to be a satisfactory method for preparing diploid inocula!

Use (4) for irradiation study in hopes that high proportion of lac⁻ is maintained

Irradiation of H226

~~676~~ 677

February 5, 1950.

Use liquid culture 676-4, ca 20 hours. O.D. 400

Assume titer of [redacted] 10^9, and work for 100 colonies/plate.

For safety, also plate at 10^{-6} and 10^{-5}.

Dilute to [redacted] 10^{-4}. Irradiate 3 ml 2 secs 20 cm low pressure uv.

A) Take .1 ml samples Crowded.

B) Dilute to ~~5 ml~~ 10^{-5} .1 ml samples

C) 10^{-6} .1 ml samples.

D) Expose* 1 ml original sample to 57°C. 10 mins.

(.7 ml) Dilute 1:10 in cold H_2O and plate out at various dilutions [redacted] plates sterile.

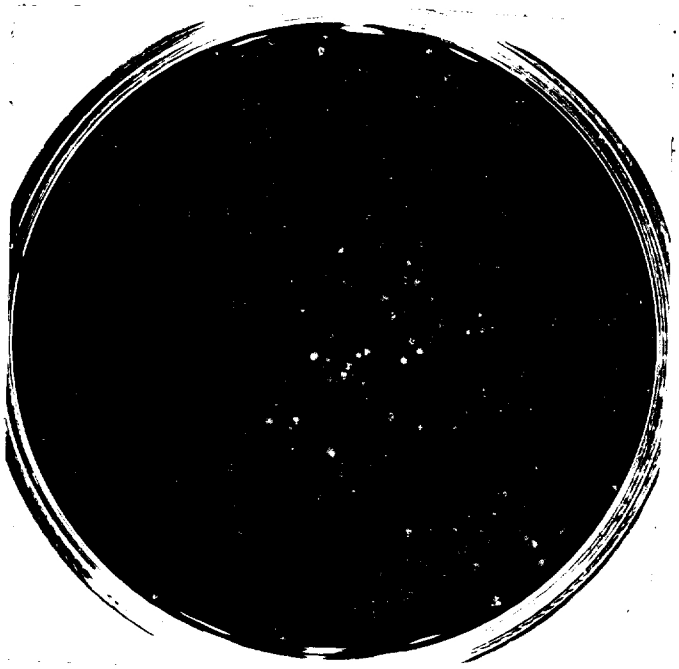
E) Plate original sample at all dilutions from 8 — 3.

	V	—	
10^{-8}	4	0	
10^{-7}	48	4	
10^{-6}	305	24	
	357	28	385.

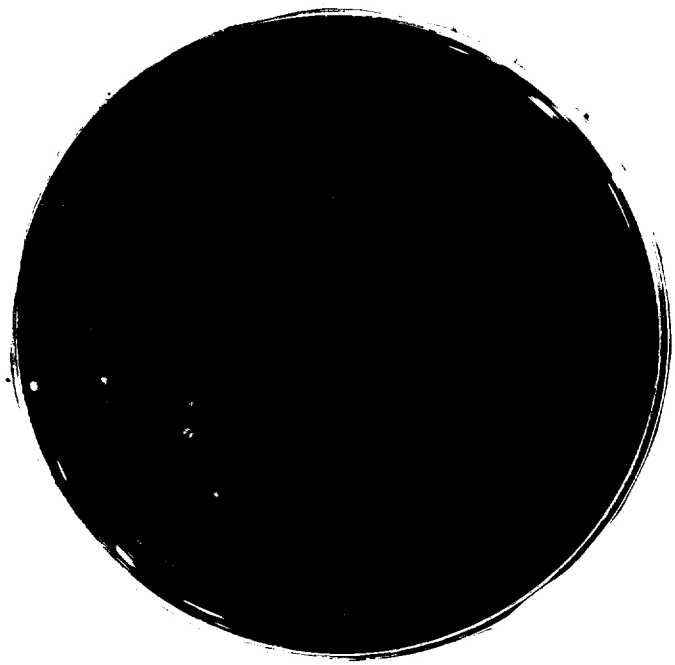
5×10^8 = titer

% ?.

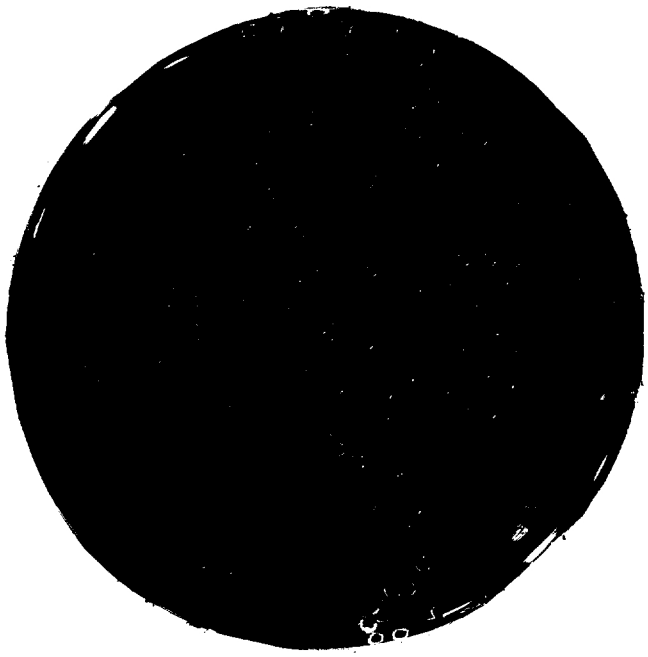
A control



A uv



B - uv



B control

Count B series.

		V	—	Σ
B (no UV)	1	170	16	
	2	174	10	
Lac	3	200	16	
	4	197	14	
	wip from 4	2	0	
		743	56	
	per ml	1857	140	1997
B_x (UV 2 sec.)		6	63	
	(wip)	12	64	
		0	2	
		18	127	
	per ml	90	635	725

Photograph sample plates of B and A, (o, x.)

Survival = $\frac{725}{1997}$ = 36%

Shift lac u from 93 ± 12%.

c no uv.		17	3	
		17	1	
		9	2	
		12	5	
		19	2	
	wip:	1	0	
		75	13	
	per ml.	150	26	176
		10	65	75
i ex		0	9	
		1	3	
		2	5	
		1	9	
		4	26	

EMB Mal counts. Most Mal plates were contaminated. Some, however, were on each in clear batch.

B: not readily scored; however, mostly Mal+ or Mal v.

B_x: Plates contaminated, but fairly numerous sectored colonies:

Mal+($\frac{+}{v}$)	Mal−	Mal v
34	50	20

The frequency of Mal v seems higher than of lac v, suggesting some diploids. See 674.

In B, a fair proportion of crescenti colonies was seen (+) (−) etc.

(on E5) a single colony was noted that, by its appearance, might be a lac+ recombinant. Streak out for check. —

Lac+ pure, Mal+, Xyl+, [redacted] Mtl+. Keep on agar & Cent.

2/10/50.

A number of colonies previously scored as lac −; lift out on desk top several days, now show central papillae. Pick and streak out as (plate photographed as 677-UV B). EMB lac.

v	−	central +
8	29	23

In general, they seem to give typical lac x!

To avoid any confusion, a special uv experiment is called for!

Treatment of H226 with Mustard (HN2)

Febr. 6, 1950.

H226, suspension 676-4 (24 h. in refrig.)

A) Control: Titrate out from buffer below.

B) Add 2 ml suspension to ~~1 ml buffer~~ ~~1 ml KH buffer pH 7.0~~ 10 ml D(lac) [as buffer]. Add 10 mg HN2. After 5 mins., dilute 1:10 in Y2 plus to inactivate HN2. Titrate. (Initial 10^{-2}) Record in terms of initial ~~dilution~~ suspension.

		V, +	—	
A)	10^{-7}	56	2	
	10^{-6}	420	22	/ 442
B	10^{-6}	46	206	/ 252

The survivors here appear generally to be lac- .

Treatment of M226 with chemicals

February 6, 1950.

Use suspensions 676-4 and 676-4A (o.d. = 750) 676-4A is loop transfer from D(lac) to D(lac). 1/100 mod = 100

A) Assay 4

B) Assay 4A

C) Dilute 4 1:5 in 6% sodium desoxycholate. 37°. 5:20 - 7:50 P.M. Titrate

D) Dilute 4A 1:5 " " " "

E) Dilute 4A 1:10 in D([redacted]) Add 10mg HN2, hydrochloride, (Room temperature) 10 min. dilute 1:10 in Penassay 1:45 exposure

F) Dilute 4A 2:10 in 1.2% Methyl Green. 8:10 PM - 9:55 PM

G) " 4 2:10 in 1.2% " " 37°.

Record dilutions subsequent to treatments. E as original, cf. B.

H. Suspension 4. Heat to 65° 5 mins..

I. " " " 10 mins.

J. " " " 20 mins.

F, G. appear all dead!

UV; HN2; kill by a nuclear mechanism.

Heat; dock; " " a non-nuclear " . ??

A.	dil	V	—	
Assay	6	393	45	
	7	33	2	
B				
assay	7	57	0	
	6	n.c.	26	
C	6	72	5	
doca.				
D	~~5~~	311	29	
doca.				
	6	9	25	
E	5	37	212 ~~249~~	Reincubate!
Mustard	4	3	n.c.	v much more numerous after after incubation
F,G	1,...	sterile		
H	4	200	22	
heat		73	5	
I	2	~~9~~	~~4~~	
J	1	0	0	

STANDARD FORM NO. 64

Office Memorandum • UNITED STATES GOVERNMENT

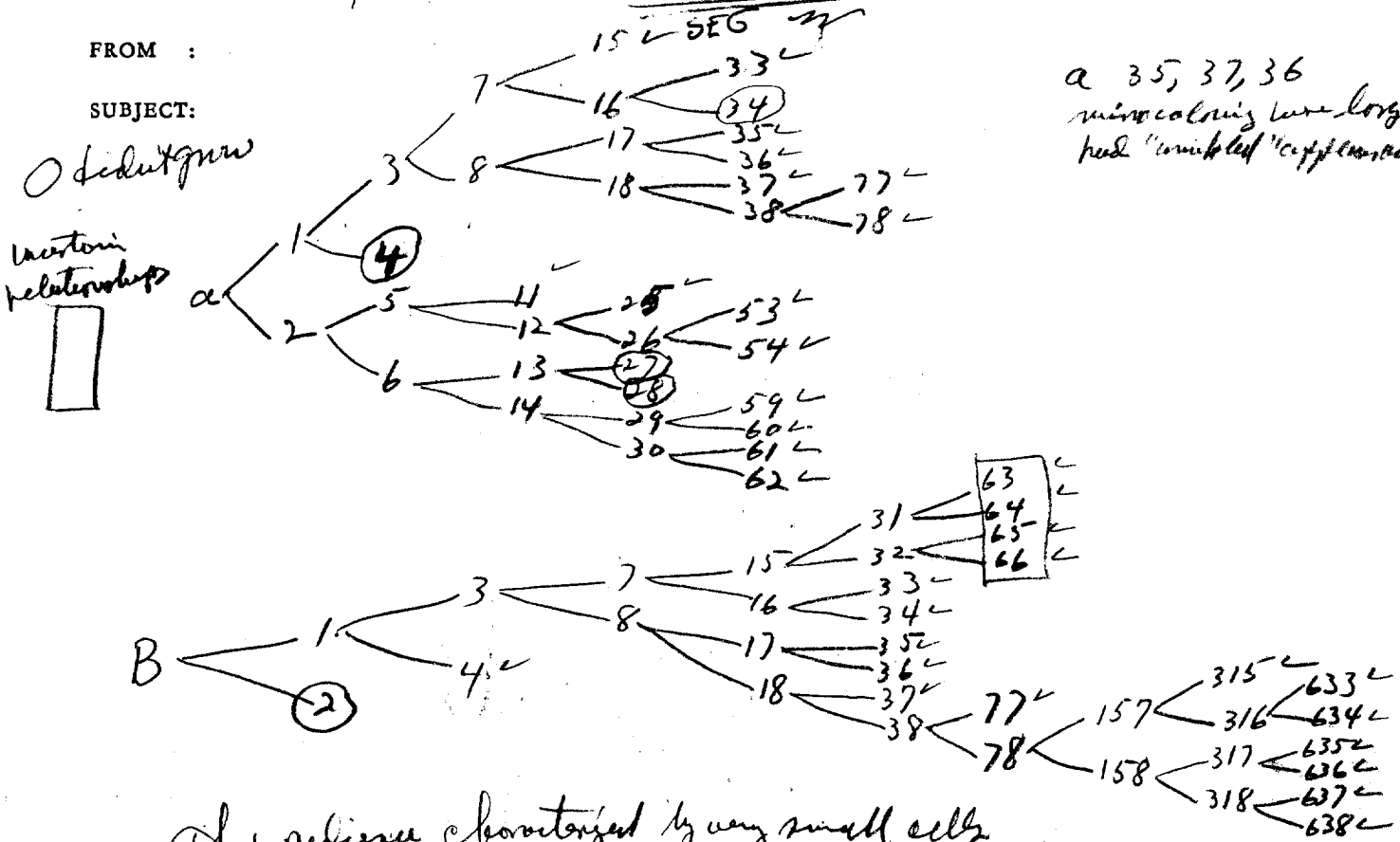
TO : # 226 DATE: 2-5-50

FROM :

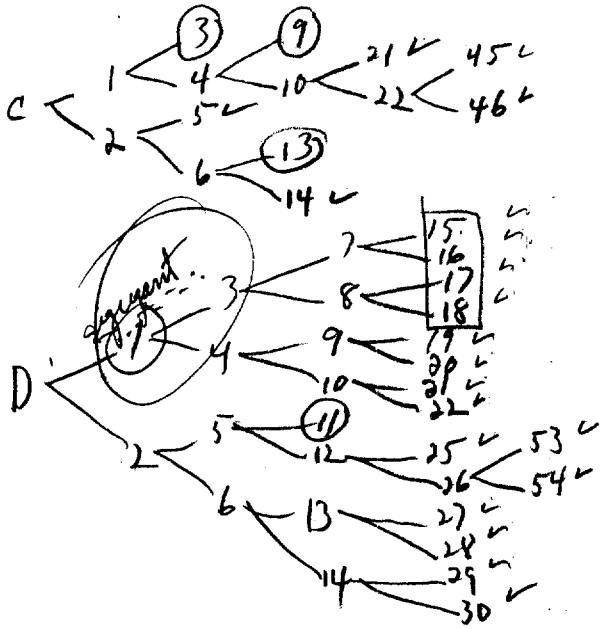
SUBJECT:

O dedutgrow

a 35, 37, 36 microcolonies were longer, had "crinkled" appearance,



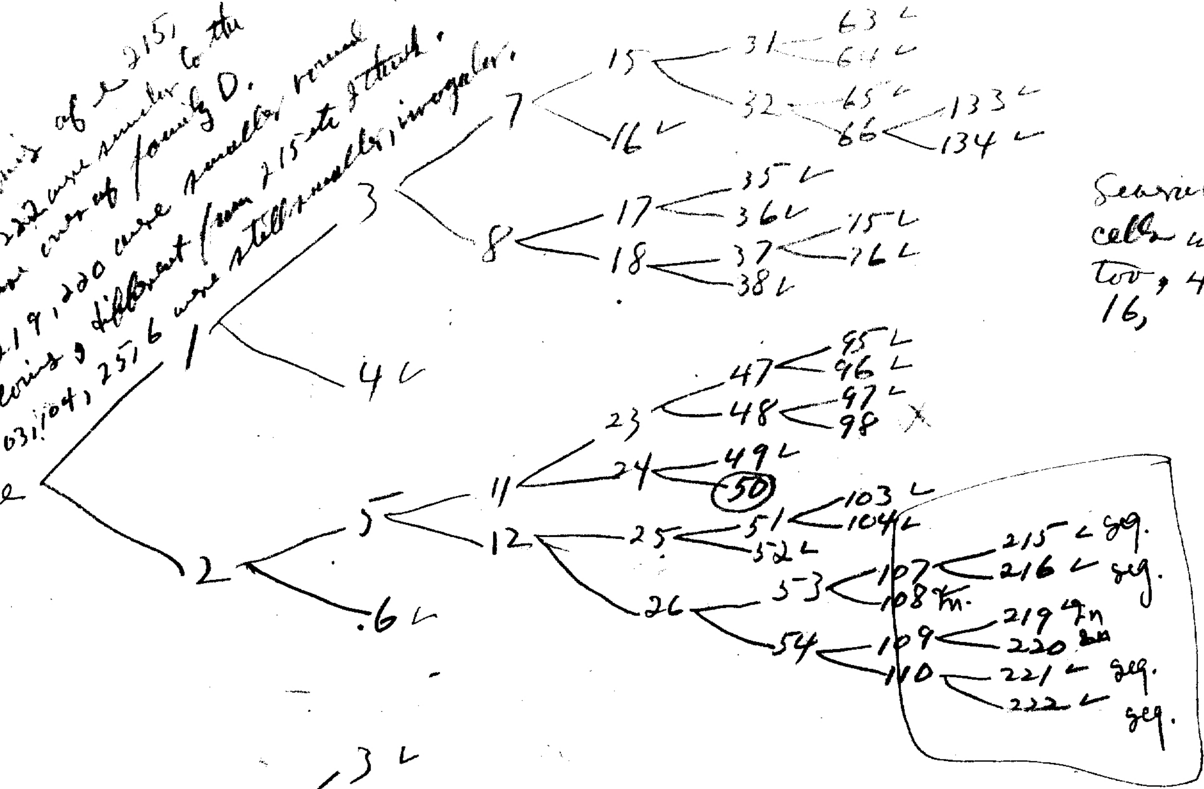
This pedigree characterized by very small cells except #4 which was filamentous + later divided. #2 became a 8-10 μ filament—stopped there.



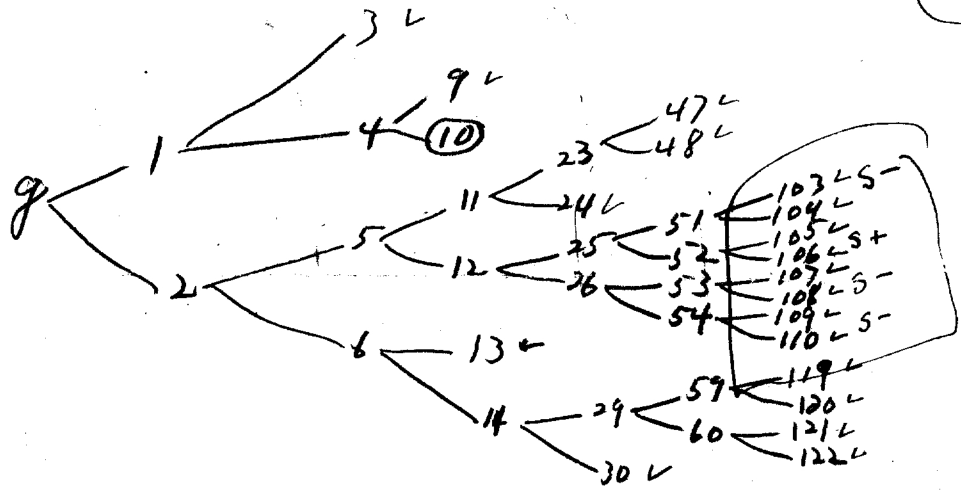
Most cells in this pedigree formed filaments, then divided somewhat irregularly.

Microcolonies of this family were rather faster growing, denser, harder to get into pipettes as if they were mucoid.

Microcolonies of e 215, 216, 221, 222 were similar to the larger dense ones of family D. e 108, 219, 220 were smaller, round colonies & different from 215 etc & thick. e 103, 104, 25, 26 were still smaller, irregular.



Several filamentous cells in this family too, 4, 6, 24, 49, 16,



Filament: 3, 9, 10, 24,

Cells descending from 12 were very small.

Microcolonies of 105 + 106 were round + smaller than 103, 104 and 107–110. 107–110 were the dense ones like the D family. 105 + 106 microcolonies were similar to e 108, 219, 220.

Zille: single cell pedigrees on H226

February 5, 1950

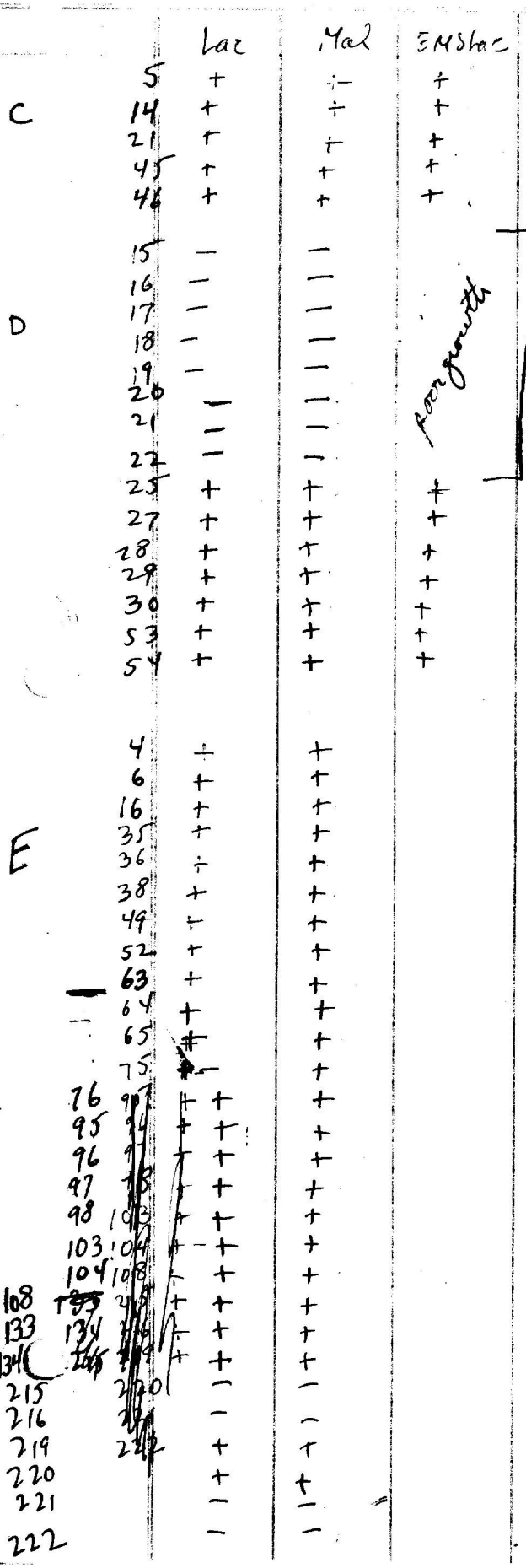
		Lac	Mal	Lac EMS
A.	11	+	+	+
	15	—	—	—
	25	+	+	+
	33	+	+	+
	35	+	+	+
	36	+	+	+
	37	+	+	+
	53	+	+	+
	54	+	+	+
	59	+	+	+
	60	+	+	+
	61	+	+	+
	62	+	+	+
	77	+	+	+
	78	+	+	+

B

4
33
34
35
36
37
63
64
65
68
77
315
633
634
635
636
637
638

All lac⁻ Mal⁺ n.g. on EMS. ∴ started with a segregant

14
21
45
46


Lac
Mal
EMshac
poor growth
C
D
E
5
14
21
45
46
15
16
17
18
19
20
21
22
25
27
28
29
30
53
54
4
6
16
35
36
38
49
52
63
64
65
75
76
95
96
97
98
103
104
108
133
134
215
216
219
220
221
222

G

	Lac	Mal
3	+	+
9	−+	+
13	+	+
24	−+	+
30	+	+
47	+	+
48	+	+
103	−	−
104	−	−
105	−	+
106	−	+
107	−	−
108	−	−
109	−	−
110	−	−
119	+	+
120	+	+
121	+	+
122	+	+

Test segregants. H226 6802

2/8/50.

G			V1	Lac	Mal	Gal	Ar	Xyl	Mtl	Stl	Nutr.	H226
103	1	R		−	−	+	+	−	−	+	TB1	pure +
104	2	R		−	−	+	+	−	−	+	TB1	m
105	3	R		−	+	+	+	+	+	+	B1	Gal; Ar;
106	4	R		−	+	+	+	+	+	+	B1	Stl
107	5	R		−	−	+	+	−	−	+	TB1	
108	6	R		−	−	+	+	−	−	+	TB1	
109	7	R		−	−	+	+	−	−	+	TLB1	
110	8	R		−	−	+	+	−	−	+	TB1	?

???
NoM−!

Chemical treatments ~~with~~ of H226

Febr. 8, 1950.

Inoculum prepared from EMS colony into D(Lac); incub. 20h.

$o.d._{4200} = 830$

A. Assay

B. Dilute 1/10 in .01% Methyl Green in D(-) 2xS pH — 5xS

C. Dilute 1:5 in 6% Na deoxycholate. "

add H_2O to 1/10 express dilutions as original.

	V	—
A7.	135	12
B6	325	37
C6	>>600	ca 10%

Methyl green: v. little killing
no haploidizations

Not enough killing!
no haploidizations.

Treatment of H226 with chemicals.

Febr. 9, 1950

Use same H226 resp. as 681.

A) Assay

B) Dilute 1:5 in 6% Na desoxycholate 11.45 } 37°
C) Dilute 1/10 in 0.1% Methyl Green/sal 11.45 }
D) " 1/10 in ~~H2~~ saline } [I am probably in error 10x dil. 3

Express as orig. conc.

	v	—
B6	230	16
C2	114	6
D "7" (8) (2 plates)	13.6 (1,2) 3	
	19	3

No appreciable killing in desoxycholate! pH of 6% solution: 8.9!
1:5 bacteria/doca: 7.1
bacteria + saline (ice):

Methyl Green kills by non-nuclear mechanism.

Chemical Treatments of H226

Preliminary data

February 10, 1950.

Add to medium stock of H226 (36h. in 50 ml DL Lac)) 1:10 D(-), plus supplement added. Incubate in tank unless indicated.

		Supp. vol.	Total Vol.
A.	—	0	11.
* B.	Acriflavine .05% dark	0.5	11.5
* C	" " under 4W fluorescent lamp	0.5	11.5
* D.	Pyronin Y .01%	0.1	11.1
E	NaCNO 1%	(NaCNO 5%) 2.5	13.5
F	hydroquinone 1%	(Hq 5%) 2.5	13.5
G	Formaldehyde .04% (=.1% formalin)	1	12.0

6 30

D — in H_2O to prevent ppts which is heavy in — alone.

* 6 30 B & C agglutinated heavy ppt in D.

9 30 1 standard loopful, spread on 1 plate serially.

All but A are sterile

Repeat 2/11/50 under less drastic conditions

11226 chem. treatment

February 10, 1950.

	Same stock as 683 (refrig.)	Supp %	vol.	Total
A	–	–		1 ml.
B	Acriflavine .005 %	1	.05	11.05
C	" " (light)	1	.05	11.05
D	Pyronine Y .001 %	1	.01	11.01
E	NaCNO 0.1%	5	.2	11.2
F	Hydroquinone 0.1%	5	.2	11.2
G	Formaldehyde ~~.01%~~ .01% (=.25% formalin)	#1	.1	11.1

4PM – Mix.

Assay at 5PM. A → D Assays at 10^{-2}; 10^{-4}; 10^{-6}

E–6 6PM.

A	7:	ca 300	90% lacv	
B	6:	ca 100	mostly lacv	} Many colonies ⊕
C	6:	ca 100	" lacv	}
D	4:	ca 200	mostly lacv	
E	6:	>500	lacv	No sign. killing ??
F	Sterile			
G	6	ca 100	80% lac– !	

∴ formalin has same mode of action as UV; mustard. Pyronin; acriflavin do not, but check for ~~latent~~ delayed lethals. Hydroquinone is extremely bactericidal.

UV Killing Curve

2/13/50.

50 cm; 5 ml samples of H226, diluted 1:100. (H226 is growm culture in flask of D(8), refrigerated 2-3 days. (see 683) Initial assay est. ca 3×10^9., After dilution, assume 3×10^7. State dilutions as of 1:100 sample.

	UV	D.I	Count (duplicate)		Survival	ρ S
A	0	5	70; 61	6.5×10^6	1.0	0
B	10	5	28	3×10^6	0.46	.34
C	20	4	144	1.4×10^6	.21	.68
D	30	4	84	$.8 \times 10^6$	.12	.92
E	40	4	25	$.25 \times 10^6$	.038	1.38
F	60	3	176	$.18 \times 10^6$	.028	1.57
G	80	1	22	.000022	3.4×10^{-6}	5.
H	100	1	5	5	$.77 \times 10^{-6}$	
I	120	1	7 - See	7	1.1×10^{-6}	
J	150	1	1 684A.	1	$.15 \times 10^{-6}$	

K Formaldehyde .05% 10 min.

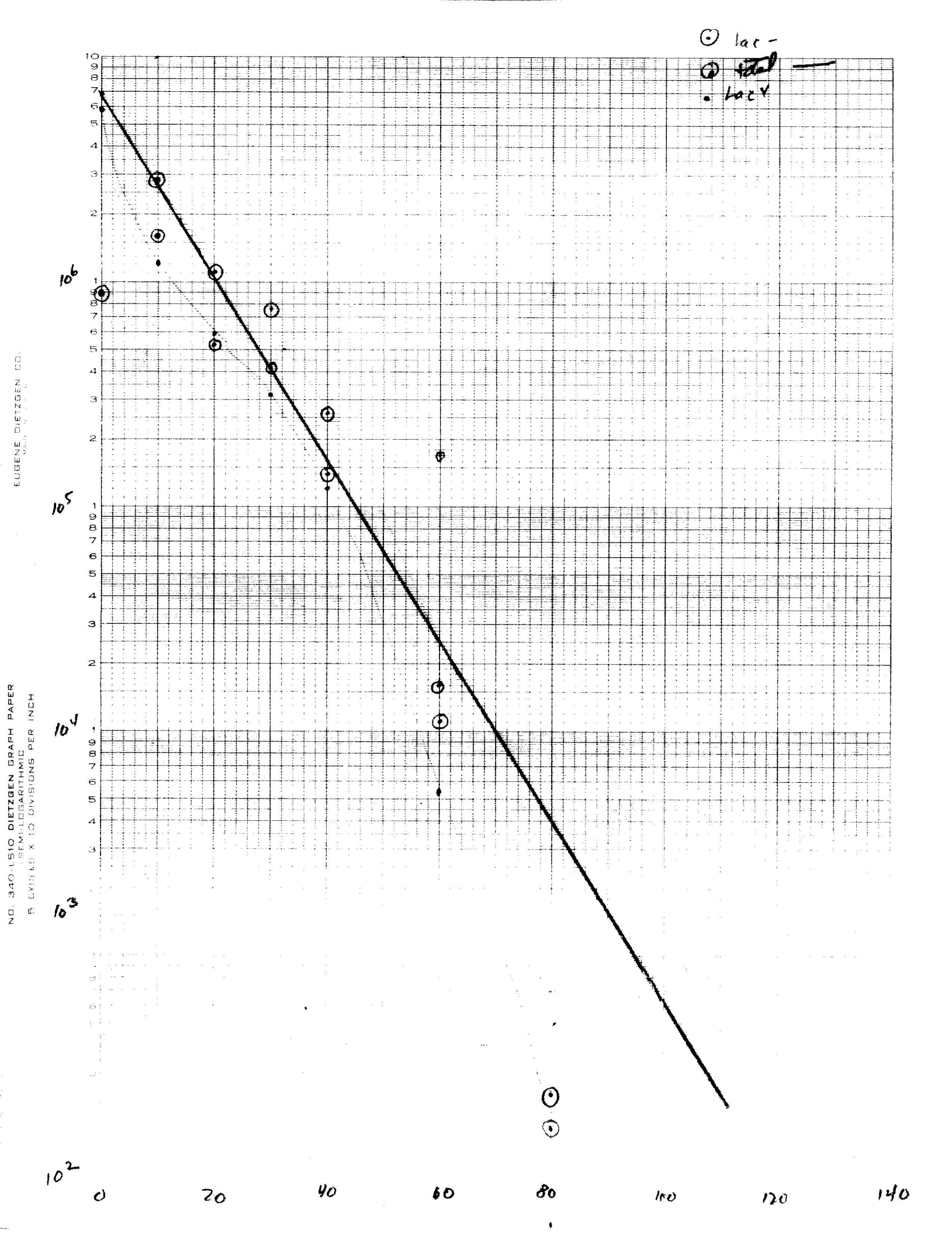
L Hydroquinone .05% ca 12 min.

Dosage	60; 68;	8 7;		6.5×10^8	
K5	31	99	130	1.3×10^8	20% survival
L5	461	98 /	559	5.6×10^9	Negligible killing. Inconclusive

Survival

	UV	Dil	lac v	lac −	Σ	Σ	v	−	% v
A	0	5	54	10	64	68			
			64	8	72				
			118	18	136	6.8^6	5.9^6	$9.[illegible]^5$	87
B	10	5	12	16	28	2.8^6	1.2^6	1.6^6	43
C	20	4	59	51	110	1.1^6	5.9^5	6.1^5	54
D	30	4	31	42	73	7.3^5	3.1^5	4.2^5	42
E	40	4	12	14	26	2.6^5	1.2^5	1.4^5	46
F	60	~~"3"~~	~~57~~	~~112~~	~~169~~	~~1.1^5~~	~~5.7^4~~	~~1.1^5~~	
		"2"	51	105	156	1.6^4	5.1^3	1.1^4	33
G	80	1	7	17	24	240	70	170	29
H	100	1	3	1	4	40	30	10	
I	120	1	1	8	9	90			
J	150	1	0	1	1				

Note that proportion of lac v does not vanish, and may reach a minimum with low doses.



H206 – UV Killing for c.o. test

February 10, 1950.

Inoculum 24h. (aer. 3h) in D(Lac) (Very dense – – est 2×10^9)

Plate out — Dilute 1:100; Irradiate UV 20 seconds, and plate out.

A) assay 5×10^{-8}

B) Irradiate and assay.

1	10^{-7}	Sterile
2	10^{-6}	
3	10^{-5}	

~~Repeat 2/11/1950~~ with same suspension.

2/13/50

A. assay s̄ irradiation.

B Dilute 1:200 and irradiate 20 sec at 20cm.

Express as 1:200 sample. assay at 10^{-5}.

4 survivors at 10^{-1}!

too high kill

2/15/50 Same suspension

Chemical Effects; UV on H226

2/14/50.

A. 1ml H226 + 9ml 0% doca in D(-). pH6.7 gel simulator. 11[45] AM. — 4[25] PM.

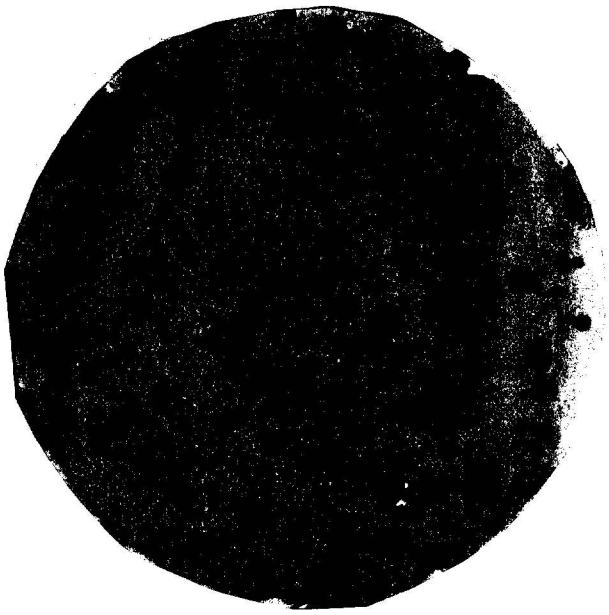
B. 1ml H226 + 2ml 0.5% hydroquinone = 0.1% HQ + 7ml D(-). 4[15] PM. — SPM

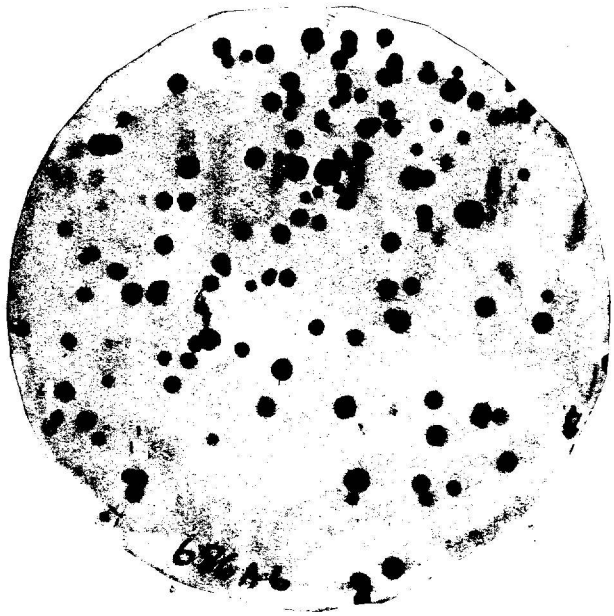
C. ~~Dilute new H226 stock 1:80 for sensitization~~ [Dil. as original stock] Assay.

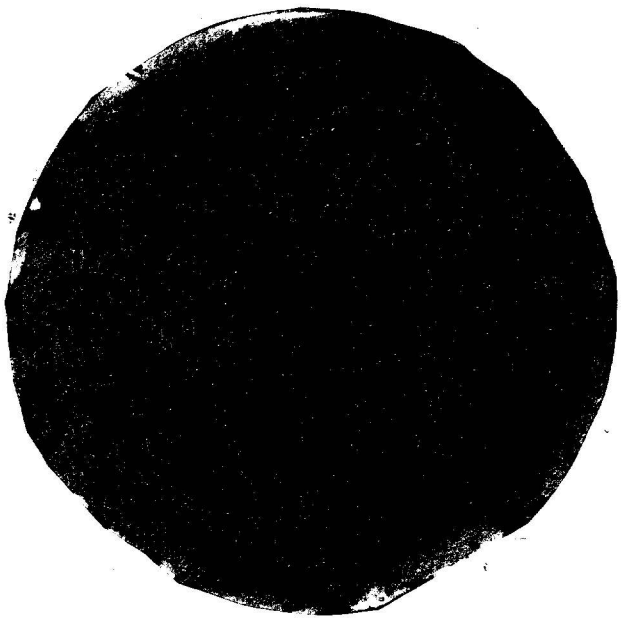
D. into 10ml H_2O, 200r/ml streptomycin 4[55] — 6[20]

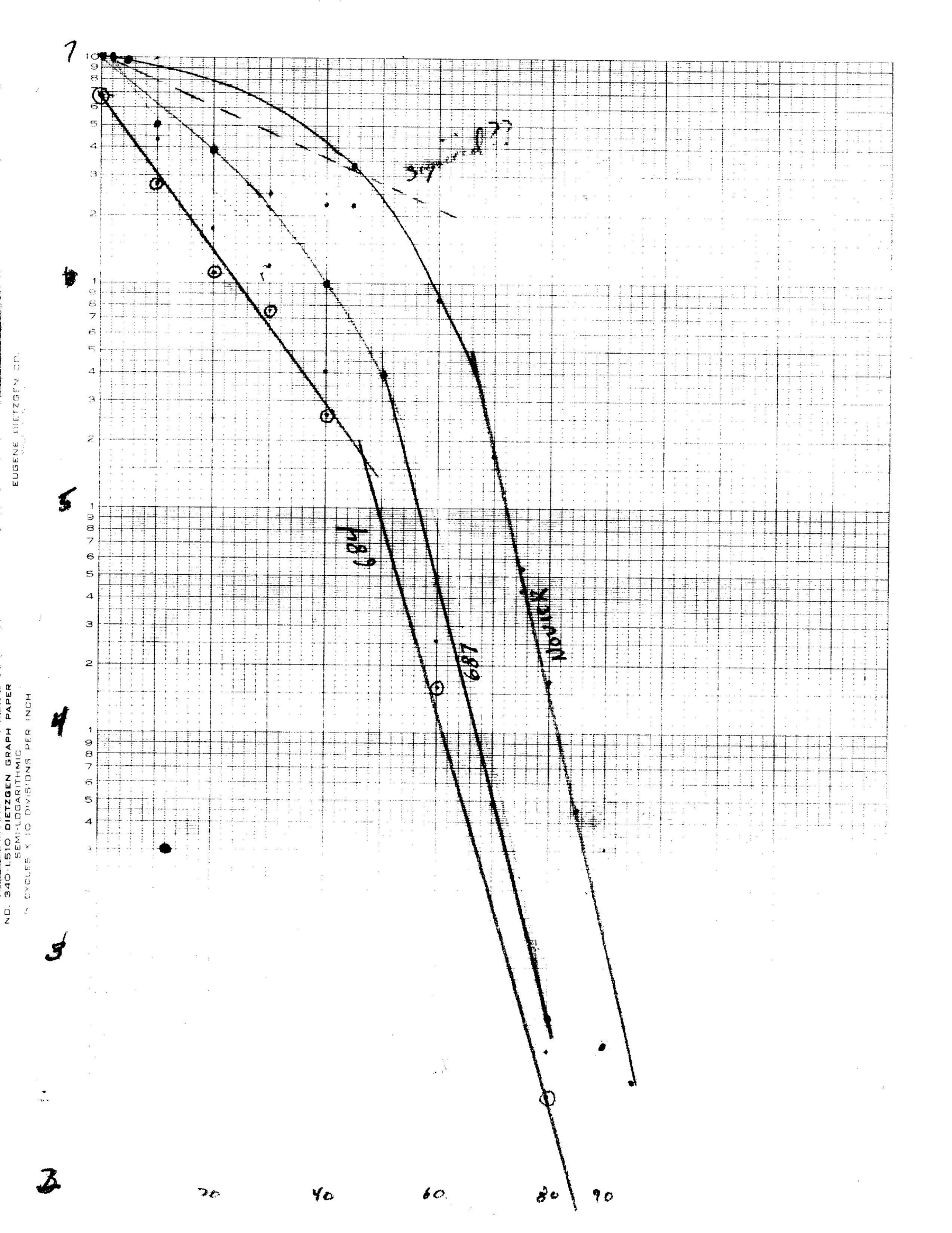
		LacU	Lac-	Σ	Σ Count	
A)	6:	57	80	137	1.4×10^8	~~with~~ ρS
B)	−2 : sterile!	~~72~~	~~15~~	~~87~~		
c)	Assay. 7	72	15	87	8.7×10^8	
D)	(Strep). 5	230	30	260	2.6×10^7	

∴ doca again shows only slight killing despite prolonged exposure (almost 5 hours). — Slight haploidization noted. Should be studied for balanced lethals.








EUGENE DIETZGEN CO.
NO. 340-L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH
Normal
1889
1891
20
40
60
80
90

UV Killing curve: H226

2/14/50.

Fresh (24; aer. D(lac)) shake of H226. Dilute 10^{-2} to give estimated 10^7/ml. Irradiate in open dishes, 50cm UV, as 684.

	uv-secs.	dil.		Lac V	Lac −	Count →		½	% V
A	0	5		173 {225 {199 174	22 {40 {14 12	195	1.9×10^7	1×10^7	89
B	2	5		[171; 167; 143	41; 41; 29 [37	197	2.0×10^7	1×10^7	81
C	5	5		~~160~~ 110	82	193	1.9×10^7	1×10^7	52
D	10	5		91 Σ102; 80	158; 76 [	208	2.1×10^7	8×10^6	44
E	20	4	Too heavy!! Count ½ pl.	(½) ~~270~~ =	(117) 510	780	7.8×10^6	3.9×10^6	35
F	30	4		123 ~~385~~ =	~~123~~ 385	508	5.1×10^6		24
G	40	3	>1800	Too heavy. ca 500	?1500?		2×10^6	1×10^6	ca 30
H	50	3	>200	184	613		8×10^5	4×10^5	30
I	60	2	>200	Too heavy.					
J	90	2	70	6; 7	98; 79	96	9.6×10^3	4.8×10^3	7
K	80	1		12; 8	128; 70	109	1.1×10^3	5.4×10^2	9
L	90	1	60	16	63	79	7.9×10^2	4.0×10^2	20

C 5 plates.

1	119	74	193
2	98	85	183
3	104	61	165
4	121	95	216
5	109	97	206
	551	412	963
	110	82	193

D 2 plates

L

served at 24h.

Aliquots plated on EMS lac

	+	−	Mean +	Mean v from 687
A	~~189~~ 189	~~2~~ 0		173
B	194	≥0		160
	178	2?		
C	120	6		110
	100	10		
D	106	12		91

Pick and streak out apparent − on EMB Mal.

	EMB lac	EMB Mal		Xyl	Mtl
1	−	v	*	+−	+−
2	−	v	*	−	+−
3	−	v	*	+−	+- ?
4	−	−			
5	−+	−+			
6	−	+			
7	−?	+			
8	−	v	*	−(+?)	+ ~~+~~
9	−	v?	*	+−	+−
10	−p+	v (not −, v)	*	+−	+−
11	−	+			
12	−	+			
13	−	−			
14	−	v	*	+−	+−
15	−+	+			
16	−	v	*	+−	+−
17	−	+, − v?			
18	−	v	*	+−	+−
19	−	+−			
20	−+	+−			

Reisolate from Mal EMB to EMS lac, EMB Mal, EMB lac: *

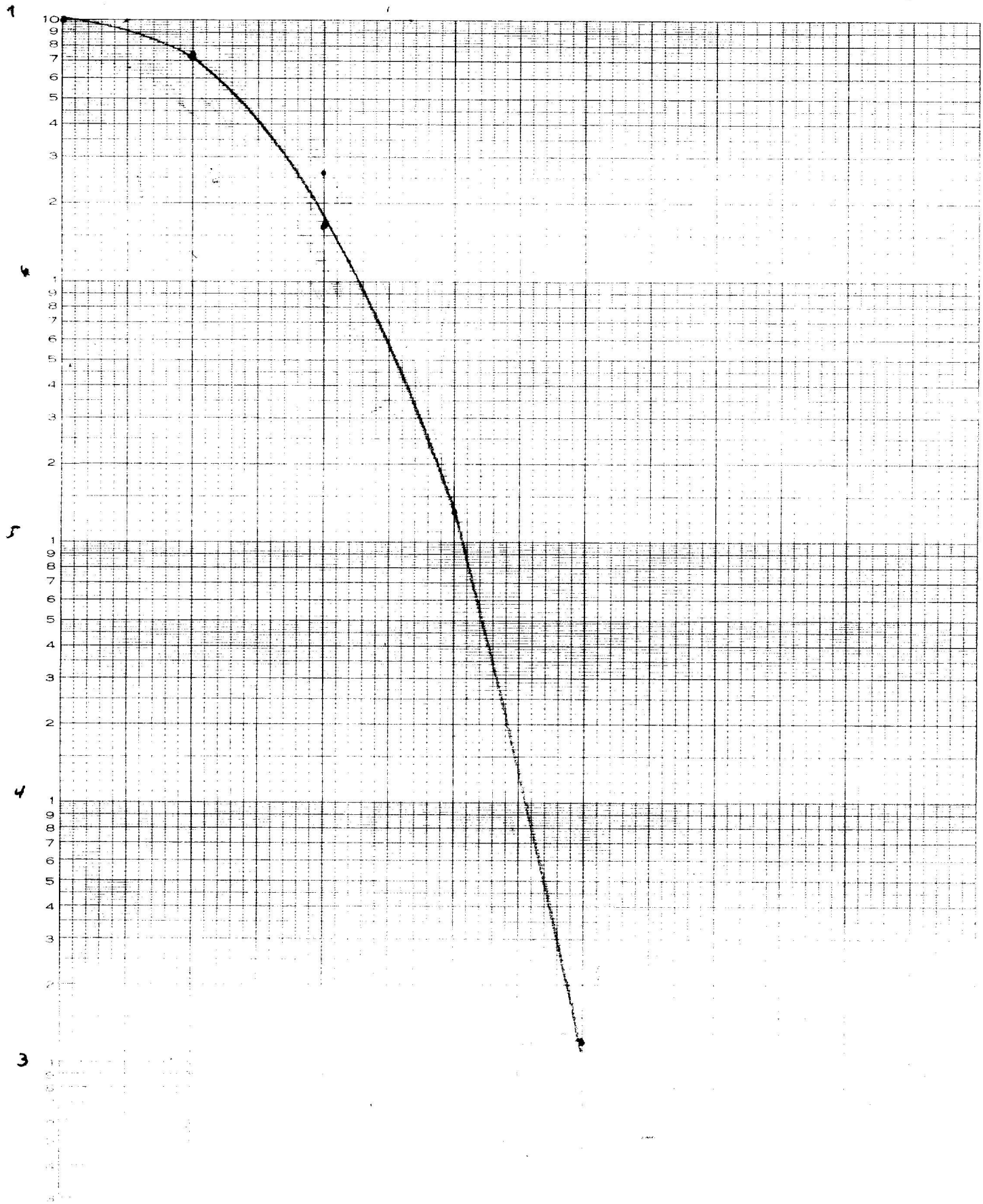
UV Killing — K-12

2-16-50.

Grown in Difco 24h. aer. 38° in refr ca 2-3 days.
Dilute 10^{-2} in saline for radiation.

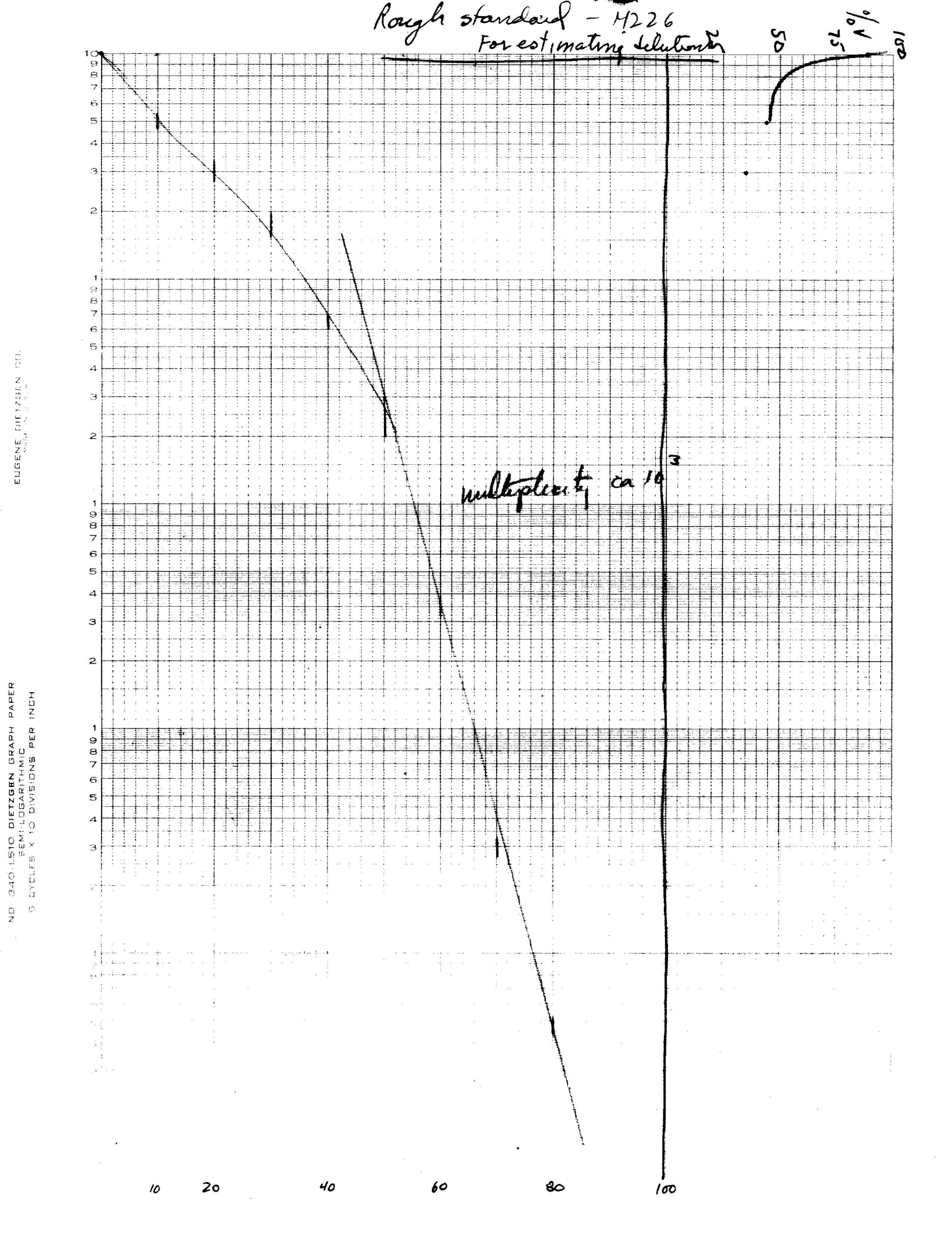
UV sec	Dil		Count		÷ 6.1
0	5		608	6.1×10^7	1×10^7
20	5		462	4.6×10^7	7.5×10^6
40	4	¼ plate × 4	1600	1.6×10^7	2.6×10^6
60	3		823	8.2×10^5	1.3×10^5
80	1		730	7.3×10^3	1.2×10^3

K-12 UV 688



EUGENE DIETZGEN CO.

NO. 340-1510 DIETZGEN GRAPH PAPER
4 FM LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH


Rough standard - 4226
For estimating dilutions
50
75
90
100
multiplicity ca 10^3
10
20
40
60
80
100
EUGENE DIETZGEN CO.
NO 340 L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH

Treat H226 with Acetic anhydride

2/12/50

Add 0.1 ml Ac_2O to 10 ml H_2O. Add 1.1 ml sterile 10% $CaCO_3$ suspension. Add 1 ml H226 and shake at room temperature. After 10 m., plate out on ± MB Lac (original H count not before assay.)
[Assume ≐ 3×10^8 ca 90% lac v]

All plates sterile. See 692 for effect of 0.1% Ac_2O.

2/20/50.

a) 1 ml H226 (2/19/50) 10 ml H_2O .1 ml 1% Ac_2O in 10% ale

c) " " .5 ml " "

10 mins 37°.

B6	Lac v 239	Lac − 203	442.	Count: 4.4×10^8	
A22				Survival = $\frac{4.4 \times 10^8}{\times 10^9}$	(see 624 assay)
				≥ 40% —	
C2.	0	3	Hold for delayed survival.		
C5,6 sterile					
C2	14	106	Hold further		

K12 vs. H226 uv sensitivity

H226 uv; chemicals

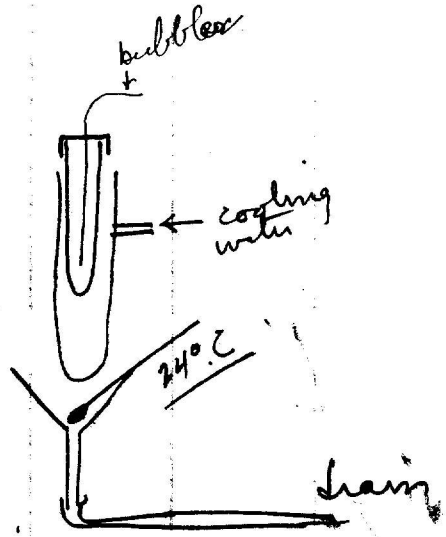
2/18/50.

Mix H226 + K12 [2/14]. Dilute to 4×10^{-7}.

Take .1ml and spread on EMB Lac

A) Control

B) UV 20 secs.



C 10^{-5} H226 dil 10^{-2} for irradiation. assay.

D UV 20 2×10^{-5} } EMB Lac

E UV 80 secs. 10^{-1} } EMS Lac, EMS Mal

F UV 80 secs. + light. (focus Spencer projection microscope lamp on suspension, in cooling jacket, 12 cm. from lamp aperture).

4 PM → 6 20 100 mins.

FORMALD: 1ml susp. + 8.5 ml H_2O + .5 ml 1% CH_2O (→ .05% final) (= dil. 1)

5 PM – 5 20 — 20 mins. → ca 10% surv. mostly haploid

H – 6 00 sterile 60 mins.

Note: after 40 hours, H3 had ca 10^3 colonies (∴ this agent may give a delayed recovery).

C. First count was done at ca 24 hours. "lac −" were marked for review at ca ~~40~~ 48 hours (10 A 20) 3 regular revisions from lac − to lac v.
This alters the means to 478/3 = 159 lac v : 20/3 = 7 lac − = ca 95% / 5%.
Control 10^{-5}

D. Lac

	v (+)	−	Σ
1	63	106	
2	54	98	
3	64	87	
	181	291	
3	60	97	157

UV 20 secs. 2×10^{-5}

Comparative titer:

c)	159	7	166
d)	30	48	78

Killing : 47% survival
39% lac v.
Compare with D − Mal: 68/166 Mal v! Not greatly different.

E. 80 secs. Lac 10^{-1}

	v (+)	−
1	15	72
2	8	72
3	16	75
4	17	106
5	19	90
	75	415
6	1	18
7	0	13

$\bar{m} = 15 : 83$, killing 5.9×10^{-5} survivors
18% lac v.
10^{-2} } high!

F = UV 80 s. + light. Lac

10^{-4}:	29	54
10^{-5}:	3	9

Survival $= \frac{8.3}{166} = .05$ 35% v
80 (L) ≠ 45 (D) p. 387 disagreement!

Formaldehyde .105% 20 mins.

G. 6 lac+,V lac− 22 45 / 67

Assay is 6.7×10^7
original was 1.6×10^9
Survival = $4.2 \times 10^{-2} \cong 5\%$
ca 33% Lac V.

2/20 Repeat a, b expt.
Mix .05 ml M226 + .05 ml K-12 grown in parallel 20 h. D(lac) aer. in 100 ml. ($=10^{-2}$ dil.)

			lac+	lac V	lac−
A.	10^{-5}	Control	150, 122	75, 59	8, 2
B	10^{-5}	20 sec uv	45, 62	9, 6	21, 23
C	10^{-1}	80 sec uv.	126, 144,	7, 7	23, 32

Control:	272 : 144	/ 416	K-12 %	65	or 1.9 : 1
uv 20	107 : 59	/ 166	"	65	1.8 : 1
uv 80	269 : 69	/ 338	"	79	3.9 : 1

i.e., ca 2-fold increase in proportion of K-12 over 4 decades of killing!

Dear Josh,

Here's another batch - I don't have too much hope for this run.

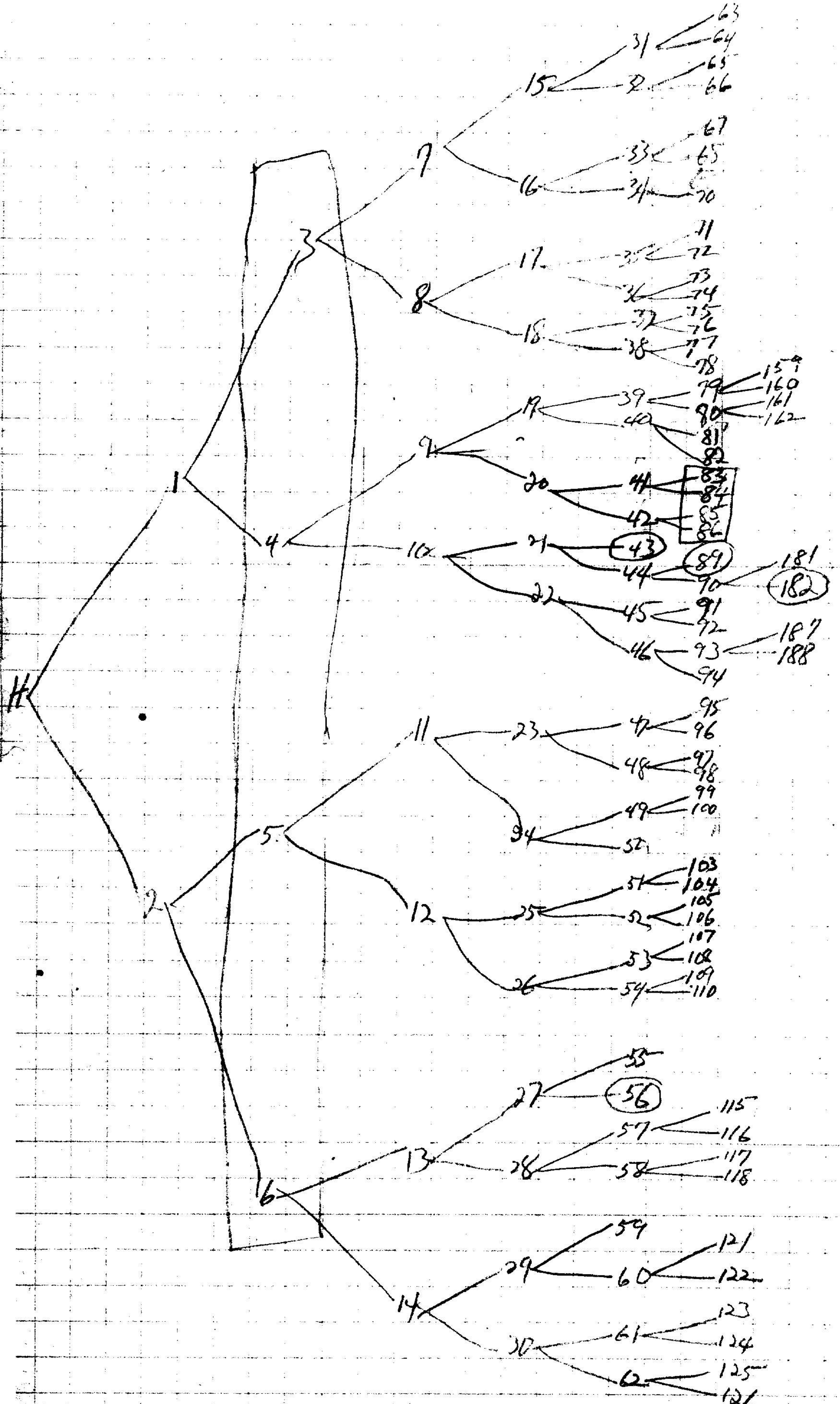
Ltr 8-5-50 pers was interesting - am anxious to hear the details.

Also anxious to learn your reaction to Pat Ltr. Please excuse me if I overstepped propriety in my last letter. Am not going to not send it but didn't get around to writing another one.

Am planning another session this Wednesday (holiday for G. Wash.) + another for the weekend. So be looking for a couple of more batches soon.

WWH

H 116 and H 123 failed to grow in both tubes.

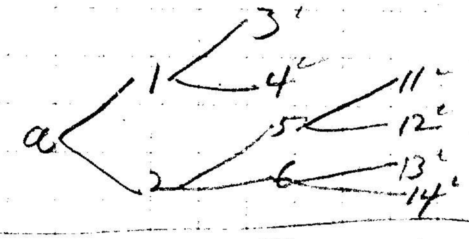


all vac - Mal -

2-18-50 H 226 strain

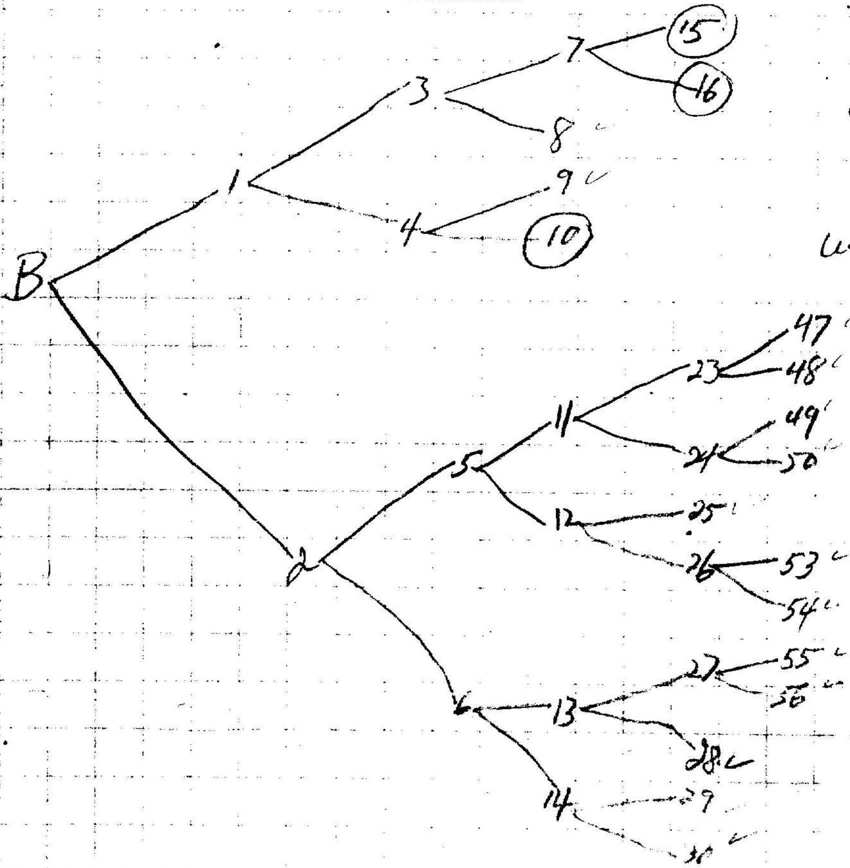
○ = didn't grow

[ ] = Unknown sib relationship



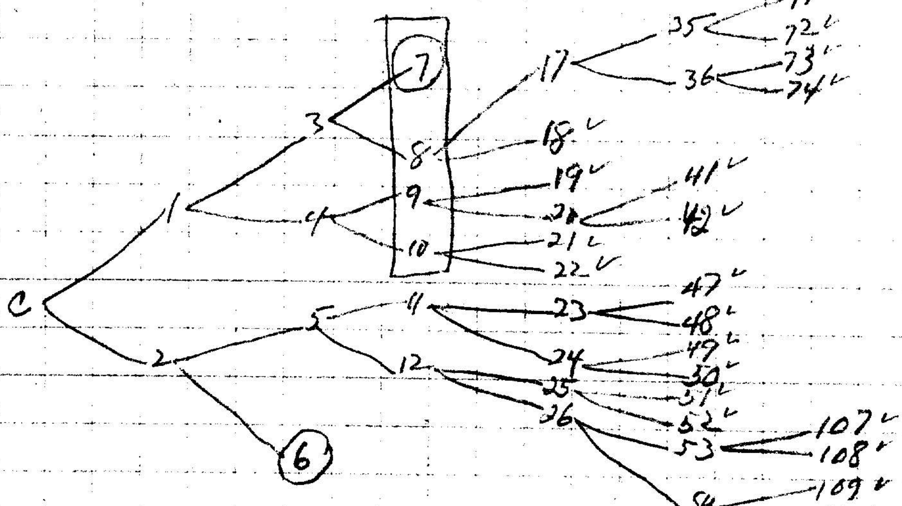
cells tended to become filamentous - stopped because of an engagement.

all het



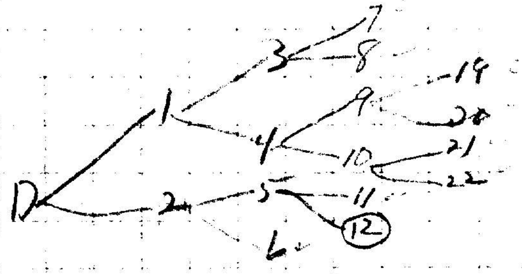
cell 3 was a filament, then split off a small cell at each end
— to 15 8 16
was arbitrarily numbered as indicated

all het

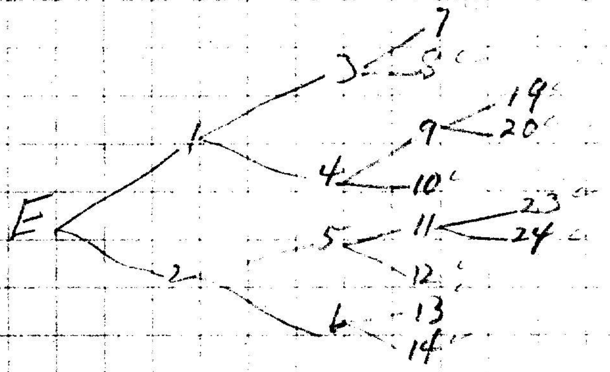


All these microcolonies were similar - round, dense, "dry" (i.e. hard to pick up in pipette) like those of clone 4 of 2-5-50

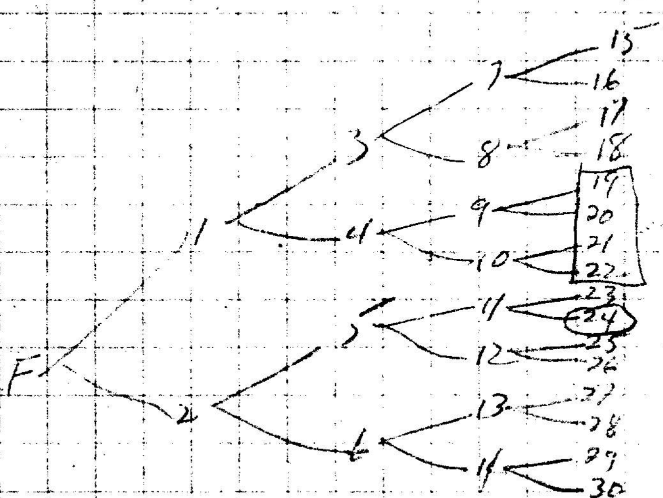
L-M



all het



all Het

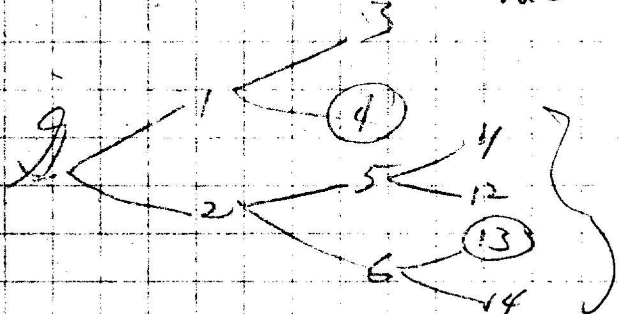


all lac+ mal+

Cells in third clone were very small from the start - I would guess cell F was a segregant. Went along with it for the hell of it.

F22 failed to grow in the broth tube due to breaking the tip off the micro-pipette.

Lac- Mal+



dip!

Platings of UV-H226 on EMS

690d

Febr. 20, 1950.

Platings on "EMS Mal" ~~probably~~ sterile. Medium probably ng.
EMS Lac:

Control C

+	−
154	0
136	0
146	0
436	0

in 145 + : 0 −

D

	+	−
	97	16
	126	25
	104	31
	109	34
4	436	106
	109	27

½ : 54 : 13

note absolute increase in Lac−, simulating "mutations".

E) Many mosaics, making counts difficult ca 20-25% Lac−.

Pick clean Lac− from D and streak out on EMB Mal.

690 exp.

	K-12	Hfr26		
	lac+	lacV	lac−	V+−
A)	104	29	11	40
	81	31	15	46
	97	29	19	48
	282	89	45	134

282	134	416
259	93	352

B

68	6	19	25
109	7	24	31
82	14	23	37
259	27	66	93

not sign. different.
31% 35%

C Assay

	lacV	lac−	
$10^{-2} \times 10^{-5}$	164	~~9~~	7
	161	~~7~~	6
	160	~~7~~	6
	~~475~~	~~23~~	~~20~~
m	158	8	7

D av 20

defer counting EMB Lac
many lacV not yet defined!
1+ noted – strain not as possible balanced lethal type
⇒ gave lacV and lac− only.

	+	V	−
EMB Mal	58	68	40 / 166

E

defer counting

F ca $10^3 \times$ E ca 10%

G

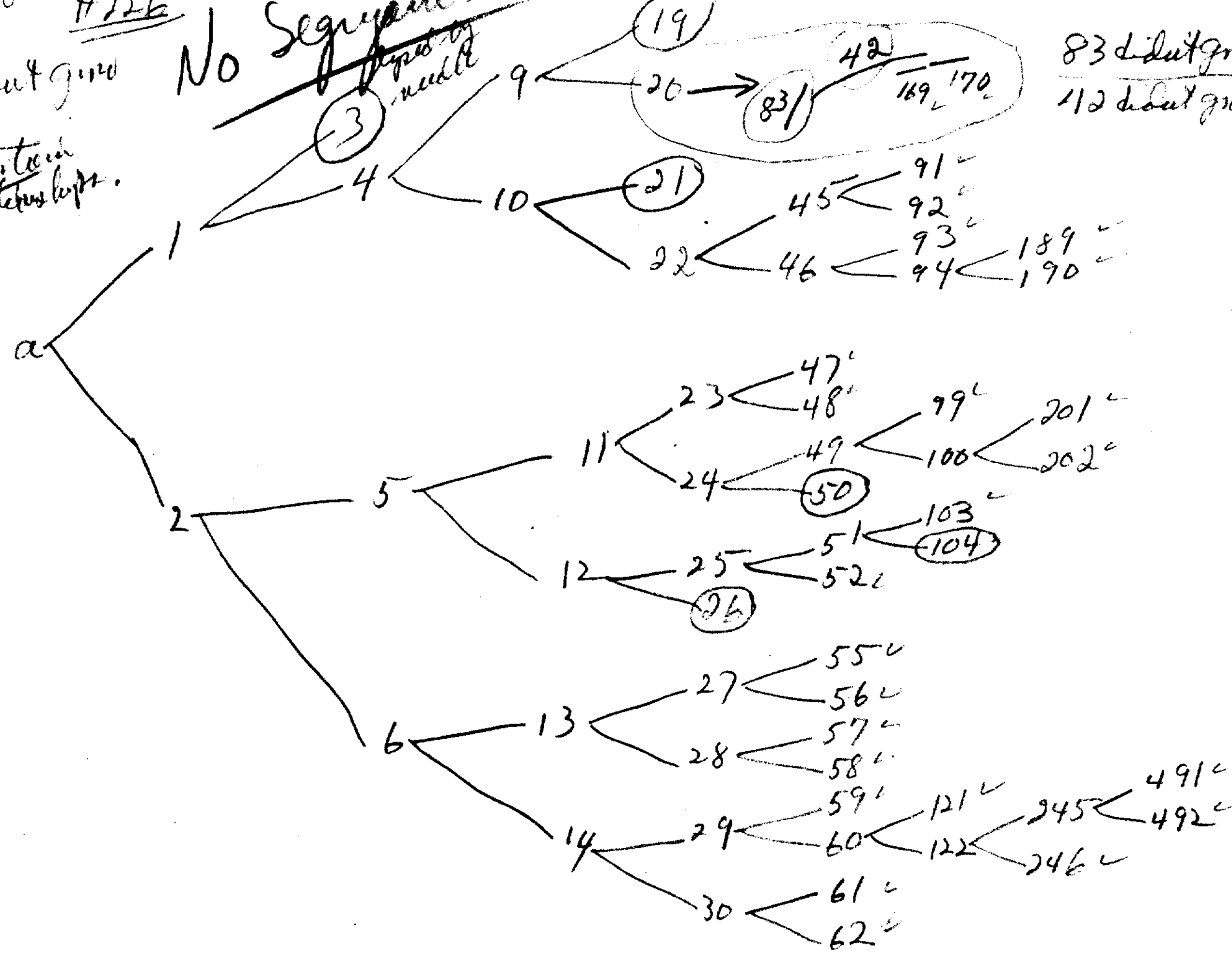
2-21-56 #226

○ didn't grow

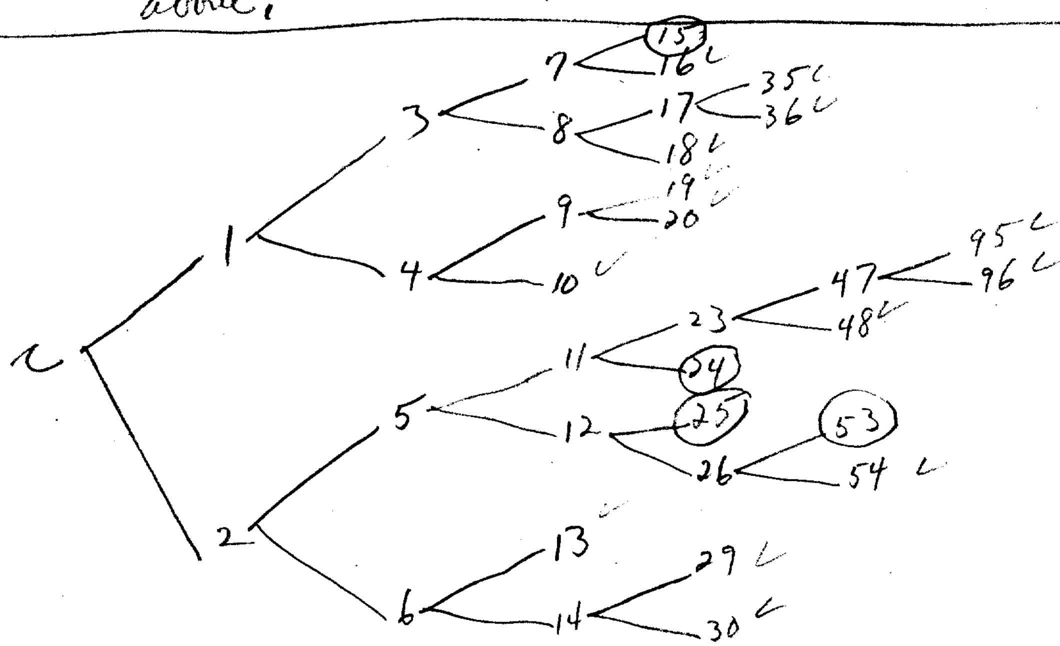
▭ uncertain relationships.

No ~~Segregants~~ spread by needle

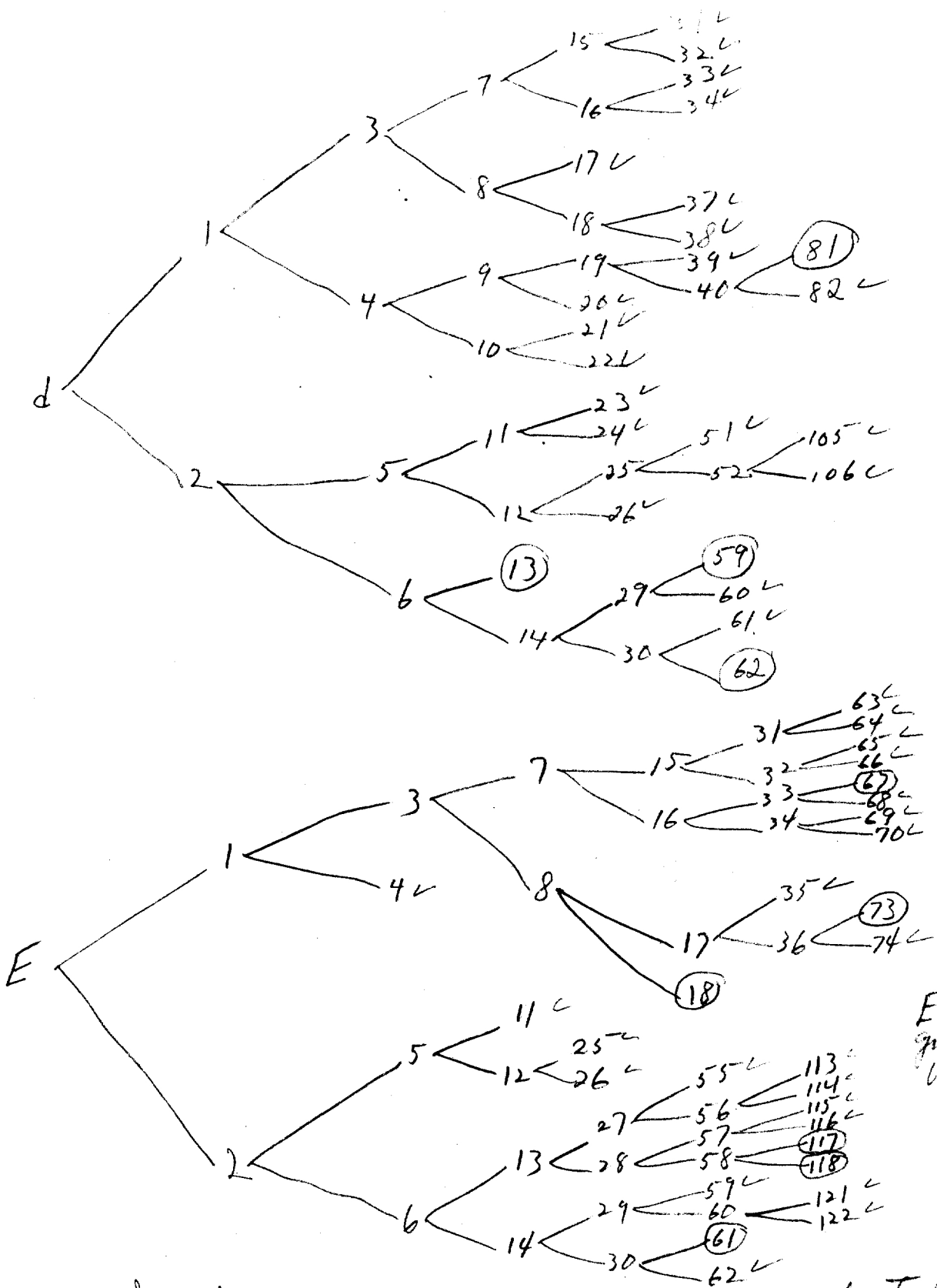
83 didn't grow
42 didn't grow



a 20 was a long filament, split off a cell at each end one of which divided. Quite arbitrarily numbered them as indicated above.



~~Hal-~~



E65 did not grow in the broth vial.

I made an error in my records & had E2 dividing to give E4+E5 Luckily the actual E4 was not subdivided so I could correctly remember the vials with no possible doubt.

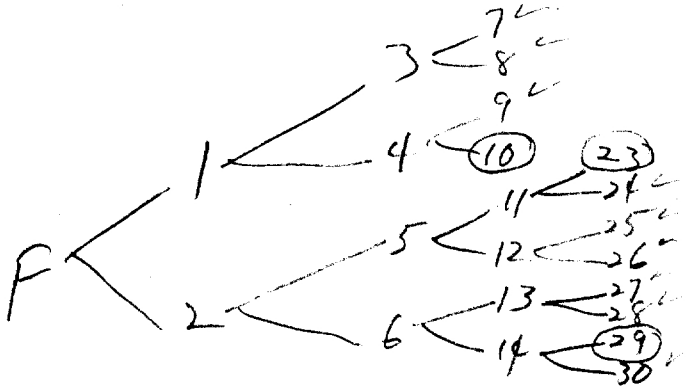
Josh,

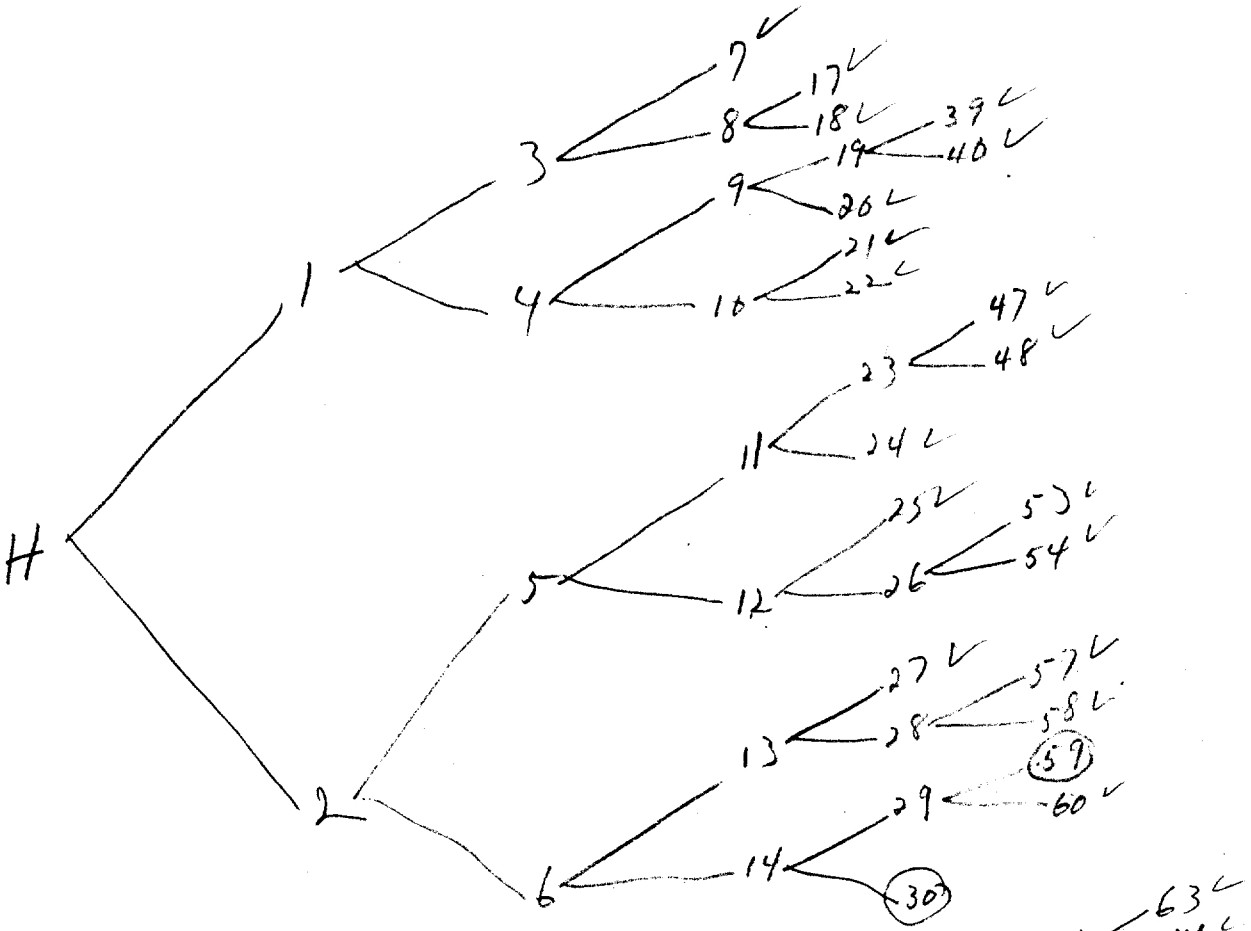
I'm still planning another batch for Friday - I don't have too many hopes for the batch either.

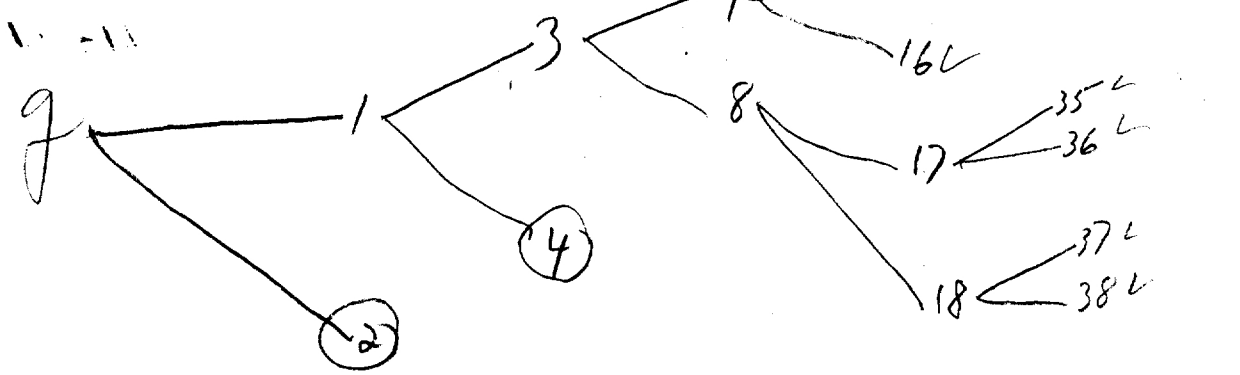
This culture tends to form filaments + then split off cells on the ends. I'm trying to keep records of all filamentous cells + sometimes rather arbitrarily number progeny of filamentous cells - indicating how they were numbered.

This is one of the hardest cultures to work yet - has a somewhat longer log period and fewer cells from young EMS colonies actually grow.

MW


F
1
2
3
4
5
6
7
8
9
10
11
12
13
14
23
24
25
26
27
28
29
30


H
1
2
3
4
5
6
7
8
9
10
11
12
13
14
17
18
19
20
21
22
23
24
25
26
27
28
29
30
39
40
47
48
53
54
57
58
59
60
63
64
65
66


J
1
2
3
4
7
8
15
16
17
18
31
32
35
36
37
38

Segregations from H-226

2/18/50

	Lac	Mal	Xyl	Mtl
1		+	+	+
2		+	+	+
3		+	+	+
4		+	+	+
5		+	+	+
6		+	+	+
7		+	+	+
8		−	−	−
9		+	+	+
10		6 +	+ +	+ −
11		+	+	+
12		+	+	+
13		+	+	+
14		+	+	+
15		+	+	+
16		+	+	+
17		+	+	+
18		+	+	+
19		+	+	+
20		−	−	−
21		+	+	+
22		+	+	+
23		+	+	+
24		+	+	+
25		−	−	−
26		+	+	+
2		27 − +	−	+ + ?
2a		28 + +	+	+ +
3		29 − +	+	+ +
4		30 + +	−	+ + ?
31		+	+	+
32		+	+	+
33		+	+	+
34		+	+	+
35	− +	+	+	+
36		+	+	+
37		+	+	+
38		+	+	+
39		+	+	+
40		+	+	+
41		+	+	+
42	− +	+	+	+
43		+	+	+
44		+	+	+
45	− +	+	+	+
46		+	+	+
47		+	+	+
48		+	+	+
49		+	+	+
50	− +	+	+	+

	Lac	Mal	Xyl	Mtl
51		+	+	+
5		+ − +	+	+ + −
60		+	+	+
61	− +	+	+	+
6		− +	−	0 +
	− +	+	+	+
		+	+	+
		+	+	+
	− +	+	+	+
		+	+	+
		+	+	+
71		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
81		+	+	+
7	− +	−	−	+ + −
		+	+	+
8		+ − +	+	0 + +
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
91		+	+	+
	− +	+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
		+	+	+
100		+	+	+

(over)

Single colonies of H226 on EMB Lac. 4/pl.
Single-colony chosen from each, and tested on the sugars indicated. Results indicate strongly the preponderance of Val+ segregants (lac_4 - ??)

Formaldehyde – prolonged exposure.

H_2O_2

2/19/50.

A Formaldehyde 8 01 → 8 37 as 690 G. Use suspension of H226 2/19. Hold! Sterile!

B H_2O_2 Expose a 10^{-2} dilution of H226 to decimal stage conc. of H_2O_2 beginning with 30% 1:10. 10ml H_2O + .5ml D(-) + .1ml susp. After 15 m. take .1ml samples and spread on EMB lac.

C) Ac_2O H226 1ml + 1ml $CaCO_3$ 10% + 0.1ml 10% Ac_2O/ETOH.

At 10^{-4}, 198 lac – 0 lac v or +! $2 \times 10^6 = 10^{-3}$ survival.

10^{-3} → >1000 lac – 10^{-2} 7 colonies only!

D) assay

10^{-7}: lac v 146, lac – 12 / 156

		% H_2O_2	
	1	3	Sterile
	2	0.3	Sterile
B →	3	0.03	Sterile near center; crowded at periphery. (lac – around center ?)
	4	0.003	Crowded!
	5	0.0003	Crowded!

Higher than .03% bacteriostatic on plate. [Should use catalase.]

A

6		lac v	lac –
P2 A22	3 same plate	3	51
		27	53
5		13	—
A22		>>13	

Survival = $\frac{5.4 \times 10^7}{1.6 \times 10^9} = 3 \times 10^{-2}$ ~~Actually 6% diploid.~~

Suggestion! Do prolonged doses of chemicals differ from UV in permitting a much lower proportion of diploid survivors, conforming to nuclear elimination theory of killing.

Segregation of H226

Febr. 20, 1950.

B. 1[30] Incr. H226 1:10 in Penassay; aerate to 7[10] (6 hours). Plate out on EMB Mal. → But mostly still lac v! (ca 80%).

A. Streak out single lac v colonies of H226 (690C) on EMB Mal. Pick single Mal+, Mal− colonies to nude and spot on EMB lac, Mal, Xyl, Mtl.

2/21 (A) 57 Mal− : all Lac− Mtl− Xyl−

62 Mal+ : #2 Lac+(v), Mtl+ Xyl+ ... 52: Lac+ Mtl+ Xyl+

3 Lac− Mtl− Xyl−

5 Lac− Mtl− Xyl+

All above tested: V_1^R. [Do H226 V_1^R/V_1^S?]

But segregants are preponderantly parental combinations.

[Tests of phage resistance; nutrition are ended.]

(B) Mal− picked (dry M.O.) to Mal, Lac, Mtl, Xyl EMB:

	Lac	Mal	Xyl	Mtl	L	M	X	Mt	L	M	X	Mt	L	M	X	Mt
1	−	−	−	−	11 (±)	−	+	±	(2) −	(±)	±	±	31 + ✓	−	+	+
2	−	−	−	−	12 ✓	−	+	✱ +	− ✓	−	−	−	− ✓	−	+	+
3	±	±	−+	−	13 ±	±	±	±	(±) −	+	+	+	±	±	−	±
4	±	±	±	±	14 ±	±	±	±	±	±	±	±	±	±	±	±
5	±	±	±	±	15 ✓	−	−+	−	○ −	(±)	±	±	−✓	−	+	+
(6)	−	(±)	±	±	16 ✓	−	−	−	○ (±)	−	+	±	−+	±	−	−
(7)	−	(±)	±	±	17 ±	±	±	±	− ✓	−	−	−	(±)	−	±	+
8	−	−	−	−	18 ✓	−	−	−	−+	±	±	±	−✓	−	−	−
9	−	−	−	−	19 ✓	−	−	−	−+	±	−+	▬	○ −	(±)	−	−+
10	——				20 ✓	−	−	−	30 ±✓	−	+	+	40 −✓	−	−	−
					21 ✓	−	−	−	±	±	+	+	−✓	−	+	+
					6 ✓	−	+	+								

Most of these are poor selections. Investigate the Lac−Mal discrepancies!

Out probable pure ✓, shows 6 ## L−M−X+Mtl+ ; #14 L−M−X−Mtl− This is a high proportion of M−X+ compared to 693A, but may be due to previous prototroph selection.

Investigate ○. Pick from Mal or Lac

Partial segregants

March 4, 1950.

In older cultures of H226, a number of "partial segregants" have been isolated. See also 687: 9 Mal$\underline{v}$ Lac- were picked up following irradiation. Later, the same was found in ~~2/22~~ control cultures.

693 (2/22) 4 Lac$\underline{v}$ Mal- and 1 possible Lac- Mal$\underline{v}$ isolated from EMB.

3/1

c. See 700. Plating of H226 after growth on EMB Mal.
10 Mal$\underline{v}$ (?) picked. Streak on EMB Mal, Lac.
all were Lac$\underline{v}$ Mal$\underline{v}$.

D. See 700 A1. On EMS Lac, Mal. $\ll 1\%$ -. Repick and purify on same medium for subsequent testing.
Mal- : 8 ; 3 Lac$\underline{v}$. #2, 3, 8. = ~~700 11, 12, 13~~ 699: 17, 18, 19
Lac- : 14 2 Mal- ; 12 Mal+ No Mal$\underline{v}$.

Segregation of M226
Partial segregation?

2/22/50.

See 693 (B).

1-4 from Lac (693B: 11, 23, 26, 37); 5-9 from Mal (693B: 6, 7, 21, 25, 35).
Streak out on EMB Mal, Lac; EMS Mal, Lac.

	Lac EMB	Mal EMB	EMS Lac	EMS Mal
1	Lac v; Lac −	Pure Mal −	+	−
2	Lac v; Lac −	Pure Mal −	+	−
3	Lac v; Lac −	Pure Mal −	+	−
4	Lac v; Lac −	Pure Mal −	+	−
5	Lac −	Mal +; Mal − v?	n.g.	n.g.
6	Lac −; Lac + (v?)	"	+	+
7	Lac −	"	n.g.	n.g.
8	Lac −	Mal + − v?	n.g.	n.g.
9	Lac −	Mal − v.?	+	n.g.

These isolations leave no doubt as to the occurrence of Lac v; Mal − types. How do they arise? They would represent a persistence of the 2 stage reversion noticed by Zelle. A Mal v Lac − has also been picked up.

H226 partial segregation.

2/24

Grow H226 1:100 to saturation in Pennassay (aerated)

2/27 Plate at 10^{-5} on EMS, EMB Mal and Lac.

EMS: ca 100 prototrophs., +, and −

EMB: Turbid!

2/28 Plate at 10^{-7}(±) on EMB.

Lac EMB: ca 1% diploid; remainder all Lac −

Mal EMB: mostly Mal+; a few Mal −, Mal v.

A. Pick − from Mal EMS. Brush on Mal EMS, streak on Lac, Mal EMB

B. − Lac " Mal EMS " " "

C. Pick Lac v from Lac EMB. Streak on Mal EMB; save suspensions.

D Pick Mal v from Mal EMB. ~~Streak~~ Brush on Lac EMB. only 2 scorable at this time! both Lac v.

See after 698

~~2/28~~+ 3/1

A. 11 "Mal-" from EMS.

	Lac	EMS	Mal
1	V		−+
2	+−		−+
3	V		−+
4	V		−+
5	−V		−+
6	−		−+
7	V		V−
8	V		−+
9	V		−+
10	V		−+
11	V		−+

The frequent occurrence of (Mal− Lac V) among Mal− prototrophs is indubitable. The residue of Mal+ papillae is not explained.

In some cases one, a single colony probably contains Mal− and Mal V, Lac V.

B. 38 "Lac−" from EMS

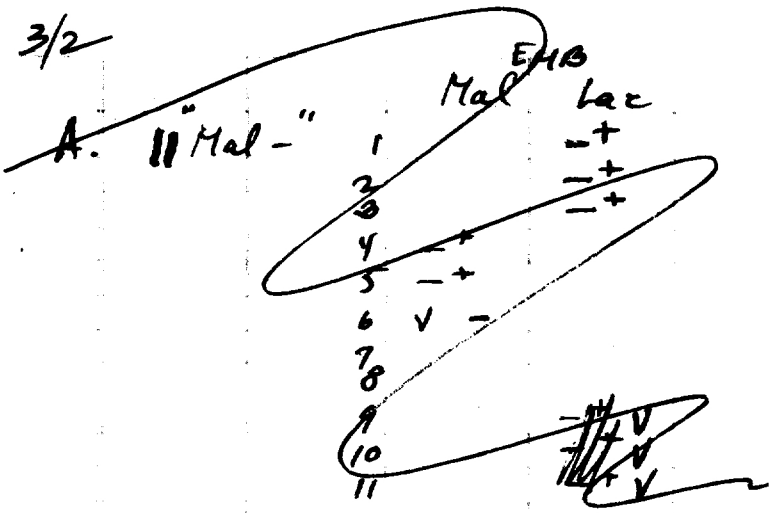
1	−	+
2	−+	+
3	−+	+
4	−+	+
5	−+	+
6	−	+
7	−+	V
8	−	+
9	−+	+
10	−	+
11	−	+
12	−+	+
13	−	+
14	−	+
15	−+	+
16	−	+
17	−+	+
18	−	+
19	−+	+
20	−+	+
21	−+	+
22	−	+
23	−+	+
24	−+	+
25	−+	+
26	−	+
27	−	+
28	−	+
29	−	+
30	−	+
31	−	+
32	−	+
33	−	+
34	−+	+

A single essentially Lac− Mal V culture has been recovered here.

Apparently, most prototrophic segregants are Lac− Mal+.

	L	17
35	−+	+
36	−	+
37	−	+
38	−	+

3/2



C. Lac v to Mal.

1	V	11	V	21	V	31	V	41	V
2	V	12	V	22	V		V		V
3	V	13	V	23	V		V		V
4	V	14	V	24	V		V		V
5	V	15	V	25	V		V		V
6	V	16	V	26	V		V		V
7	V	17	V	27	V		+		V
8	V	18	V	28	V		+-		V
9	V	19	V	29	V		+-V		V
10	V	20	V	30	V	40	V	50	V

Not well streaked out. The expt. should be repeated.

∴ No Lac V Mal- found in this sample.

Photorecovery of uv effects

2/20/50.

See 690 for set-up. Irradiate 2/19 H226 10^{-5} 5 seconds.

A) No uv

B) uv 5 sec.

C) uv 5 sec + light 110 mins. (temperature not well controlled)

C: no survivors.

Count A + B at 364.

	Lac v	Lac -
(A)	63	16 !
	91	30
	74	18
	228	64
(B)	65	31
	79	37
	30	14
	174	82

Increased prop of ⊙ types.

Relatively little effect at 5 secs. (use 10 for following expts.)

2/22/50:

	Lac v	Lac -	
A:	114	9	
	134	2	
	124	6	
	372	17	/ 389
B	124	6	
	74	37	
1+	61	57	
	44	33	
	62	41	
	241	168	
	60	42	/ 102

C.

Lac v	Lac -	
69	10	
74	16	
91	25	
234	51	
78	17	/ 95

Tests for balanced lethals

2/21/50 ff..

Picks lac+ colonies from a variety of treatments to examine for balanced lethals. Streak out on EMB lac, and examine Lewis + for + stability. If any are more or less unstable, pesticals. Emphasize intact lac+ (so this frequency is exaggerated)

690D+ : was lac+.

690D: 28 colonies. 10 Rechecks

E. 17 cols. 10 Rechecks. 1 v.v. = 695-3

F. 12 cols. 4 (1a,b) Rechecks. 1 v.v. = 695-2

G. 4 cols. 0 checks.

692A 12 cols. 1 pure lac++ transferred to check. as 695-92A1. others lac+.

689B

Repicks from first streaking if any colony not obviously sectoring is seen.

Retests for possible partial segregants

March 6-7, 1950.

			Lac	Mal	Mtl	Xyl
A.	1	= A 169		V	+ −	V
	2	A 189		++	+ −	V
=2/21/50	3	C 30		V	+ −	V
	4	E 116		V	O	↓− T
Series.	5	E 121		++ −	V	V
	6	H 11				
	7	A 15		++	V	V
2/24	8	B 21		V	V	++ −
	9	B 36 ✓		++	++	++
	10	C 25		V	V	V
B	11	D 12		V	V	V
	12	D 13 ✓		+(−)	++	++ , 1−
	13	H 22 ✓		++	++	++
	14	I 26		+ −	+ −	− +
	15	J 16		+ −	+ −	+ −
	16	K 16 ✓		++	++	++
	17	K 22		++ −	V	++ −
	18	L 13		++ −	V	++ −
	19	F 10		++	++	++
	20	F 12		++	++ −	++
	21	F 15		++	++	++
	22	F 16		++	++	++
	23	F 19		++ −	++ −	++ −
	24	F 24		++	++ −	++
	25	F 27		++ −	V	++ −
	26	F 28		++	V	++ −
	27	F 29		++ −	++	++ −
	28	F 30		++	++	++
	29	F 42		+ −	+ −	++ −
	30	F 47		+ −	+ −	++ −
	31	F 48		++	++	++

F series (19-31) is peculiar in Lac V Mal+ (very few − segregants). Reisolate and compare reisolated diploid with H226.

Of the others: 2/24: B36, D13, H22 and K16 warrant detailed attention. In 2/21, A189 and E121 should be reisolated.

EMB Mal, Lac

EMB Lac

Zelle 2/18.

Most of these pedigrees are uninteresting.
However, keep B28, 55, 56; Series D; E 7, 8, 10, 20;
~~[redacted]~~ Series G;

Also keep 680: A15; A33
D: 15–22; 53; 27; 29
E 215–222; 108; 49; 52; 75, 76
G: entire pedigree

abl-
bar-Mal-

Zelle - single cell pedigrees

Zelle 2/18

		Lac	Mal	
	7	+	+	Spur? Ors Rechts: − −, V ++, V, −
	8	+	+	− − V ++, −
	10	+	+	
	12	+	+	
	13	+	+	
	14	+	+	
	19	+	+	
	20	+	+	
	23	+	+	
	24	+	+	
F		−	+	
		−	+	
	17	−	+	
	18	−	+	
	19	−	+	
	20	−	+	
	21	−	+	
	22	−	+	
	25	−	+	
	26	−	+	
	27	−	+	
	28	−	+	
	29	−	+	
	30	−	+	
G	3	−	+	
	11	+	±	
	12	+	±	
	14	+	±	
H	5			
	6			
	66			
	67			
	68			
	71			
	72			
C		all Lac −	all Mal −	

Zelle 2/18

		Lac	Mal	
A	3	+	±	all het
	4	+	±	
	11	+	±	
	12	+	±	
	13	+	±	
	14	+	±	
B	8	+	±	all het
	9	+	±	
	25	+	±	
	26	+	±	
	29	+	±	
	30	+	±	
	47	+	±	
	48	+	±	
	49	+	±	
	52	+	±	
	53	+	±	
	54	+	±	
	5	+	±	
	1	+	—, +	Probably Lac +
C	18	—	—	all mut
	19	—	—	
	21	—	—	
	23	—	—	
	24	—	—	
	4	—	—	
	5	—	—	
	28	—	—	
	3	—	—	
	22	—	—	
	23	—	—	
	27	—	—	
	15	—	—	
	32	—	—	
	109	—	—	
	110	—	—	
D	6	+	+, − ? (pens)	Both are —, v; and Mal +, −.
	7	+	+	
	8	+	±	
	11	+	±	
	19	+	±	
	20	+	±	
	21	+	±	
	22	+	±	

3/1 3.2/24

	Lac	Mal	Xyl	Mtl
I 17	+	±	+	±
18	+	±	+	±
19	+	±	+	±
— 20	+	±	±	±
21	+	±	±	±
22	+	±	±	±
24	+	±	±	±
25	+	±	±	±
26	+	±	±	±
27	+	±	±	±
28	+	±	±	±
31	+	±	±	±
32	+	±	±	±
33	+	±	±	±
— 34	+	±	±	±
47	+	±	±	±
48	+	±	±	±
59	+	±	±	±
60	+	±	+	±
61	+	±	±	±
62	+	±	±	±
J 14	+	±	±	±
15	+	±	±	+
— 16	+	±	±	+
17	+	±	±	±
18	+	±	±	±
19	+	±	±	±
20	+	±	±	±
21	+	±	±. ?	±
22	+	±	±	±
23	+	±	±	±
24	+	±	±	±
25	+	±	±	±
26	+	±	±	±
27	+	±	±	±
28	+	±	±	±
K 5	−+? Rev	± Rev	−+	±
15	+	±	−+	±
16	+	+	+	+
18	−	−	−	−
19	+	±	±	±
20	+	±	±	±
21	+	±	±	±
22	+	±	±	±
— 29	+±+	−+	−+	−+
55	+	±	±	±
56	+	±	±	±
57	+	±	±	±
58	+	±	±	±

L	Lac	Mal	Xyl	Mtl
2	±	±	±	±
13	+	+	±	±
14	−	−	−	−
23	+	±	±	±
24	+	±	±	±
25	+	±	±	±

Restreak

Mal A15, F, [illegible], H22, I26, K16, K27, L13

Xyl A9, A15, F, B36, K25, H22, D12, 13, K16,

Mtl F, H22

Restreak small sugars:

A15, B36, A21, C25, D12, D13, F −,

H22, I26, J16, K16, K22, L13

K5 K18 L13, 14.

Lacv Malv

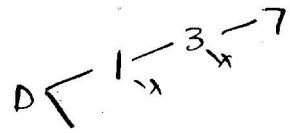
[SIB IS LETHAL.]

Lacv Malv

32/24

H226

		Lac	Mal	Xyl	MHe
C	61	+	+ ±	±	±
	62	+	+ ±	±	±
D	7	+	+ −	−	−
*	12	+	+ ±	+	±
	13	+	+ ±	+	+
	23	+	+ ±	+	±
	29	+	+ ±	+	+
	30	−	+ +	+	+
F	10	+	+ +	+	+
	12	+	+ +	0 ±	+
	15	+	+ +	+ +	+
	16	+	+ +	+ +	+
	19	+	+ +	± +	± ?
	24	+	+ +	+ +	± ?
	27	+	+ +	0 +	+
	28	+	+ +	+ +	+
	29	+	+ +	0 +	+
	30	+	+ +	+ +	+
	42	+	+ +	+ +	+
	47	+	+ +	+ +	+
	48	+	+ +	+ +	+
G	10	+	0	+	±
	13	+	±	+	±
	15	+	±	+	±
	16	−	−	−	0
	17	+	0	+	±
	19	+	0	+	±
	20	+	±	±	±
	24	+	±	±	±
	25	+	±	±	±
	26	+	±	±	±
	29	+	±	±	±
	30	+	0	±	±
	38	+	±	±	±
	47	+	±	±	±
	48	+	±	±	±
	76	+	±	±	±
H	8	+	±	±	±
	16	+	±	±	±
	20	+	±	±	±
	21	+	±	±	±
	22	+	+	+	+
	23	+	±	±	±
	31	+	±	±	0
	32	+	±	±	±
	39	+	±	±	0
	40	+	±	±	±
	50	+	±	±	±



D29, 30, 73.

No Segregants but save F10.

G15, 16.

3/1 3.2/24 Pedigree H226 Zelle

		Lac	Mal		Xyl	MM
A	9	+	±	±	+	±
	13	+	±	0	+	±
	15	+	++	+	+	±
	16	+	++	±	+	±
	18	+	++	±	+	±
	21 ~~23~~	+	±	±	±	±
	23 ~~24~~	+	±	±	±	±
	24 ~~43~~	+	±	±	±	±
	26	+	±	±	±	±
	29	±	±	±	±	±
	30	+	±	±	±	±
	35	+	±	±	±	±
	36	+	±	±	±	±
	46	+	±	±	±	±
	51	+	±	±	±	±
	52	+	±	±	±	±
B	15	+	±	±	±	±
	16	+	±	±	±	±
	18	+	±	±	±	±
	19	+	±	±	−	±
	20	+	±	±	±	±
	21	+	±	±	+	±
	22	+	±	±	±	±
	28	−	−	−	−	−
	29	+	±	0	−	±
	30	−	−	−	−	−
	35	+	±	±	±	±
	36	+	+	±	+	+
	47	+	±	±	±	±
	48	+	±	±	±	±
	49	+	±	±	±	±
	60	+	±	±	±	±
C	15	+	+	±	±	±
	17	+	+	±	±	±
	18	+	+	±	±	±
	19	+	+	±	±	±
	20	+	+	±	±	±
	22	+	+	0	±	±
	23	+	+	±	±	±
	24	+	±	±	±	±
*	25	+	+	±	+	±
	26	+	+	±	±	±
	27	+	+	±	±	±
	28	+	+	±	±	±
	33	+	+	0	±	±
	34	+	+	±	±	±
	59	+	+	±	±	±
	60	+	+	±	±	±

Relationships uncertain.

Detailed tests on single cell segregants

March 7, 1950

696 A.

A189 } Streaker
E121 } EMB Mal, Lac. Both are mostly segregated Lac−, Mal+ but show some Lac ✓ and Mal ✓ colonies.

696 B.

	Lac EMB	Mal EMB	Reisolates (Lac+ [EMB])
B36	mostly −	++	✓
D13	mostly −	++	✓
H22	"	++	✓
K16	"	++	+ ✓ −
	recover from EMB Lac or EMB Lac		Mal
Fsmic	"	"	
10	− +	++ −	++ ✓ −
12	− +	++ −	+ ✓ −
15	− +	++ −	✓ ✓
16	− +	++ −	✓
19	−	− +	✓
24	− +	++ −	✓
27	− +	++ −	+ ✓ −
28	− +	++ −	✓
29	− +	++ −	✓
30	− +	++ −	✓
42	− +	++ −	+ ✓ −
47	− +	++ −	✓
48	− +	++ −	+ ✓ −

Restreak Lac ✓ from EMB Lac where available.

Fsmic may have a higher proportion of Mal+, but this is doubtful.

Keep F10

Feb. 24, 1950.

M226 (2/24) brought to Chicago (Rest Radiobiology and Bio physics).
15 ml in small crystallizing dish, shaken gently, exposed to unfiltered X-radiation. 2 ml samples removed at intervals of 1 min., 10 min., 20 m., 30 m and 40 min. 1 minute = 942 r [(81; 76)]
Plate at various dilutions on EMB Lac. Plates carried from Site B to Site A in cold weather: temperature shock should be considered.
~~Also~~ Residue of aliquots stored in refrigerator in screw vials.

X-ray effects

2/27-28/50.

Material of 4 of C. X-ray experiment No. ___. 40 minute (ca 40,000 r) H2x

2/27. Plate 10^{-5} and 10^{-4} on EMS. 10^{-5} on EMB.

EMB Lac

Lac V	Lac -
0	13
8	14 -
1	8
1	12
10	47

Mal:

+	−	V
2	5	5
4	0	5
1	4	4
1	4	1
6	5	1
14	18	16

EMS Lac hold!

2/28/50.

10^{-4} ("x40-3)

Lac

V (+)	−
13	115
17	126
30	241

Mal 10^{-5}

+	−	V
67	42	55
5	11	7
5	1	2
3	1	5
2	4	4
3	6	4

Mal is difficult to count, but preparation of high Mal+/- colonies is clear.

A) Pick clear Mal+/- colonies and streak on Lac, Mal EMB; ~~Lac~~ Mal EMS

B) Possible Lac+ were picked in Chicago for streaking out in Madison. By error, different doses were not separated. 7: 10,000 r. 9: 20,000 34: 30,000 4: 40,000 r

A:

	Lac	Mal
1	−	+-
2	+-	V
3	−	+-
4	− +	+-
5	−	?
6	−	+-
7	−	+-
8	−	+-
17	− +	+-
18	− +	+−

	Lac	Mal
9	−	+−
10	−	+-
11	−	V?
12	−	+-
13	−	+-
14	−	+-
15	+-	+V-
16	+-	+V-
19	−	+−
20	−	+-

B: 6 cultures were scored as stable Lac+ after two restreakings. They do not appear to be typical Lac+/Lac+ haploids. Store on slants.

3/3/50

C. Picks 40 Lac - (no apparent +) colonies from plating of 40 min (≐ 40,000 r) X-ray H726. Streaks on EMB Mal, and brush on EMS Mal.

	EMB Mal			EMB Lac		
1	+	11	+	21	+	31	−	
2	−	12	+		−		−	
3	+	13	+		−		−	
4	−	14	+ −		+		−	Tc.
5	+	15	+ −		−		−	
6	+	16	−		+		+ −	
7	+	17	−		+		−	ca 10% of
8	+	18	−		+ −		−	Lac - are Mal? +/-
9	+	19	−		+		−	
10	−	20	+	30	+	Vp	+	

A+

$0 - 10^{-7}$

Lac V	Lac −				% V
108	7				94
111	8				
119	4				
107	7				
445	26	471	118	118	94

1 min X-Ray ≐ 1000 r.
10^{-7}

74	29				
68	29				
80	23				
74	30				
296	111	407	102	102	72

10 min.
2×10^{-7}

17	67				
15	59				
17	71				
11	57				
60	254	314	39	39	19

20 min
10^{-6}

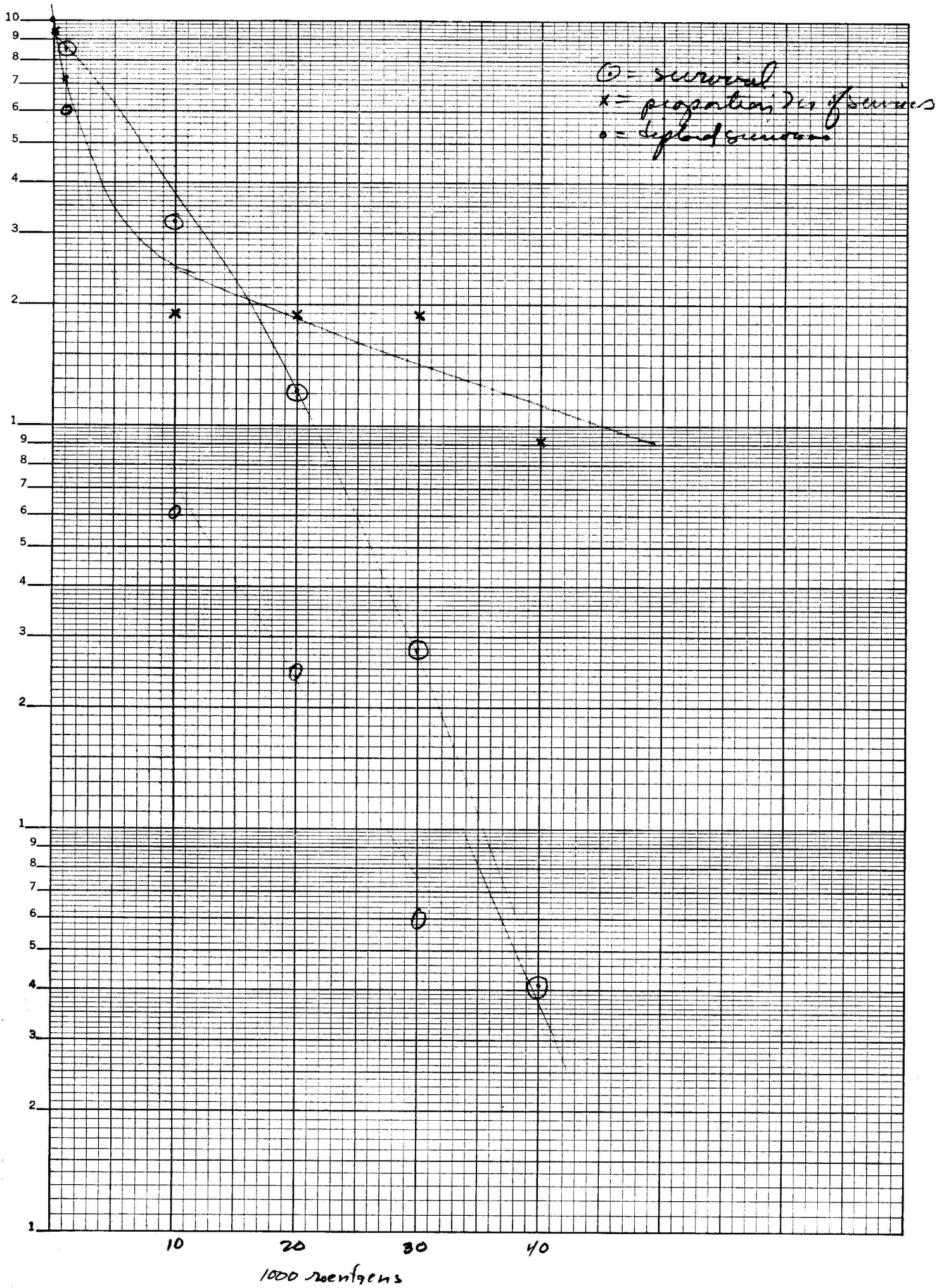
19	112				
30	102				
27	121				
34	140				
110	475	585	~~85~~		↑
		146	1,46	14.6	
75	610	685	.86		19
				8.6	19

30 min
1×10^{-5}

40 min.
10^{-5}

9	73				
4	31				
3	37			4.9	9.3
3	36				
18	177	195	.49		

		Sensorial % v
$10^{-7} \times \frac{471}{4}$	1.2×10^{9}	1.0
$10^{-7} \times \frac{407}{4}$	1.02×10^{9}	.85
$\cancel{4} \times 10^{-7} \times \frac{314}{4 \times 2}$	3.9×10^{8}	.32
$10^{-6} \cdot \frac{585}{4}$	1.46×10^{8}	.121
$10^{5} \cdot \frac{685}{2}$	3.4×10^{7}	.028
$10^{5} \cdot \frac{195}{4}$	4.9×10^{6}	.0041



Partial segregations: verifications and purifications

March 4, 1950.

Many(?) from 693a 2/22/50. 1-4 contains lac⁻ Mal⁻. Pick single lac⁺ from EMS lac and streak out on Mal; put on plants. = ~~699: 1-4~~

6, ~~5~~ may also be lac⁻ Mal⁻ ~~699.5~~

3/1 693 B. Streak on EMS Mal. ~~699-6.~~

693 A. 1, 3, 4, 5, 7, 8, 9, 10, 11. EMS lac ~~699: 7-15~~

H227 (See 668J) Lac⁻ Mal⁻. ~~699.16~~

Repurify all cultures on EMS lac or Mal.

699:	From	EMS Mal	Purported: Mal		Lac		Xyl	Mtl	Mal Lac
1	# 693a 6	all +	V	V	—	V			
2	693B	+, −	V	+	—	—			
3	H227	−	—		V	V ✓			
4	693A 1	−, +	—		V	V ✓			
5	3	−	—		V	− ✓			
6	4	−	—		V	−			
7	5	−	—		V	V ✓			
8	7	−	—		V	V ✓			
9	8	−	—		V	V ✓			
10	9	−	—		V	V ✓			
11	10	−	—	−	V	✓ ✓			
12	11	−	—	−	V	✓ ✓			
13	693a 1	−	—	−	V	✓ ✓			
14	2	−	—	−	V	✓ ✓			
15	3	−	—	−	V	✓ ✓			
16	4	−	—	−	V	✓ ✓			

P6: Pick 1 colony from EMS and streak out to establish purified stocks for reversion tests, etc. Results in pencil

#1 and 2 Require further purifications for Mal⁺ lac⁻ type

#5 and 6 " " " " Mal⁻ lac⁺.

Abundance of lac⁻ Mal⁺ is seen.

Partial segregants.

March 10, 1950.

#1 Pick several (EMS) Mal+ colonies to EMB Mal. Of 12, #2, 7, 8, 12. Restreak on EMB Mal as potential Mal v Lac −: Confirmed.

#2 Only Mal+ and Mal − found on restreaking.

#~~5~~ 6 6 Lac+ (v?) Mal − ~~#6~~. N.G.
#~~6~~ 5 ~~#~~4 Lac v Mal − OK Lac v Mal −

Retest these cultures:

		Lac	Mal	Mtl	Xyl		
1	1	—	v	v	v	Type 1	
3	3	v	—	v	v	2	
4	4	v	—	v	v		
5	5	v	—	v	v		
7	7	v	—	v	v		
8	8	v	—	v	v		
9	9	v	—	—	—	3	
10	10	v	—	v	v		
	11	v	—	v	v		1. Mal v Lac v! − pied? v for all ...
	12	v	—	v	—	4	
	13	v	—	v	v		1. Mal + Lac −
	14	v	—	v	v		
	15	v	—	v	v		
	16	v	—	v	v		
	17	v	—	v	v		
	18	v	—	—	—		
	19	v	—	v	v		
=712A1	20	—	v	v	v	1	
A2	21	—	v	v	v	1	

Mal + reversions:

P15 699 − 11 Mal+ R Carry as 699 − 11R1. Lac v Mal v−

P17 11b. Lac − Mal +. Segregant reversion.

3a Lac v Mal v+ + Relatively stable. few sectors! 699 − 3R1
4a Lac v Mal v Not unusually stable 699 − 4R1

Balanced lethals

700
C

March 4, 1950.

a) Inoculate 698-0 (control) and 698-40 (X-ray 40,000r) 1:1000 in penassay and aerate: effort to induce segregation.

b. 698B1-7 are 7 stable lac+ from X-rayed H226.
695-92A is a single pure lac+ from UV H226.

Test balanced lethals. 700=[1-7]; 695: 1-3. Streak on EMB lac, Mal, Xyl, Mtl. Brush EMS Lac.

	Lac	Mal	Xyl	Mtl	
1	+V	—	+, [redacted]	++,±	} disqualified as stable diploid!
2	+V	—	+, —	++	
3	+	+	+	++	
4	+	—	++ sheen	++	Only #3 is uniform +; others show
5	+	—	++ sh	+	various changes.
6	+	—	++ sh	+	
7	+	+	—	+	
695 "8	+	—	—	—	All grow very poorly on EMS lac.
695 9	+	—	+	+	
695 10	++ sheen	—	—	—	

[redacted] See 693d.

#1 has sane colonies clearly almost pure lac+, others lac v! Restrain lac+: These give predominantly apparently pure + colonies. A very rare colony may appear -. Pick a type + and use as 700-2.

P7: Plate .1 ml grown culture of 700-3 with T6; also K12
Check nutrition of 700-3.: 3/8 – Meth + [redacted] (~~not prototrophic~~)

700-3 is probably not diploid: a) ~~is~~ gives pure Mal+ non-crossing
b) V_6^R mutants are pure lac+

Search for balanced lethals

700a
A-B

March 5, 1950.

A B.
Inoculate H226 and H226 - X Ray 40,000 into ~~X~~ Permassay and etc.

P5: Plate 1st culture. 3P5 Inoculate ca 1:1000 into fresh Permassay.
10P5 Reinoculate (3d culture). also B2.

B1 and B2 on EMB lac < ½% Lac+. Pick few + from B1 and B2
All appear to be Lac+ = 700B: 1-6
A1 Ca. 10%+. Pick 40 + and streak out.