From here to 412
B gal SM platings were
from method

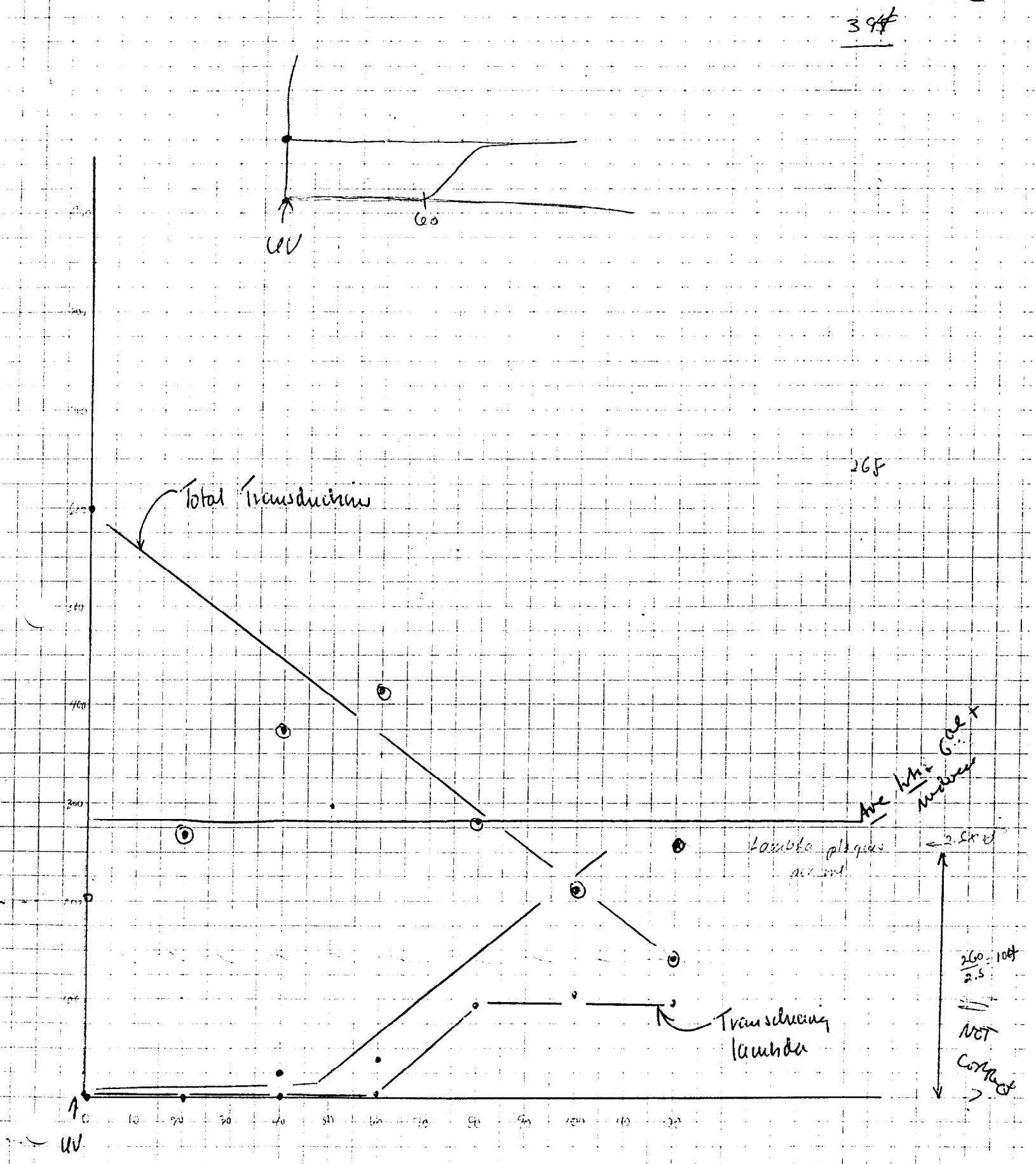
DATE: 2/15/56 REF: 394

1	2	3	4	5	6	7	8	9	10
2580 – overnight, incubated 2.5 hrs with air. Centrifuged resusp'd in solvent –							+3079	22 / 13.0 / 11.0 / 0.51	59% survival
	Pre-irrad. cell assay			✓ Bgal		Bgal SM			
			dil 10^6 – 0.05	① 107					
				✓ ② 110					
				217 × 10^7 = 2.2 × 10^9					
	" "	trasd assay undiluted →				✓ ③ 0	1/10 cx-res plaques	✓ on B gal	
						✓ ④ 0		✓ 30 12	
								40 21	
		Sus + 9 ml 2x pen							
10	Irradiate 40 sec								
Pure culture sus. pen. 1 × 2 ml	Post. irrad. cell assay		dil 10^6 – ✓	⑤ 62	λ assay	B gal ✓ ⑦ 26 plaques		71 × 10^7 = 7.1 × 10^8 plaque cultures	
			✓	⑥ 70		✓ ⑧ 45			
				132 × 10^7 = 1.3 × 10^9		71		5/14 · 71 = 355/14 = 25.5 × 10^8	
	Post. irrad. trasd. assay undiluted			⑨ on 30 plates 400 cells		✓ ⑪ 0	Almost confluent		
				✓ ⑩ " "		✓ ⑫ 0		This column B gal SM	
0 / 1.20									
time in 24 hrs									
20 / 1.40				⑬ 279		✓ ⑮ 0	1/16 = cx-res plaques		
				✓ ⑭ 252		✓ ⑯ 0			
				531/2 = 266					
							dil 10^6 .05 + 3079		
							✓ ⑰a 137		
40 / 2.00				✓ ⑰ 350		✓ ⑲ 0	1/16 cx-res plaques	✓ ⑮a 162	
				✓ ⑱ 397		✓ ⑳ 0		299 × 10^7 = 2.9 × 10^9	
				747/2 = 374					
								✓ ㉑a	
60 / 2.20				✓ ㉑ 419		✓ ㉓ 1		✓ ㉗a 163	
				✓ ㉒ 488		✓ ㉔ 3		189	
30				830/2 = 415				352 × 10^7 = 3.5 × 10^9	
80				✓ ㉕ 312		✓ ㉗ 108 (many small)			
				✓ ㉖ 248		✓ ㉘ 81			
				560/2 = 280		189/2 = 95			
							centrifuge 3 ml isgal + 3079		
100		0.05 ml + 3079 on D(0) ④ ✓ ⑧ ✓ column at 20 hrs		✓ ㉙ 195		✓ ㉛ 95 (many small)		✓ ㉙a 57	
				✓ ㉚ 214		✓ ㉜ 109 (many small)		✓ ㉚a 67	cultures
				409/2 = 205		204/2			
120									
40				✓ ㉝ 125		✓ ㉟ 68 (many small)			
				(150) ✓ ㉞ 149		✓ ㊱ 127 (many small)			
				274/2 = 137		195/2 = 98			
							dil 10^7 .05 + 3079		
							✓ ㊲ 123		plaque
							✓ ㊲a 139		
				Total = 1677				262 × 10^8 = 2.6 × 10^{10}	
				Ave Total/6 = 1677/6 = 279					
600 × 20 × 10/3 = 24000 = 2.4 × 10^4 / 2.2 × 10^9 cells = 1/10 cells									
50									

104
48
147/3 = 99

99/279 = burst size

279 | 99.0 (0.36) / 837 / 1530



UV
60
Total Transduction
Transducing lambda
UV

DATE: 2/16/56 REF: 395

1. Cross done $\boxed{2/13/56}$ of 300 9 x 341.9
Hfr Gal, lp^v ⊗ TLB$_1$ ≡ 2$^-$/2$^-$ lp$^+$

1. 1.0 ml of each parent added 9.0 ml per, incubated 3 hours, diluted 1-100 with tetrated Hott, 0.1 ml

	(+)	(−)
2 (a)	~~X~~ 7	ca 250
(b)	5	ca 300
(c)	5	ca 250

10$^-$ colonies picked streaked B gal - grew up as Gal on B gal - 10 col B gal picked to D(0) - all grew therefore prototrophic

The 10$^-$ tested against HFT 1$^-$ HFT 2$^-$
10 + 0 = Gal$_2$$^-$

3 Gal + . after 2 streaks tested Genotype

seq. ?	/λ	/3079		
1. 0	R	+		lp$^+$
2. 0	R	+		lp$^+$
3. 0	R	+		lp$^+$
4. 0	R	+		lp$^+$
5. +	R	+	lp$^+$	lp$^+$
6. 0	R	+		lp$^+$
7. +	R	0	lp^R	lp$^{R/S}$?
8. 0	R	0		lp$^{R/S}$?
9. 0	R	+		
10. +	R	+	lp$^?$	
11. 0	R	+		
12. 0	R	+		
13. 0	R	+		
14. 0	R	+		
15. 0	R	+		
16. 0	R	+		
3 +	16 R	14 +		
13 0		2 0		

$2^+ 1^- S \times \frac{2^- 1^+ 1^?}{2^- 1^+ 1^?} \rightarrow \frac{2^+ S}{2^- R}$

3 seq obtained	/λ	/2	λ
5	+	0	R
7	+	0	S
10	+	0	R

DATE: 2/17/56 REF: 396

2637 derivatives from 390
labelled - after some re-arrangement, and re-arrangement

	Presumed	3079	Growth Q(0)		Crossing
W3091	Gal1 - Lp+	nl	+	partially S	Lp^R
92	Gal2 " +	nl	+	R	Lp^S
93	—				
94	Gal4 - Lp+	nl	+	R	Lp^R
95	—				
96	Gal6 - Lp+	lysog.	+	R	Lp^r
97	Gal7 - Lp+	lysog.	+	R	Lp^+
98	—				
99	—				
W3100	Gal+ Lp+	lysog.	+	R	Lp^+
3101	Gal1 - Lp^S	~~[illegible]~~			
3102	Gal2 - Lp^S	~~[illegible]~~			
3103	~~[illegible]~~				
3104	Gal4 - Lp^S	—			
3105	—				
3106	Gal6 - Lp^S	~~[illegible]~~ lys.	+	S	Lp^S
3107	Gal7 - Lp^S	~~[illegible]~~ lys.	+	S	Lp^S
3108	—				
3109	—				
3110	Gal+ Lp^S	~~[illegible]~~ lys.	+	S	Lp^S

Frequency of homogenotes among stable (+)
Lft lysate = K12 8/10/55

1. 2580 spont = 8
   o.l.w. = 573

2. 3079 spont = 0
   o.l.w. 517

3. 750 spont = 3
   o.l.w. 356

DATE: 2/28/56 REF: 397

1	2	3	4	5	6	7	8	9	10

2580 one step using 3077 as indicator.
overnight cult diluted 1-10, aerated 3 hours.

Survival 318 / 49.0 (0.154; 31.5; 1720; 1590; 1300) = 15.4%

II

PRE-UV — 1 ml 10⁶ — 0.05 ml — 58

O.D. / water = 1/10 dil = 0.206

	Bgal		Bgal SM
(1)	148	(3)	0
(2)	170	(4)	0
	318		

cell count Array 00's — Pre transd. Array, plaque array — Same plate.

00 .26

1-10 dil

U.V. 60 seconds — add 3 ml + 9.0 ml Pen 2X — w. indicator pre-coat

Post U.V. — not added to Pen.

10

dil 10⁶ — 0.05 ml

1:10 PM 0 →

	Bgal	
(3)	32	survival
(4)	27	
	49	
(5)	887	transd. centers
(6)	1097	
	1984	

	Bgal	
(7)	58	for 0 plaque
(8)	64	
	61	
(7)	0	(ca 1000 plaques)
(8)	0	(ca 1000 plaques)

pen 1:10

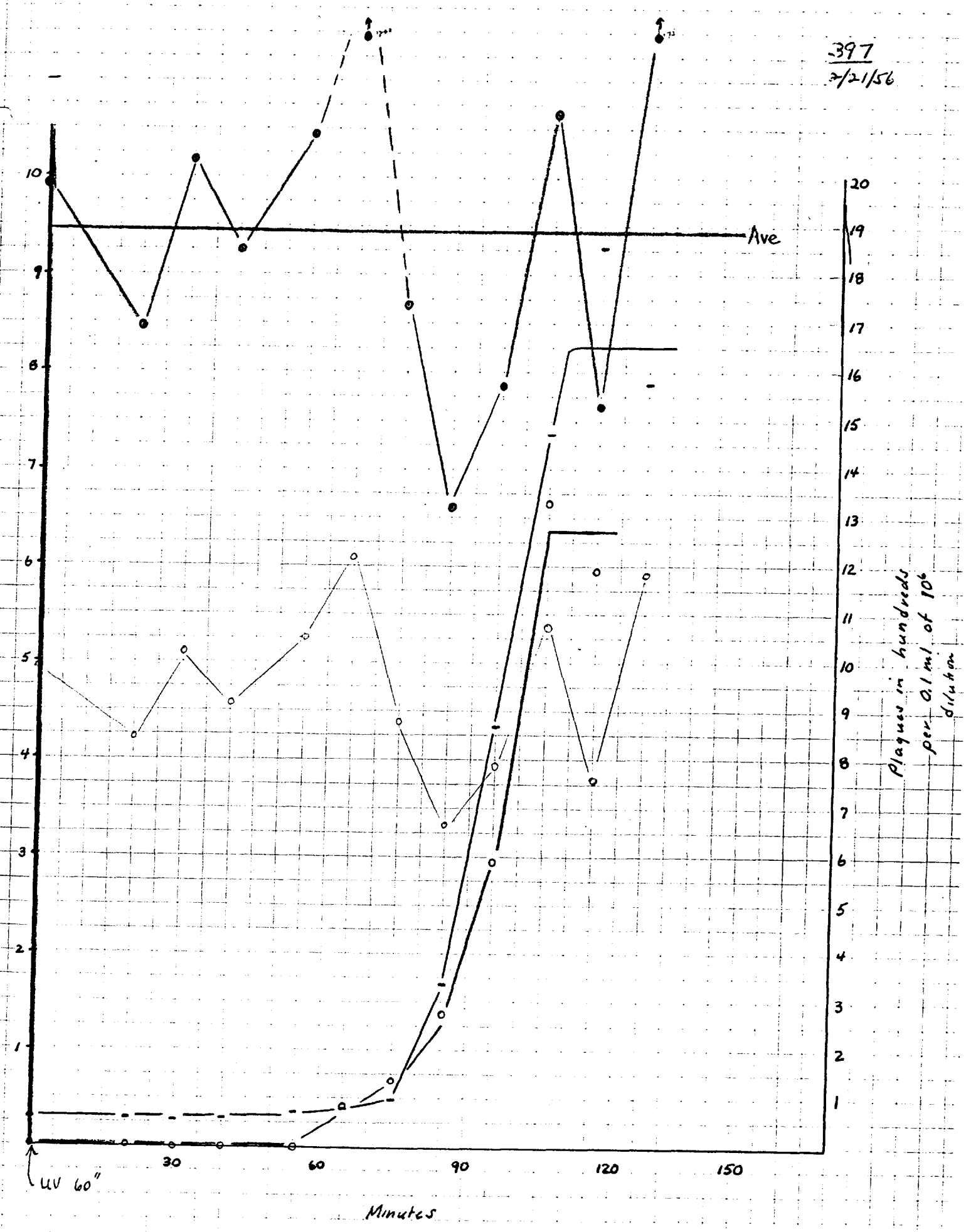
992

0 1/2 used

dil 10⁶ — take samples. Time

	(9)	27	1:30 PM	20	(9)	476	(11)	0	1/2 = 993 plaques
20	(10)	29			(10)	368	(12)	1	ca 700 plaques
		28	56			844			
pen step	(11)	32	1:40	30	(13)	467	(15)	0	1/2 = 195 plaques
	(12)	21			(14)	552	(16)	0	ca. "
		27	53			1019			
	(13)	38		40	(17)	462	(19)	0	ca 1000 plaques (> #15, 16, 20)
	(14)	32	1:50		(18)	461	(20)	0	" "
		35	60			923			
25	(15)	33	2:05	50 (55)	(21)	528	(23)	0	
	(16)	36	69		(22)	517	(24)	0	
30		35				1045			
	(17)	39	2:15	60 (65)	(25)	595	(27)	23	
	(18)	38	77		(26)	621	(28)	18	41
		39				1216			
	(19)	35	2:25	70 75	(29)	427	(31)	29	61
	(20)	63	98		(30)	441	(32)	39	68
		49				868			
	(21)	109	2:35	80 85	(33)	296	(35)	46	
	(22)	221	335		(34)	364	(36)	91	
		168				660		137	
40	(23)	452	2:45	90 95	(37)	358	(39)	136	
	(24)	413	865		(38)	395	(40)	159	
		433				753		295	
	(25)	772	2:55	100 105	(41)	505	(43)	313	
	(26)	701			(42)	562	(44)	352	
		737	1473			1067		665	
	(27)	960	3:05	110 115	(45)	408	(47)	306	
	(28)	873			(46)	350	(48)	291	
			1833			758		597	
	(29)	873	3:15	120 125	(49)	612	(51)	212	
50	(30)	700			(50)	563	(52)	125	
			1573			1175		337	

397
7/21/56


Ave
10
9
8
7
6
5
4
3
2
1
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
30
60
90
120
150
UV 60"
Minutes
Plaques in hundreds per .01 ml of 10^6 dilution

DATE: 2/22/56 REF: 398

Better transduction indicators?

Lysate = 364A1 diluted 10^{+} .05 ml taken

1. Effect of starving. Overnight cultures of 3079, 3080 sedimented resuspd in saline, sedimented, resuspd in D(M). Aerated 6 hours, 0.1 ml used as an indicator. Broth cultures consisted of freshly grown cultures (6 hours from overnight, both unaerated) probably lower cell density than starved cells

Platings on B gal SM

1. Platings	Pour	Surface	Broth	Starved	Counts	
3079	+	o	o	+	139	ca 1000 plaques each
	+	o	o	+	158	
	+	o	+	o	31	ca 1000 plaques
	+	o	+	o	24	
	o	+	+	o	52	
	o	+	o	+	164	
3080	+	o	o	+	200	
	+	o	o	+	322	
	+	o	+	o	65	
	+	o	+	o	149	
	o	+	?	?	138	plates not labeled
	o	+	?	?	127	

These results suggest that starved cells may be better as an indicator. Cell densities were not the same and it will be better to repeat using a density check.

DATE: 2/24/56 REF: 399

One step with 945 (archive from AR) technic and numbering as in 397 with the exceptions. Surviv = 5/93 = ~5%

A. Expt.

1. Culture of 945 (mal- Ly2^R) instead of 2580 (mal+ Ly2^S)
2. Density of susp. (irradiated) adjusted to 0.2/58 "UV.-60"
3. Diluted 1-10 instead 3/12 (.05 ml samples)
4. Times (absolute) the same - samples taken at 50, 60, 70, 80 ck. instead 5, 15...

pre-cell (1) 44, (2) 49 (93); post cell (3) 3, (4) 2 (5); (3) 0 (4160 plaques), (4) 0; (5) 179, (6) 147, 326; (7) 0 (4545 plaques), (8) 0 (4153 plaques) post UV Free transd.

transd. centers measured; dilute 1-10

B. Results

Time		2		3	4		7	8
					2 Gal		B gal SM	
0	(7)	0		(9)	12	(11)	0	57 plaques
	(8)	0		(10)	15	(12)	0	31 plaques
					27			
20	(9)	6		(13)	27	(15)	0	71 plaques
	(10)	11		(14)	20	(16)	0	77 plaques
		17	(17)		47			
30	(11)	7		(17)	66	(19)	0	149 plaques
	(12)	11		(18)	69	(20)	0	146 plaques
		18	(18)		135			
40	(13)	8		(21)	47	(23)	0	1/2 = 66 plaques
	(14)	4		(22)	53	(24)	0	"
			(12)		100			
50	(15)	9		(25)	80	(27)	0	91? (small) almost confluent
	(16)	8		(26)	35	(28)	0	74? (small) " "
			(17)		115			
60	(17)	8		(29)	84	(31)	0	40? (small) confluent
	(18)	4		(30)	92	(32)	0	73? (small) "
			(12)		176			
70	(19)	8		(33)	93	(35)		1 large
	(20)	7		(34)	98	(36)		1 large
			(15)		191			2
80	(21)	10		(37)	60	(39)	22	
	(22)	9		(38)	63	(40)	9	
			(19)		123		31	
90	(23)	12		(41)	58	(43)	28	
	(24)	8		(42)	38	(44)	28	
			(20)		96		56	
100	(25)	62		(45)	88	(47)	78	
	(26)	23		(46)	47	(48)	84	
			(85)		135		162	
110	(27)	74		(49)	80	(51)	84	
	(28)	84		(50)	60	(52)	90	
			(158)		140		174	
120	(29)	184						
	(30)	145						
		329/16 = 20						

Surface plating? ... plaques

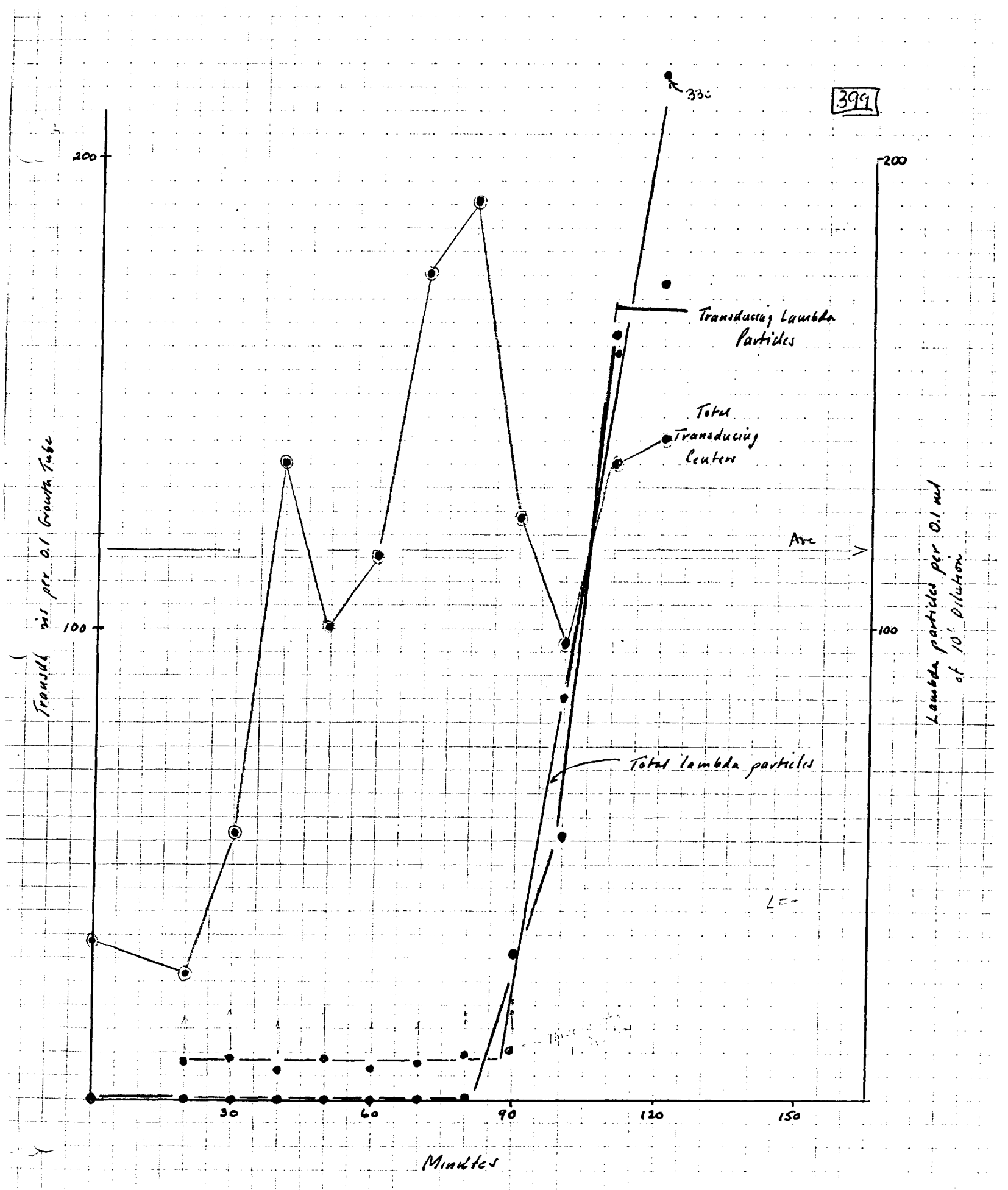
Total Transd.

117 / 1 ... 18

Free transd.

W3079

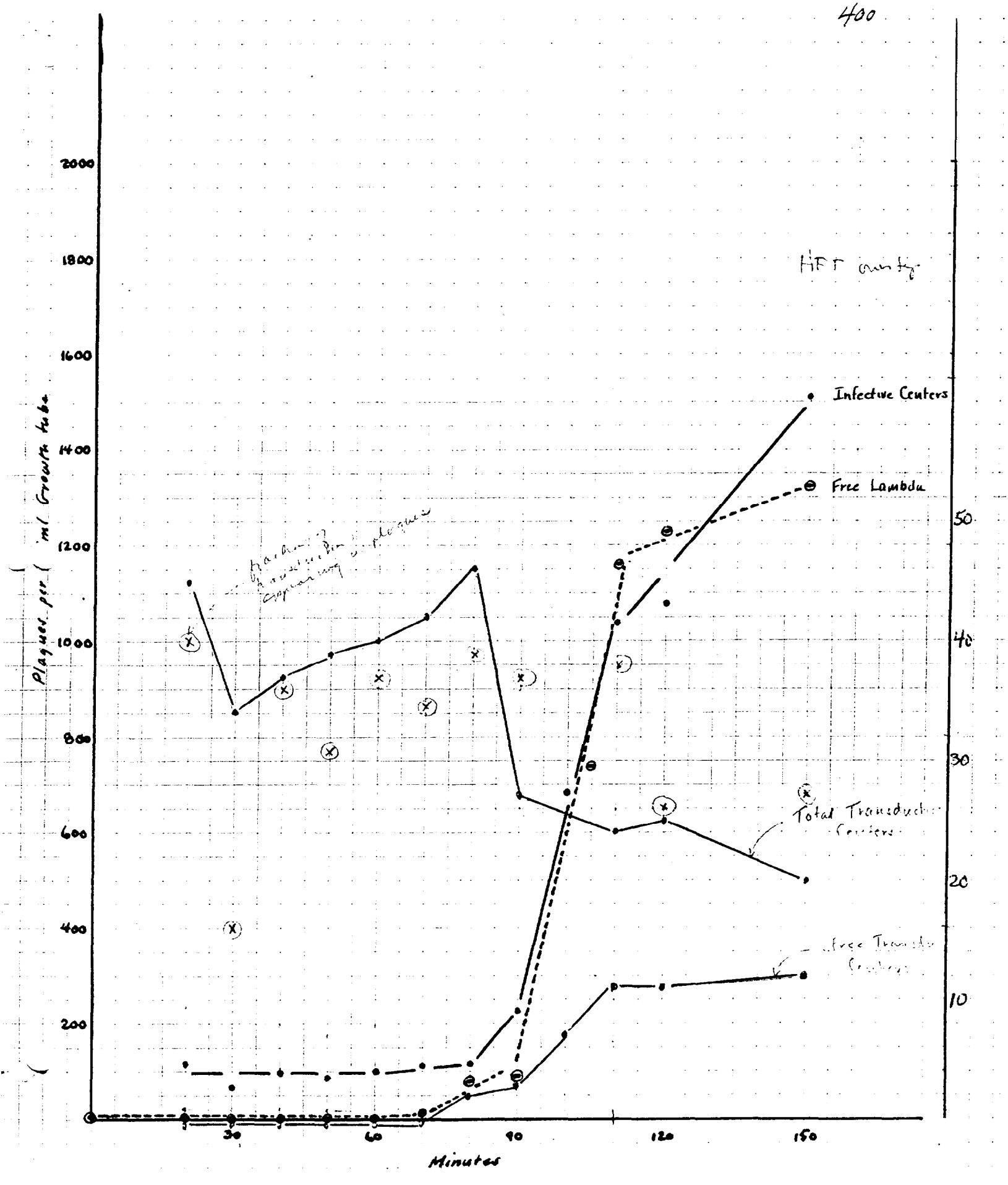
Pure plaques testing


330
200
100
Transduc. per 0.1 Growth Tube
Transducing Lambda Particles
Total Transducing Centers
Ave
Total lambda particles
Lambda particles per 0.1 ml of 10⁻⁵ Dilution
30
60
90
120
150
Minutes

DATE: 2/27/56 Survival = 16/40 = 0.40 REF: 400

1	2	3	4	5	6	7	8	9	10
	One step with 341-9							Plaque count in 9 ml =	
45 65, 44 69 after med		1. Overnight culture diluted ca 1-10, incubated in rotator at 37c for 2 hours. Centrifuged, resusp. volume to O.D. = 0.58							each of these 2 of LFT
15/17 18/21 at 0		2. Phi assay (A) dil 10^2-10^4-10^6 → 0.05 ml spread per volume: ASSAY THESE COL. FOR HFT →				(1) ✓ (2) ✓	Bgal (23) (17) 40	Bgal SM — 4/40 = 10%	
10		(B) transd. assay 10^6 dil 0.05 plaque } / 3079				0 plaques ✓ (3) 1; 0 plaques ✓ (4) 0	✓ (5) 0; ✓ (6) 0 transd. occurring ✓ plaques	transd. occurring ✓ plaques	0 plaques; 0 plaques
	3. Incubate 60 sec				(10 LFT)				
~~0~~ 12:15	4. Dilute 10	10^2-10^4 → 10^5 = 0.05	Incubate - plated at various time intervals	surpose ✓ for HFT		✓ (7) 76; ✓ (8) 51 / 157	✓ (9) 0 plaques; (10) 0		0 plaques; 0 plaques
~~20~~ 12:35	20		15/157 = ca 10%	(5 LFT)		✓ (11) 22 19/22 57; ✓ (12) 23 19/22 59 / 45 116	✓ (13) 0; ✓ (14) 0	0; 0 / 0	
20 ~~40~~ 12:45	30		Cell disappearance, 40-16 = 24; Infect. centers produced 20' = 12	(bad plate) partially solidified in pouring, but went on		✓ (15) 20 4/20 27; ✓ (16) 14 9/14 37 / 34 64	✓ (17) 0; ✓ (18) 0	0; 0 / 0	
~~50~~ 12:55	40		30 = 6; 40 = 10; 50 = 9; 60 = 10; 70 = 11; 80 = 11; Ave = 8 5/6 = 10			✓ (19) 11 10/11 42; ✓ (20) 26 13/26 56 / 37 98	✓ (21) 0; ✓ (22) 0	0; 0 / 0	
~~60~~ 1:05	50					✓ (23) 12 6/12 32; ✓ (24) 21 10/21 53 / 39 85	✓ (25) 0; ✓ (26) 0	0; 0 / 0	
~~70~~ 1:15	60					✓ (27) 18 9/18 50; ✓ (28) 22 5/22 71 / 40 99	✓ (29) 0; ✓ (30) 0	0; 1 / 1	
~~80~~ 1:25	70					✓ (31) 23 16/23 64; ✓ (32) 19 8/19 49 / 42 113	✓ (33) 0; ✓ (34) 0	10; 6 / 16	
~~90~~ 1:35	80					✓ (35) 25 12/25 55; ✓ (36) 21 11/21 55 / 46 110	✓ (37) 1 1/1; ✓ (38) 1 1/1	16; 66 / 82	
~~100~~ 1:45	90					✓ (39) 13 11/13 63; ✓ (40) 14 10/14 22 / 27 225	✓ (41) 1 1/1; ✓ (42) 2 2/2	35; 58 / 93	bad plating solution
1:55	100					plated Bgal ✓ (43) / 264; ✓ (44) / 412 / 676	✓ (45) 3 3/3; ✓ (46) 4 3/4	435; 306 / 741	
2:05	110					✓ (47) 7 7/7 352; ✓ (48) 17 7/17 688 / 1040	✓ (49) 6 3/6; ✓ (50) 5 3/5	536; 626 / 1162	
2:15	120					✓ (51) 13 6/13 631; ✓ (52) 12 7/12 445 / 1076	✓ (53) 7 4/7; ✓ (54) 4 3/4	612; 621 / 1233	
2:25	150					(55) 9 5/9 771; (56) 11 5/11 740 / 1511	(57) 4 3/4; (58) 8 3/8	686; 638 / 1324	

transform plaque


HFT counts
Infective Centers
Free Lambda
Total Transductants Centers
Free Transductants Centers
Plaques per (ml Growth tube
Minutes
2000
1800
1600
1400
1200
1000
800
600
400
200
30
60
90
120
150
50
40
30
20
10

DATE: 3/8/56 REF:

Preparation of prototrophic F stocks with the gal markers – "Kalchai" stocks

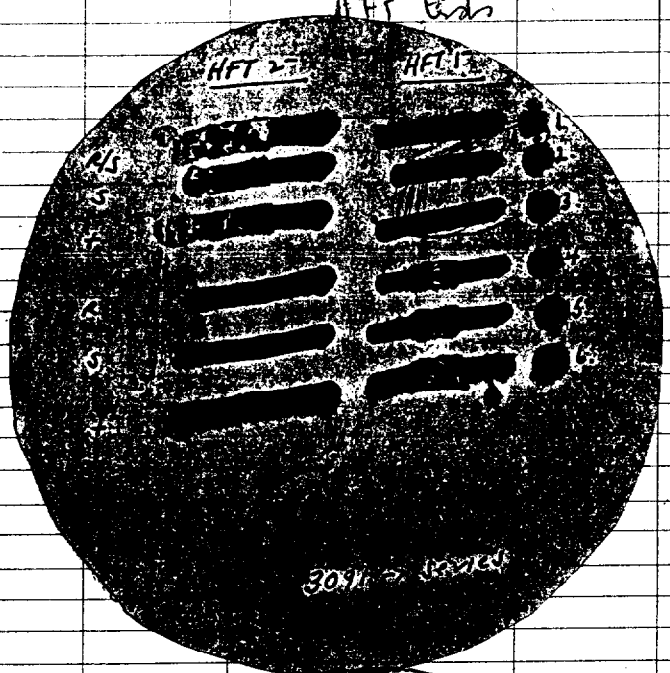
1. The numbers proposed for these are 3091 → 96 (Lp^+), 3101 → 3107 (Lp^S), 3100 → ? (Lp^R (observed))
   3100 = Lp^+ Gal^+
   3110 = Lp^S Gal^+

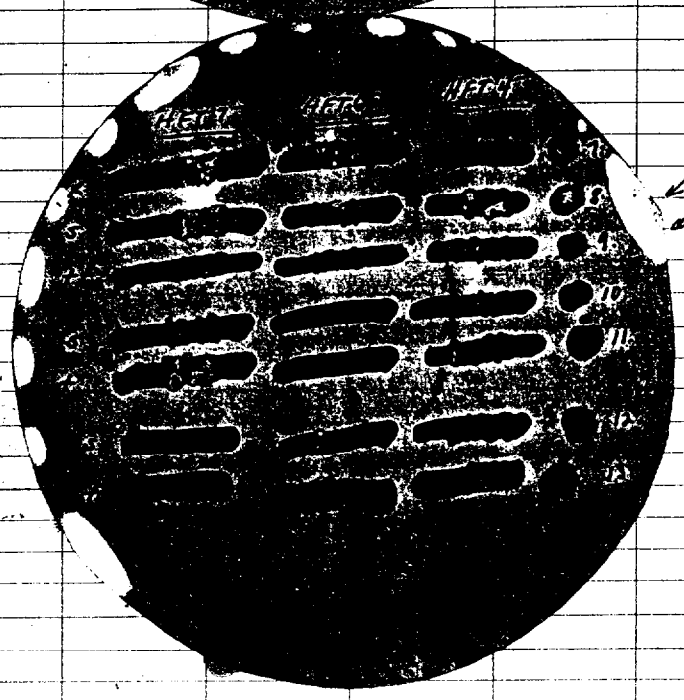
2. Beginning with 3110 = (F^- 1485) – the cells from an overnight culture expand to HFT's and diluted and plated. See page 390 also 396

3. After all cultures obtained, they were tested:

	3079	λ	Growth D(0)	Original gen. designation
1.	ul	R	+	1^- R/S
2.	ul	S	+	1^- S
3.	lys	R	+	1^- +
4.	ul ?	R	+	2^- r
5.	ul	S	+	2^- s
6.	lys	R	+	2^- +
7.	ul	R	+	4^- r
8.	ul	S	+	4^- s
9.	lys	R	+	4^- +
10.	lys	R	+	6^- s
11.	ul	S	+	6^- +
12.	ul	S	+	7^- s
13.	lys	R	+	7^- +
14.	ul	S	+	+ s
15.	lys	R	+	+ +

HFT tests





Notes
① The Gal_2^- derivatives appear to be revertable
② The cultures obtained by mat. 6^- Lp^+ (to obtain Lp^S) appears no longer to be Gal_4^-; and the Lp^+ derived is also not Gal_4^-

Redo Gal_2^-
Gal_4^- Lp^S, Lp^+

THE CULTURES FIRST ISOLATED FOR Gal_2^- and Gal_4^- STOCKS #'s 4, 5, 6, 7, 8, 9 above given numbers 401-1, 4, 5, 6, 7, 8, 9 etc

DATE: 3/11/56 REF: 402

Comparison of lysates from homogenate 34A1 - a homogenate obtained in a heterogenote line originating in an Lg^s recipient. If the higher titers of HFT lysates on Lg^+ recipients (as compared with Lg^s recipients) is due to selection of phage particles entering Lg^+ recipients (as HFT homogenates have been obtained in Lg^+ recip. lines), then there may not be higher titers on Lg^+ recip. when the line originates in Lg^s recip.

1. 6 lysates made starting from single HFT colonies (in pure culture) incub. 60 sec., incubated 4 hours. diluted 10^2, 10^4, 0.05 ml plated with 3079 (1-4-Lg^s 5^R), 3080 (1-4-Lg^+ 5^R), in B gal STM.

Lysate	3079 Plaques*	3079 Transduct.	3080 Transd.
1	ca. 15	2 (11)	29
2	20	7 (17)	28
3	29	4 (15)	7
4	26	3 (29)	25
5	17	5 (23)	14
6	7	5 (12)	19

It appears that HFT lysates obtained in Lg^s recip. clones also assays higher on Lg^+ recipients

2×10^5 / 17

$3.4 \times 10^5 = 3 \times 10^6$

$\boxed{3.4 \times 10^4}$ / 20

$7 \times 20 \times 10^4$

140×10^5

1.4×10^6

* plaque assays low because plates were dry

$27 \times 20 \times 10^4$

$\frac{3.4 \times 10^6}{3}$

1.1×10^6 transd

1.7×10^3 / 20

$.8 \times 10^3$ pfu

Observation that Gal- grow on M Gal - tests needs to see what the cause may be.

Comparison - using 1° proto homogenate, 1° seg. derived from st., 2° Lg^+ hom. derived in Lg^s (1) c- homogenate 34A-9, and a very long seg of this 2-hom.

Medium	
M (no gal)	no growth - crystals deposited along streak
M gal 0.01% glu	growth by all - colonies up to 1 mm in diam
M gal (air-dried by fellbach)	growth by all - 2's better than ones - colonies up to 2 mm in diameter
M Gal Strch	growth by all 2's better than 1's - colonies up to 2 mm

DATE: 3/13/56 REF: 403

The Kalekar stocks — Rpt.

3094, 3104 — 3092, 3102 needed.

1. Cuckoo 390-4 = 4 — x 3100 (Cal + Lp^s) → heterogenate. This heterogenate not tested directly but previous segregants found Lp^R (see 401) in addition of this seg. gave a non-only — Lp^s, also evidence of some pleage.

2. Segregants re-obtained from 390-4. 4 picked from first streak and therefore clonally related.

	HFT(+)	HFT 4	Lp^RX	
1.	+	0	Serv	← 403-1 = Lp^s (3104)
2.	+	0	"	
3.	+	0	"	
4	+	0	"	

W3104

Serv. genotype not expected — indicates that this heterogenate is Lp^R/Lp^s — If so where did pleage and serv. come from in first expt.? Contam.? What is genotype of 401-8?

DATE: 3/15/56 REF: 404

~~[illegible]~~

Irradiation of HFT lambda - lysate, approx. titer 3.4×10^6 total λ/ml - see page 402 lysate no 5 from 364A1. Dilute the lysate, 0.1ml + 10ml D(O), inadiate and add 0.1ml samples to 1.9ml Pen. - Samples (0.05ml) removed from these broth dilutions and plated, by pouring on Bgal with indicator

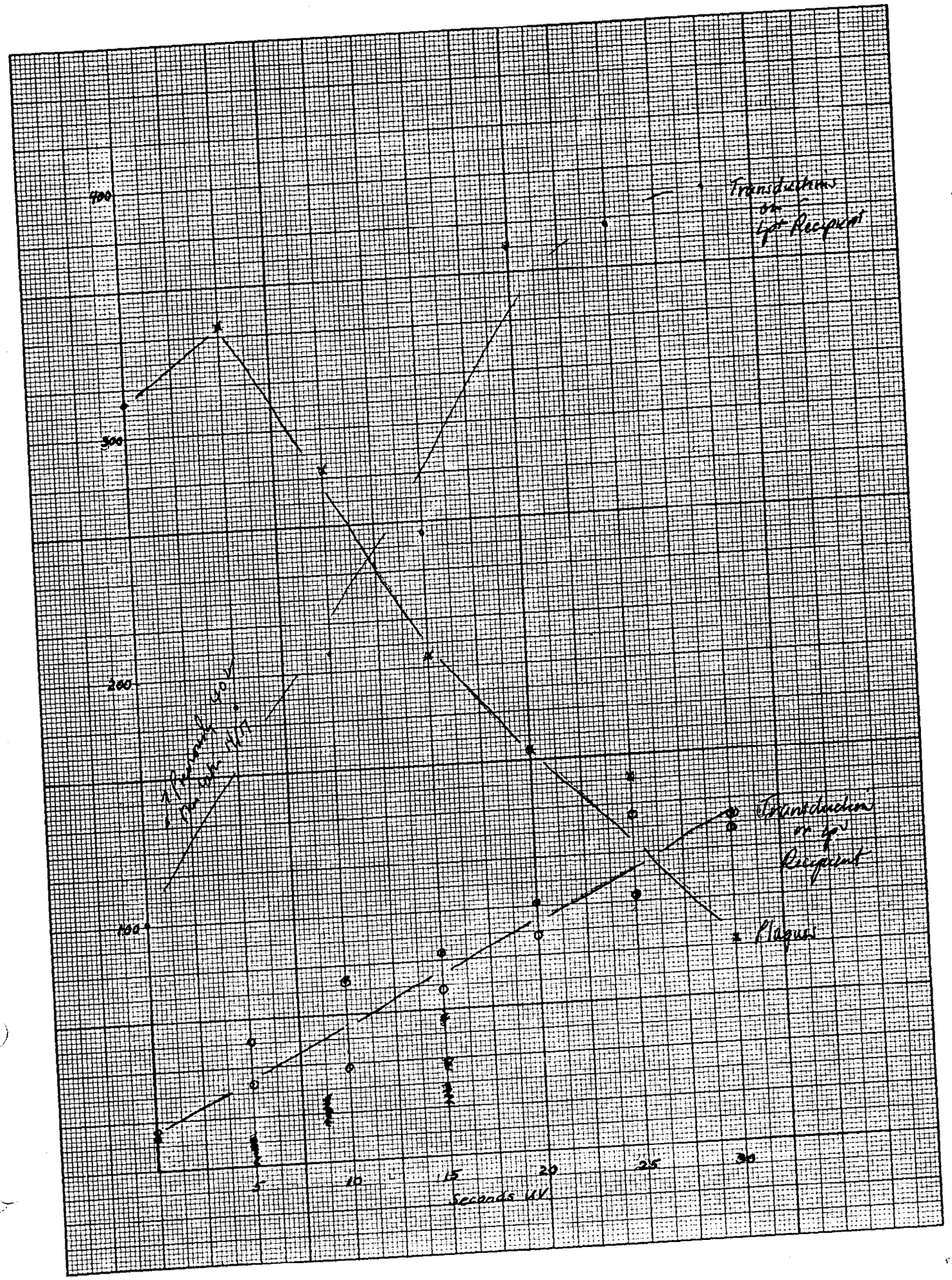
	Dose	Plaques/3079	Transd/3079	Transd/3080	plaques Bgal SM /3079	/3080	transd/3079
1	0	314	13	101 (poor plate)	164	~~60~~	155
2	5 sec.	344	34	188 "	265	~~150~~	58
3	10 sec.	283	74	208 "	170	~~126~~	38
4	15 sec.	205	83	256 "	142		68
5	20 sec.	164	101	371 "	99	~~221~~	89
6	25 sec.	151	136	378 "	98		103
7	30 sec.	83	128	ca 390 "	136		133
~~8~~	~~35 sec~~						
		L_p^s	L_p^s	L_p^+		3080 apparently contam.	

Rpt. 3/26/56

1		36		16	4
2		68		56	0
3		14	11	25	0
4		26			0
5		14	16	50	0
6		11	8		0
7		31	32	99	7

Reason for the variability unknown.

These lysates have been exposed to a variety of conditions - including moving, etc. - storage at room temp, refrig, etc.



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NO. 341-M DIETZGEN GRAPH PAPER
MILLIMETER

$\frac{3\times10^{3}}{3\times10^{7}} = 1\times10^{5}$

DATE: 3/16/56 REF: 405

Production of Transducing λ - LFT - Dosage study.

2.945 grown overnight on aerator - dil 1-4 Pen, reincubate 2 hours - Centrifuge, resuspend in saline. Adjust density to 0.58 dilute 1-20, irradiate with UV 50 cm.

All platings 0.05 on BGal

Controls 1. 945 neg. 2. 3079 3. 3080

Dose	Undiluted platings 3079		Undiluted platings 3080		Dilute 10^2, 10^4 plate 3079		cells	
0	(1) 3	(2)	5	(3)	0	(4)	128	1.0
15	(5) 26	(6)	23	(7)	14	(8)	78	0.61
30	(9) 27	(10)	12	(11)	57	(12)	42	0.33
45	(13) 14	(14)	15	(15)	60	(16)	13	[illegible]
60	(17) 5	(18)	3	(19)	49	(20)	2	0.016
75	(21) 2	(22)	2	(23)	31	(24)	1	0.008
90	(25) 2	(26)	4	(27)	21	(28)	1	
105	(29) 1	(30)	6	(31)	7	(32)	0	

Fract. cells yielding transducing activity

$\frac{\text{no transd}}{\text{no cells}} = \frac{5.4\times10^{2}}{2.6\times10^{7}} = 2.1\times10^{-5}$

Rpt modification - overnight, totaled 3-4 hours after 1-10, centrifuged, resuspd. saline, O.D. = 0.6

3/28/56 dilute 2/9 - irradiate as before - remove 0.05 ml + 4 sal, $10^2 \rightarrow 0.05$ for diluted; 0.05 as before for undiluted - Numbers as above.

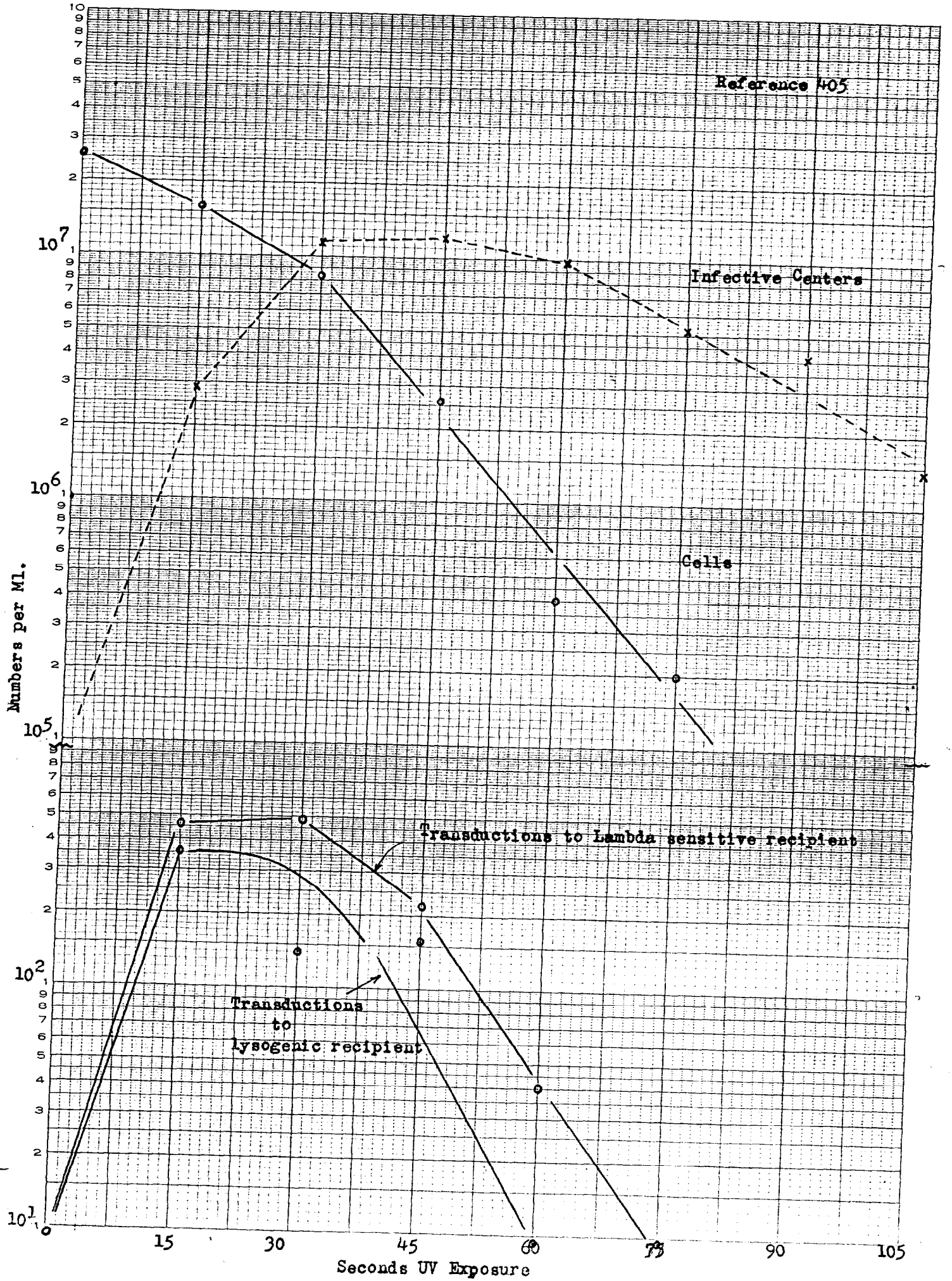
3079		Dose	Und. transd 3079		Und. transd 3080		drawn 3079 = plaque		0 = cells	3.1×10^{8}	Fraction as plaques
0/3.1×10⁸	0/3.1×10⁸	0	(1) 13	(2)	19	(3)	1	(4)	790	1.0	1/790 0.0013
0.61×10^{-5}	0.34×10^{-5}	15	(5) 108	(6)	77	(7)	18	(8)	402	0.52	2.2×10^{-2}
1.4×10^{-5}	1.0×10^{-5}	30	(9) 226	(10)	179	(11)	77	(12)	291	0.37	9.8×10^{-2}
1.8×10^{-5}	0.61×10^{-5}	45	(13) 249	(14)	118	(15)	170	(16)	173	0.22	2.1×10^{-1}
1.0×10^{-5}	0.35×10^{-5}	60	(17) 175	(18)	74	(19)	171	(20)	56	0.07	2.2×10^{-1}
[illegible]	[illegible]	75	(21) 130	(22)	26	(23)	71 low?	(24)	23	0.029	9×10^{-2}
[illegible]	-	90	(25) 40	(26)	9	(27)	44	(28)	5	0.0063	5.6×10^{-2}
[illegible]	-	105	(29) 67	(30)	7	(31)	8	(32)	0	0 -	1.0×10^{-2}

this plate from diff. batch B gal

$20\times10^{2} \times 2\times10^{2}$

Fraction of cells yielding transductions

4×10^{5} $\frac{\text{no. transd}}{\text{no cells}} = \frac{4.72\times10^{3}}{3.1\times10^{8}}$


Reference 405
Infective Centers
Cells
Transductions to Lambda sensitive recipient
Transductions
to
lysogenic recipient
Numbers per Ml.
Seconds UV Exposure
15
30
45
60
75
90
105
10^7
10^6
10^5
10^2
10^1
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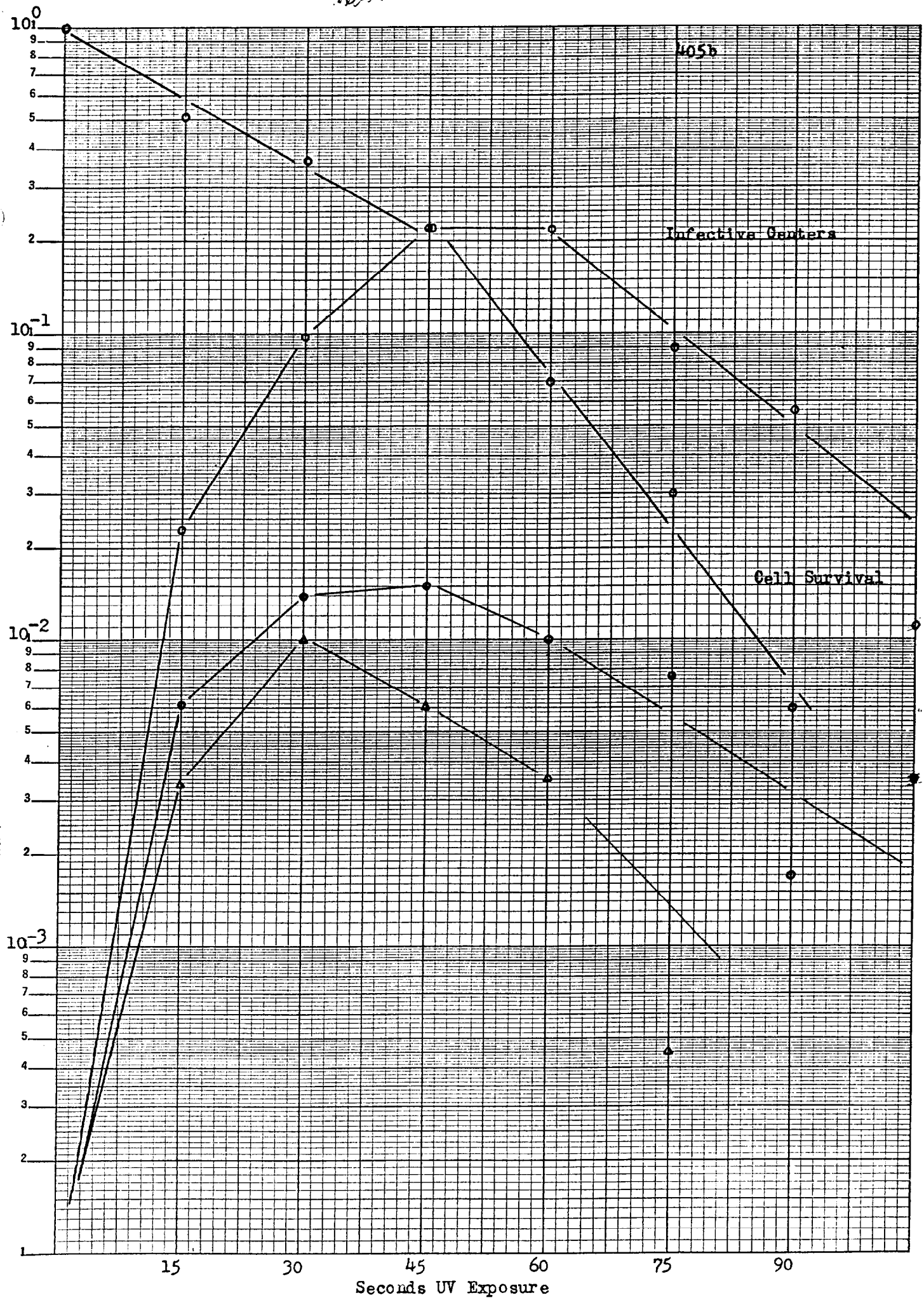
1602


405b
Infective Centers
Cell Survival
10^0
10^{-1}
10^{-2}
10^{-3}
15
30
45
60
75
90
Seconds UV Exposure
KEUFFEL & ESSER CO., N. Y. NO. 358-61
Semi-Logarithmic, 4 Cycles X 10 to the Inch, 5th lines accented.
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DATE: 3/24/56 REF: 406

Comparison of U.V. Resistance of haploid and heterogenetic clone.

1. Set up. 341-9, a 2/2- homogenote streaked out, and a LFT segregant obtained. A galt reversion of this segregant by selection on B gal. The comparison for U.V. survival is between this segregant and 341-9.

2. Experimental.

(A) 341-9 started from HFT + clone (detected on B gal - HFT test) 341A(+) from single colony.

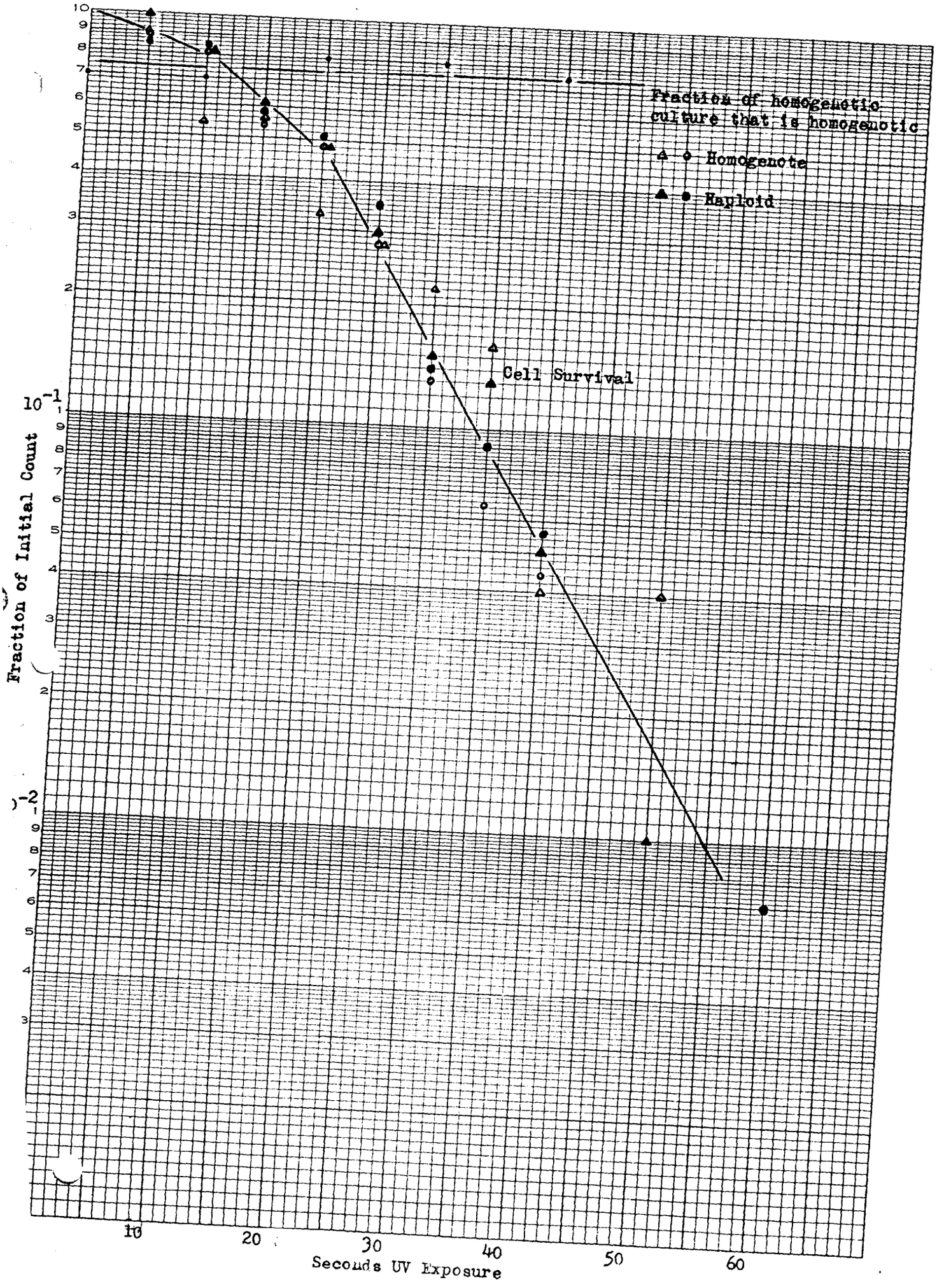
(B) Overnight cultures diluted ca 1-20 incubated 37 in rotator for ca. 3 hours. Diluted 0.2 + 10 ml saline - each into the first tube, and then 10^2, 10^4 additional. This final tube irradiated, and samples, 0.05 ml taken out and plated on B gal.

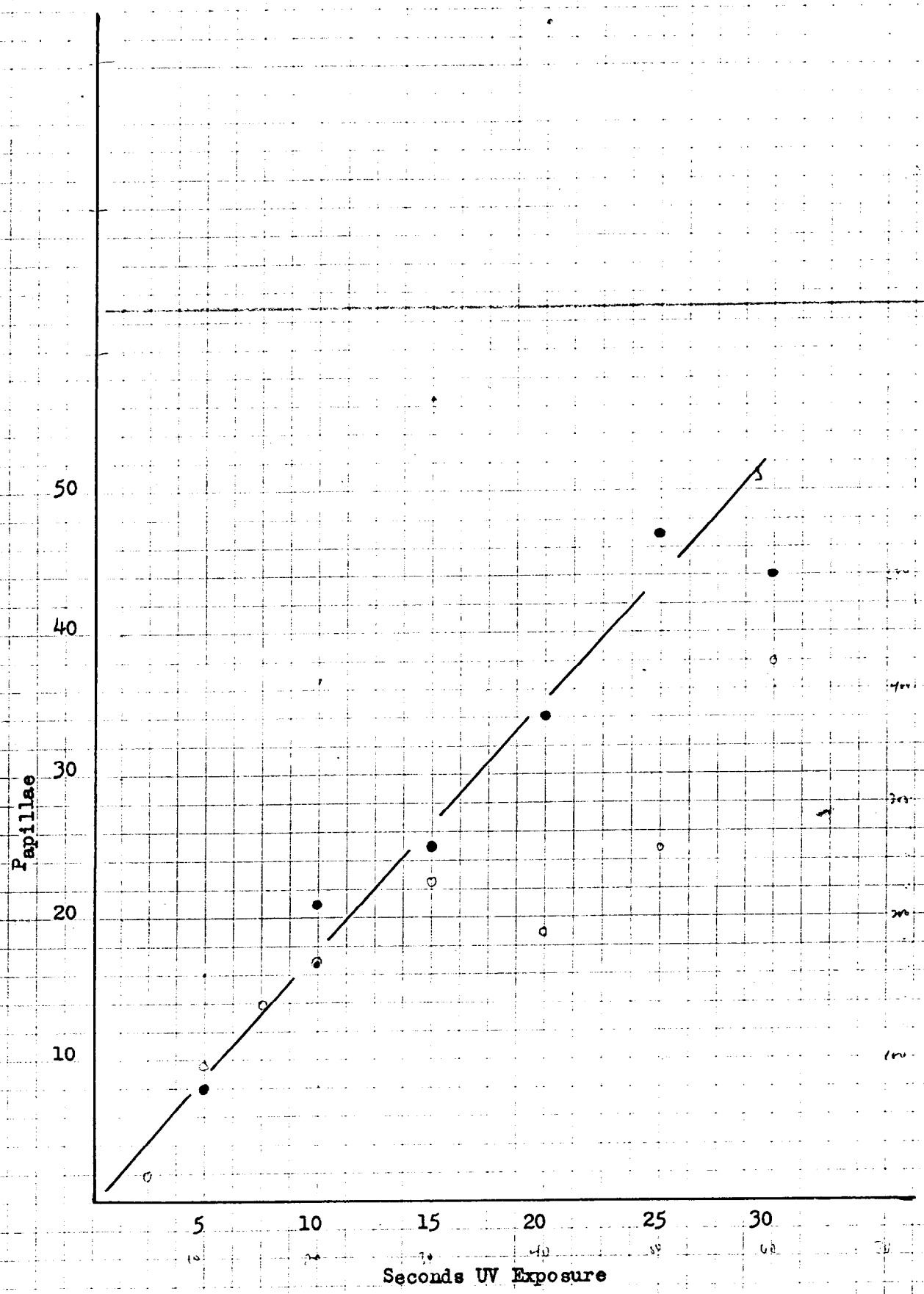
Time	Galt colonies (same scale) ①	②	Total	Fraction	Gal - colonies ①	②	Total	Fraction	Fraction of Gal - Colonies HFT +/3080	
0	226	234	460	1.0	147	129	276	1.0	14/20	0.7
5	193	214	407	0.89	116	119	235	0.85	~~14/20~~	~~0.7~~
10	173	203	376	0.82	116	117	233	0.84	14/20	0.7
15	138	110	248	0.54	66	95	161	0.58	—	
20	110	111	221	0.48	76	64	140	0.51	16/20	0.8
25	55	73	128	0.28	53	43	96	0.35	—	—
30	38	22	60	0.13	27	11	38	0.14	16/20	0.8
35	16	14	30	0.065	17	8	25	0.091	—	
40	4	16	20	0.044	5	10	15	0.016	15/20	0.75
60	0	0	0	0	1	1	2	0.007	1/2	0.5

A second analysis (3/4/56) Using a +R of 341-9 and comparing with a (-) segregant of this

Time	Galt colonies	Fraction	Gal - colonies		In the ~~Gal~~ + culture, the ratio of +/- was:	
0	325	1.0	151	1.0	+/- 2.15	Increase 0.47
5	296	0.9	152	1.0	1.95	0.52
10	176	0.54	124	0.82	1.42	0.7
15	179	0.55	92	0.61	1.95	0.52
20	108	0.33	72	0.48	1.5	0.67
25	90	0.28	45	0.30	2.0	0.50
30	72	0.22	22	0.15	3.1	0.32
35	53	0.16	20	0.13	2.7	0.37
40	14	0.04	8	0.05	1.75	0.57
50	13	0.04	2	0.01	-6.5	0.16

1. 23/1
2. 19/3
42/4


Fraction of homogenotic culture that is homogenotic
Homogenote
Haploid
Cell Survival
Fraction of Initial Count
Seconds UV Exposure
10
20
30
40
50
60
10^{-1}
10^{-2}


50
40
30
20
10
Papillae
5
10
15
20
25
30
Seconds UV Exposure

DATE: 3/29/56 REF: 407

1	2	3	4	5	6	7	8	9	10

$0.58 = 2.6 \times 10^7$ cell/ml $\times 20$ / (15×10^8) 0.64

Test of conditions required to obtain "maximum" induction in B gal

A ① Overnight aerated 948 – diluted in 1-20 incubated in rotator in 3 hours, centrifuge, resuspend volume. Adjust density to 0.6 diluted 1-20 (A)

② Susp A incub. 45 seconds, stored would contain ca 500 bacteria/ml

(A) Test – incub 45 seconds, plate 0.05 + 30 79 – ① 77 ② 49 — 63

③ Plate 0.05 ml of suspension on surface of B gal – Incubate varying doses, add 0.1 ml of 30 79 repeated.

Dose			
30"	③	99	112
	④	124	
45"	⑤	115	116
	⑥	117	
60"	⑦	86	62
	⑧	38	
75"	⑨	57	56
	⑩	54	

Are some of these contaminated?

④ Plate 0.05 ml of suspension of surface B gal – Incubate ~~30~~ hours – incubate 60", add 30 79, repeated ~~B gal plates~~ 2:30' 50:0'

Wash two plates dilute and assay for total growth – ~~ca~~ wash 1.0 ml pulmi ~~[illegible]~~ once, 0.5 ml obtained, added to 10 Pm

⑪	lost	
⑫	ca 300	
⑬	ca 300	
⑭	ca 300	
⑮	—	
⑯	—	
⑰	27	61/2 = 30
⑱	34	
⑲	34	40 =
⑳	46	

Repeated plus with dispersion may have been contam.

dil 10^2, 10^4 → 0.01 ml

$20 \times 10^4 \times (\frac{10}{2}) \times 20$ $4 \times 10^6 = 120 \times 10^6 = 1.2 \times 10^8$ cell/plate

1.6×10^8 cell/plate

B.

① Also broad	Dose	0	5	10	15	20	25	30	35
inverse	positive	5	13	26	30	39	52	49	28
response –	controls	0	8	21	25	34	47	44	23
w. X									
hours									

C.

② Incubation

(a) 6h dil 1-10 } dil 45", + 0.1 ml 30 79 = 7

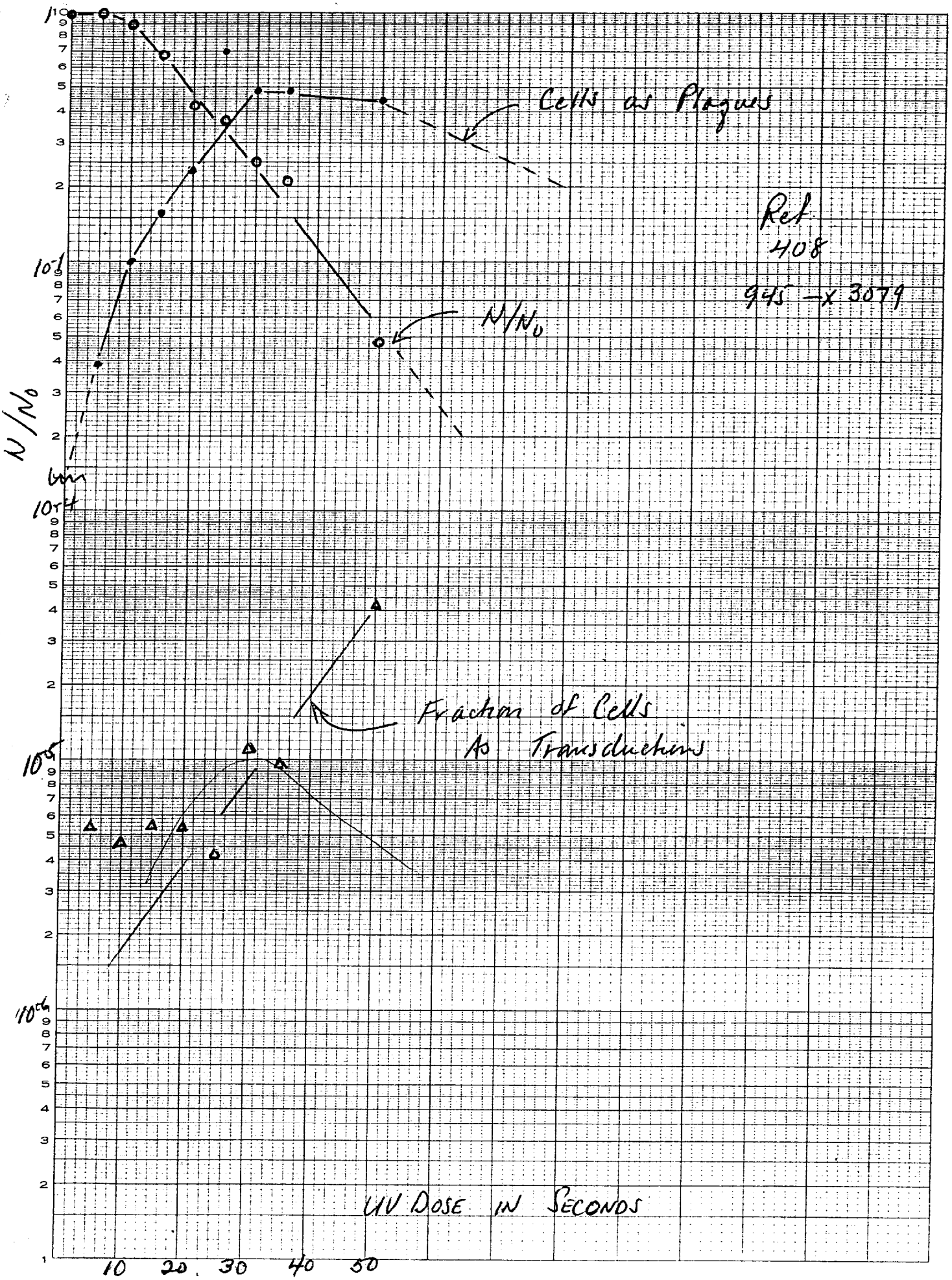
" 1-100 × 1-10 } = 0 (no Phase plaque)

$\frac{4\times10^4}{.12} \times 10^{-3} = 4.8\times10^4 \times \frac{1}{7.5}$ $\frac{0.1\times10^2}{2} = 5$

DATE: 4/1/56 REF: 408

1	2	3	4	5	6	7	8	9	10
	945 for response to UV. Linearity vs. exponential								
	Fresh culture - 3-4 hours incubation after being on the bench several days. Adjust density to 0.5 Saline (1) dilute 1:2+10 and plate for transd. response. (0.6 = 5.0 x 10^8 cells/ml) (2) Dilute 10^-1 x 10^-3 for plaque-producing response - This last, incub. (1-20 x 10^-2) and then add 0.1 to 7.5 ml. Incubate samples about 30 min. before pouring.								
incub. in plate and 0.1 ml 3179	Indicator = 3079				Est. cell	0.6 = 5X10^8 0.5 = 4X10^8			
	(A) Transduction					B. Induction of λ			Too Cells many
	Time	Plate 1	Control	Control		Time	Plate	Plaque	
	0	1	14	0		as given	10	0 15	8516 ca 2500
	5	2	28	14			11	143 158	3620 also ca 2
	10	3	26	12			12	349 364	3155 type
	15	4	28	14			13	549 564	2437 Plaque Amp Semi
	20	5	28	14			14	816 831	1580 Plaque
	25	6	25	11			15	0.1:2557 / 2.0 = 1267	1293 Cell Neg.
	30	7	43	29			16	1661	896
	35	8	39	25			17	1654	738
	50	9	120	106			18	too many ca 1600	170
						Total No cells/ml			
		Transduction = 0.1 ml X $\frac{11.2}{1.2}$ = 100			= 3.5X10^4 X $\frac{7.5}{0.1}$ X 10^2 = 3.5x75x10^8 = 2.6X10^8 cells/ml				
	2.6X10^8	Fraction of Cells as Transd.		Transd	FRACTION OF CELLS AS PLAQUES	Time	Tplaque/ml	N/N₀	N/ml
		0		0	0	0	0	1.0	3.5X10^4
		5.4 X 10^-6		1.4X10^3	0.039	5	1.4X10^3	1.0	3.6X10^4
		4.6 X 10^-6		1.2X10^3	0.099	10	3.5X10^3	0.9	3.2X10^4
		5.4 X 10^-6		1.4X10^3	0.155	15	5.5X10^3	0.68	2.4X10^4
		5.4 X 10^-6		1.4X10^3	0.230	20	8.2X10^3	0.42	1.5X10^4
		4.2 X 10^-6		1.1X10^3	0.700	25	2.5X10^4	0.37	1.3X10^4
		1.1 X 10^-5		2.9X10^3	0.48	30	1.3X10^4	0.25	9.0X10^3
		9.6 X 10^-6		2.5X10^3	0.48	35	1.7X10^4	0.21	7.4X10^3
		4.2 X 10^-5		1.1X10^4	0.45	50	1.6X10^4	0.048	1.7X10^3

Fraction of cells yielding transducing act. = $\frac{2.9\times10^3 \text{ transd}}{2.6\times10^8 \text{ cells}}$



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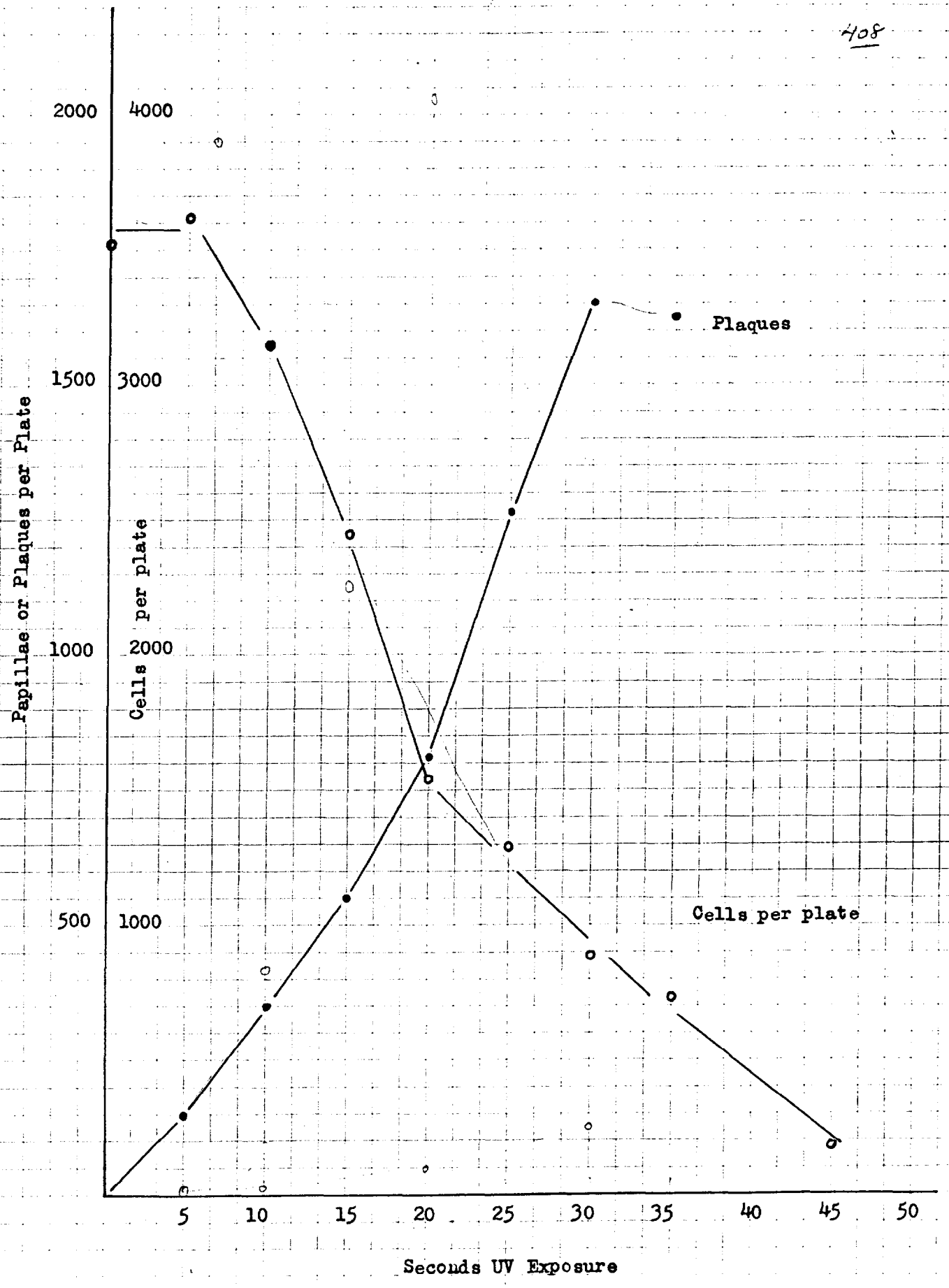

2000
4000
1500
3000
1000
2000
500
1000
Papillae or Plaques per Plate
Cells per plate
Plaques
Cells per plate
5
10
15
20
25
30
35
40
45
50
Seconds UV Exposure

DATE: 4/3/56 REF: 409

Examination of 4⁻ xx 7⁻ heterogenotes

1. 4⁻ — x 7⁻ - Overnight culture diluted 10^2, 0.1 ml + 0.2 ml lysate.
293-12 lysate | W2308 | Incubated room temp for 10 minutes, diluted 10^2, 10^4
0.1 ml plated 3 plates B gal.

A

	(−)	partially lysed	wk (+)
1.	31	(7)	1
2.	37	(5)	0
3.	29	(4)	1

3 additional possibilities appeared second day — 3B

3A

5/7/56 18 (−) seg obtained from the interval "+" typed - 18/18 Gal$_7^-$

12 (−) seg obtained from the ~~interval~~ face "+" and typed 12/12 Gal$_7^-$

2. 7⁻ — x 4⁻ As above - 1st run no papillating or semi (+) colonies observed.
2nd run - 2 presumptives observed - slight papillation in the centers of two colonies noted. Picked and streaked total colonies ca 40, all phage contaminated.

C

DATE: 4/3/56 REF: 410

1	2	3	4	5	6	7	8	9	10
One step with W945, against 3079									
1. From overnight culture, diluted 1:10 incubated ca 3 hours									
2. Adsorb Spun reg. 4 O.D. ~0.6, ~~dilute 1:10 count~~ ~~[illegible]~~									
dilute 1:5 (3:19); incubate 60 seconds, Die 1-2 with both (Per)									
3. Pre assay. 53						Cells			
O.D.=.6a - die 10^7, 10^7, 10^6 assay					Bgal	1. 38		7.4×10⁸ cells in OD=0.53 suspension	
						2. 38			
						Plaque free ✓ Transd.			
In a different expt at this time approx. 300 colonies 945 tested, all found inducible					SM	3. 0		no indicator?	
						4. 0			
						Trans			
4. Post-U.V.									
(A) die 10^7, 10^8-10^5 both			Incubate	(B) Undiluted					
cells				Bgal		Bgal SM (true transd.)			
Otani 5.	0			11.	12	16	11. 0	ca 200 plaque	Further cells yielding transd. = 3×10⁷ / 7.4×10⁸
6.	0			10.	19		12. 0 (100 mm)	"	
λ Centers (3:19)									
7.	19								
8.	18								
TIME 15'									
13.	20			15.	7	14	17. 0 (many small)	ca 200 plaque	
14.	20			16.			18.		
30									
19.	23			—			21. 0	ca 200 plaque	
20.	12			—			22.		
45									
23.	19			25.	20	20	27. 0	ca 200 plaque	
24.	33			26.			28.		
60									
29.	25			31.	13	13	33. 0 many small		
30.	28			32.			34. ca 10⁴ plaque		
75									
35.	29			37.	7	14	39. 0		
36.	29			38.			40.		
85									
41.	25			43.	1	5	45. 1		
42.	19			44.	4		46. 0		
95									
47.	75			49.	4	10	51. 2	2	
48.	83			50.	6		52. 2		
105									
53.	130			55.	2	4	57. 8	4	
54.	141			56.	2		58. 4		
120									
59.	238			61.	9	21 pound	63. 5	7	
60.	not pure			62.	33	surface	64. 9		
130									
65.	313			67.	14	26 pound	69. 10	15	
66.	326 surface plate			68.	38	surface	70. 20		

Mean / Total transd.

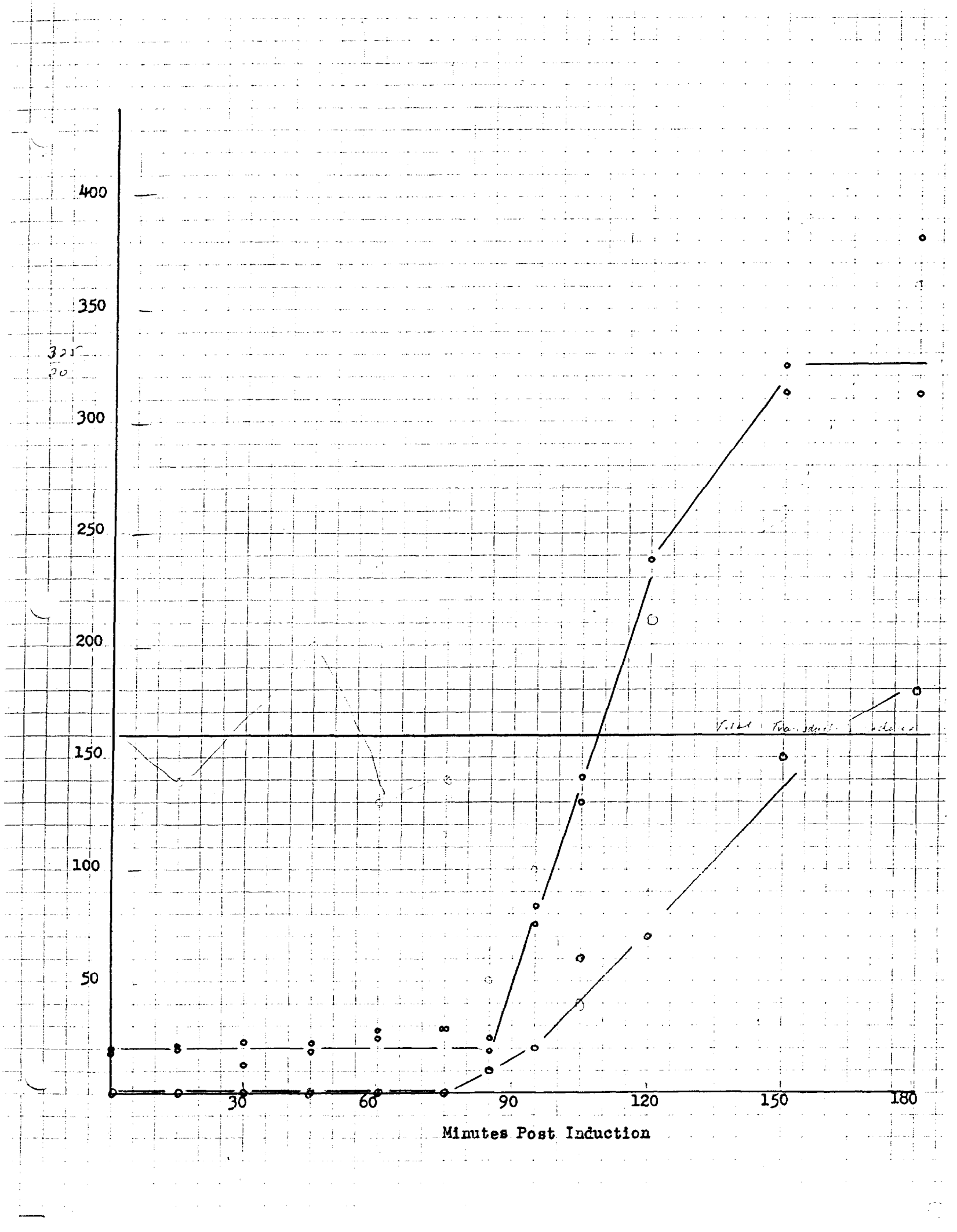
16	16
14	7
20	20
13	13
14	7
5	4
0	5
4	2
21	21
26	26
26	38
159 / 11 = 16	157 / 11 = 14

Then were all surface plaques

Cleared		Bgal	
100.	71. 38 ✓	72. 37	36
	72. 31 ✓	74. 36	

Bgal SM

75. 19	18
76. 17	


400
350
300
250
200
150
100
50
30
60
90
120
150
180
Minutes Post Induction

Gal+ R/S
vs
Gal- R/S

DATE: REF: 411

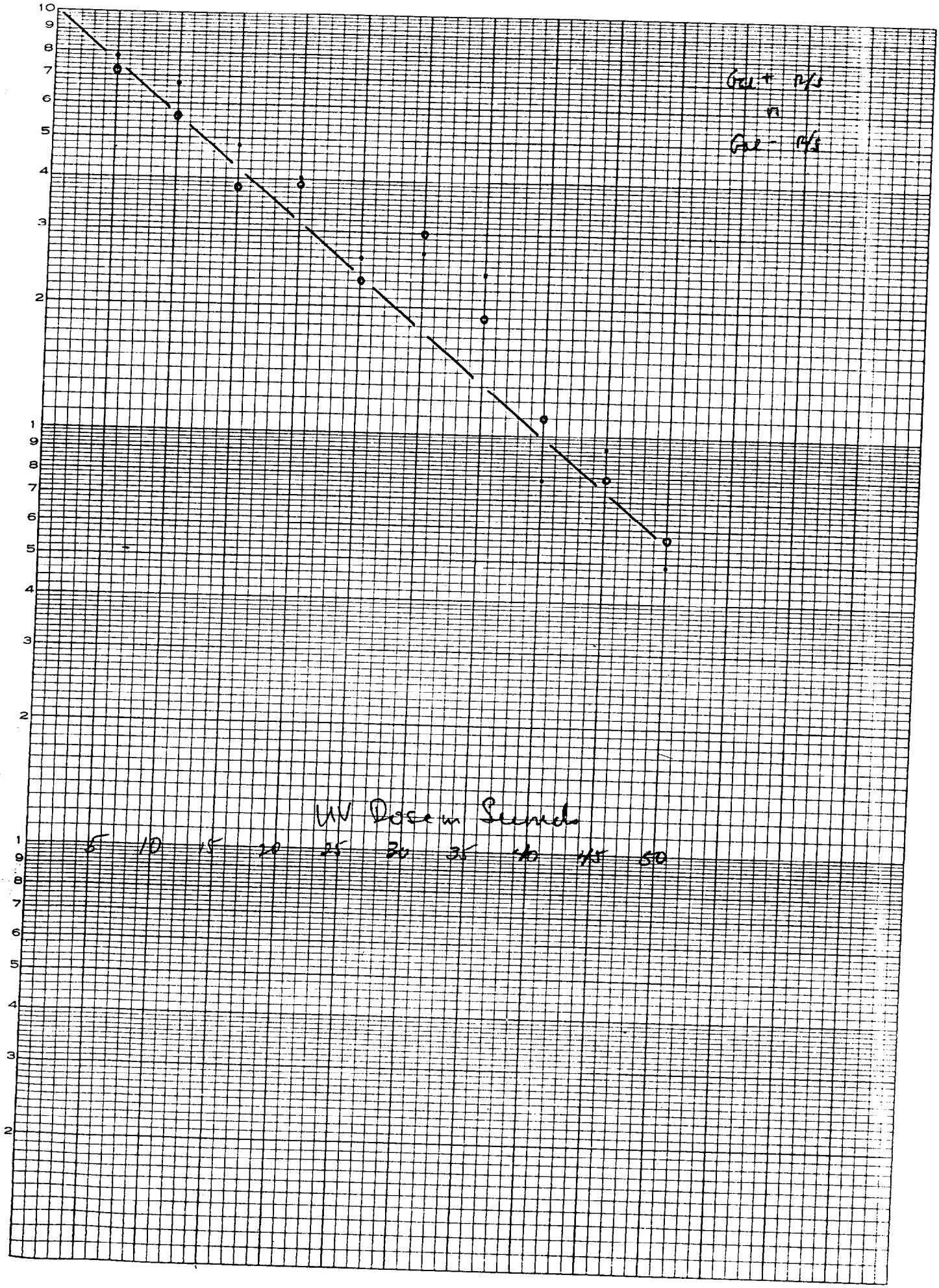
UV. Resistance R/S heterogenote vs S seg.
W2868 taken - and a +1 seg of it. On testing against λ, Lp^s
the segregant found R/S as well as the heterogenote

Both cultures diluted ca 1-10 incubated 2 hours, diluted 50 x 50 x 50 in saline. The heterogenote first, 0.05 ml to 2 B gal plates to obtain Gal+/Gal- ratio; then seg diluted in same tubes. The last tube irradiated and samples taken (OS) at the following times.

Time	Gal+	Gal-	Fraction Gal+	Fraction Gal-
0	600	204	1.0	1.0
5	431	158	0.72	0.78
10	336	139	0.56	0.68
15	226	99	0.38	0.49
20	236	81	0.39	0.40
25	138	54	0.23	0.26
30	178	55	0.30	0.27
35	115	48	0.19	0.24
40	66	16	0.11	0.078
45	48	19	0.08	0.093
50	34	10	0.057	0.049

Rate Gal+/Gal- in 2868

Plate	Gal+	Gal-
1.	229	10
2.	259	1
	488	11



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DATE: 3/10/56 REF: 412

One step with 945
Overnight culture diluted 1-5 incubated ca. 1 hour.
Centrifuge, resuspend in saline OD. .60
(~~Adsorb 1 hr~~), incubate 60 seconds, dilute 1-2 with 2X peptone

Assay 1. Pre UV. Dilute $10^2, 10^4, 10^5 \rightarrow 0.05$ ml

	Cells	(1)	163	Bgal			1.75 × 2×10^6	3.50 × 10^6
		(2)	186					3.5 × 10^8 cells/ml OD 0.6
			349					
10	Plaques	(3)	0 (ca 1000) plaques	Bgal SM				
	Transd	(4)	0 (ca 1000 ")					4.2×10^3 / 3.5×10^8 cells/ml

Post UV - dilute $10^2, 10^4, 10^5$ per incubate — Unadd. Incubated

				Bgal		Bgal SM		
9:25	0 time							
	Cells	(5)	0					
		(6)	0	(9)	104	(11)	1 ca 1000 plaques	no cells induced to yield transd
				(10)	101	(12)	0 " " "	
				103				ave = 210 × 20 × 2 =
	Isocentis	(7)	42					0 = no growth
20		(8)	32					+ = growth

Some plaques checked

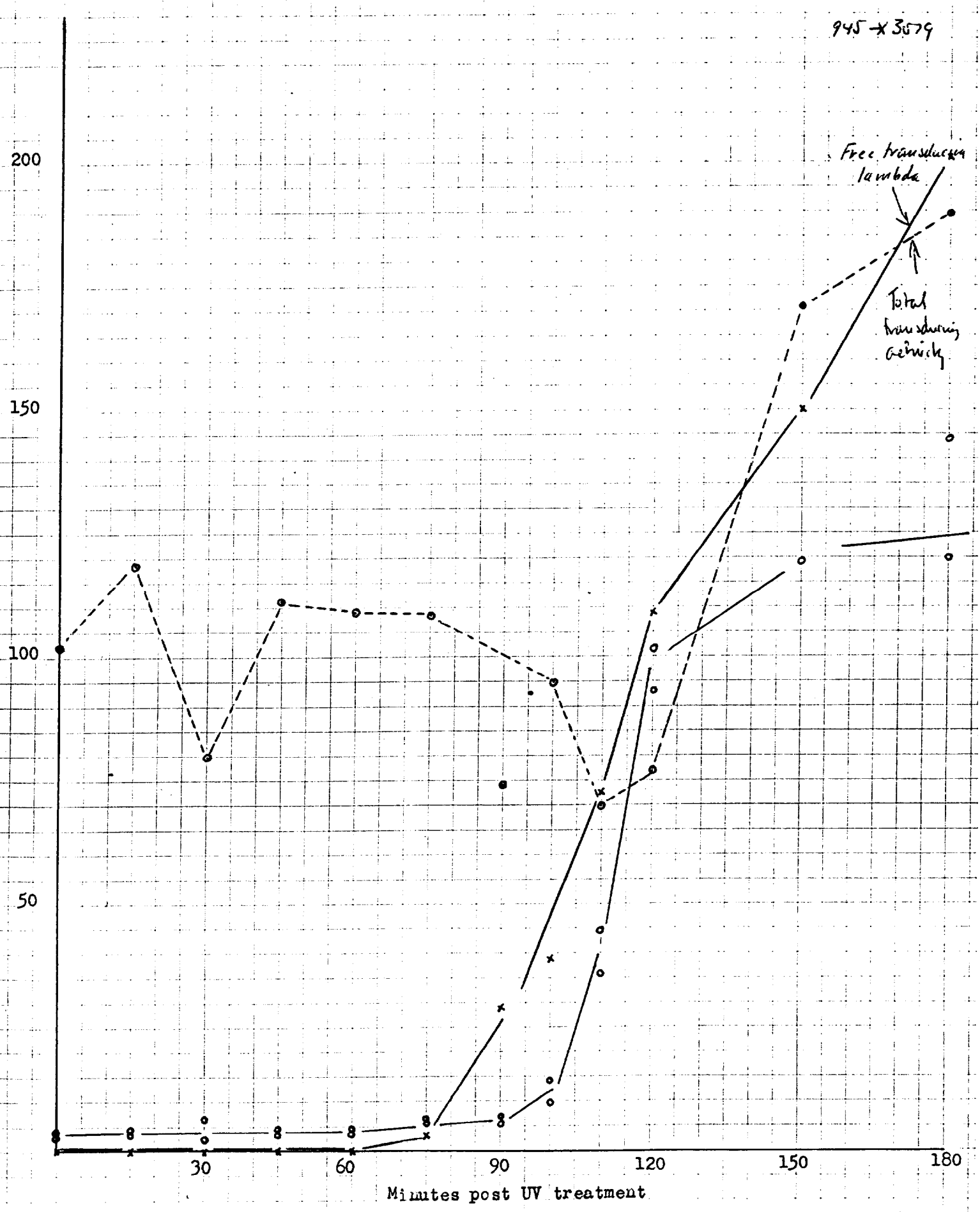
9:40	15	(13)	43	(15)	85	(17)	0 ca 1000 plaques																			
		(14)	40	(16)	150	(18)	0 ca 1000 plaques																			
				11.7																						
9:55	30	(19)	21	(21)	72	(23)	0 ca 1000 plaques																			
		(20)	56	(22)	98	(24)	0 " " "																			
10:16	45	(25)	37	(27)	113	(29)	0 ca 1000 plaques																			
		(26)	47	(28)	112	(30)	0 ca 1000 plaques																			
30																										
10:35	60	(31)	37	(33)	93	(35)	0																			
		(32)	39	(34)	124	(36)	0 (ca 10^3 plaques)																			
				AVE: 105.2																						
10:40	75	(37)	53	(39)	88	(41)	2 ca 1000 plaques																			
		(38)	57	(40)	127	(42)	5																			
10:55	90	(43)	54	(45)	80	(47)	31																			
		(44)	64	(46)	(67)	(48)	24																			
11:05	100	(49)	99	(51)	71	(53)	29																			
		(50)	143	(52)	118	(54)	46																			
11:15	110	(55)	357	(57)	59	(59)	67																			
		(56)	448	(58)	81	(60)	78																			
11:25	120	(61)	1024	(63)	87	(65)	91																			
		(62)	930	(64)	65	(66)	124																			
11:55	150	(67)	1442	(69)	181	(71)	150																			
50		(68)	937	(70)	161	(72)	149																			
			2379	1189																						
12:25	180	(73)	1422	(75)	203	(77)	193																			
		(74)	1196	(76)	174	(78)	208																			

Plate 46	Bgal	Bgal SM	45	75	
1	+	+	+	+	+
2	+	+	0	+	+
3	0	0	0	+	+
4	0	0	0	+	+
5	+	+	0	+	+
6	0	+	0	+	+
7	0	0	0	+	+
8	0	0	0	+	+
9	0	0	0	+	+
10	0	0	0	+	+
11	+	+	0	+	+
12	0	0	0	+	+

	77
1	+
2	+
3	+
4	+
5	+
6	+
7	+
8	+
9	+
10	+
11	+
12	+

412

945 → 3579


200
150
100
50
30
60
90
120
150
180
Minutes post UV treatment
Free transducing lambda
Total transducing activity

DATE: 4/14/56 REF: 413

Recheck on induction — as plaques — is it exponential?
— as transductions — . . . linear?

1. Plaque induction — 945 rotated 2.5 hours post overnight, diluted 1-10. Centrifuged made up to O.D. .60 in saline — diluted 1-2, $\times 10^5$, irradiated UV standard dist, etc., assay for cells: B gal (pour method
for plaques: B gal + 3079, also pour method.

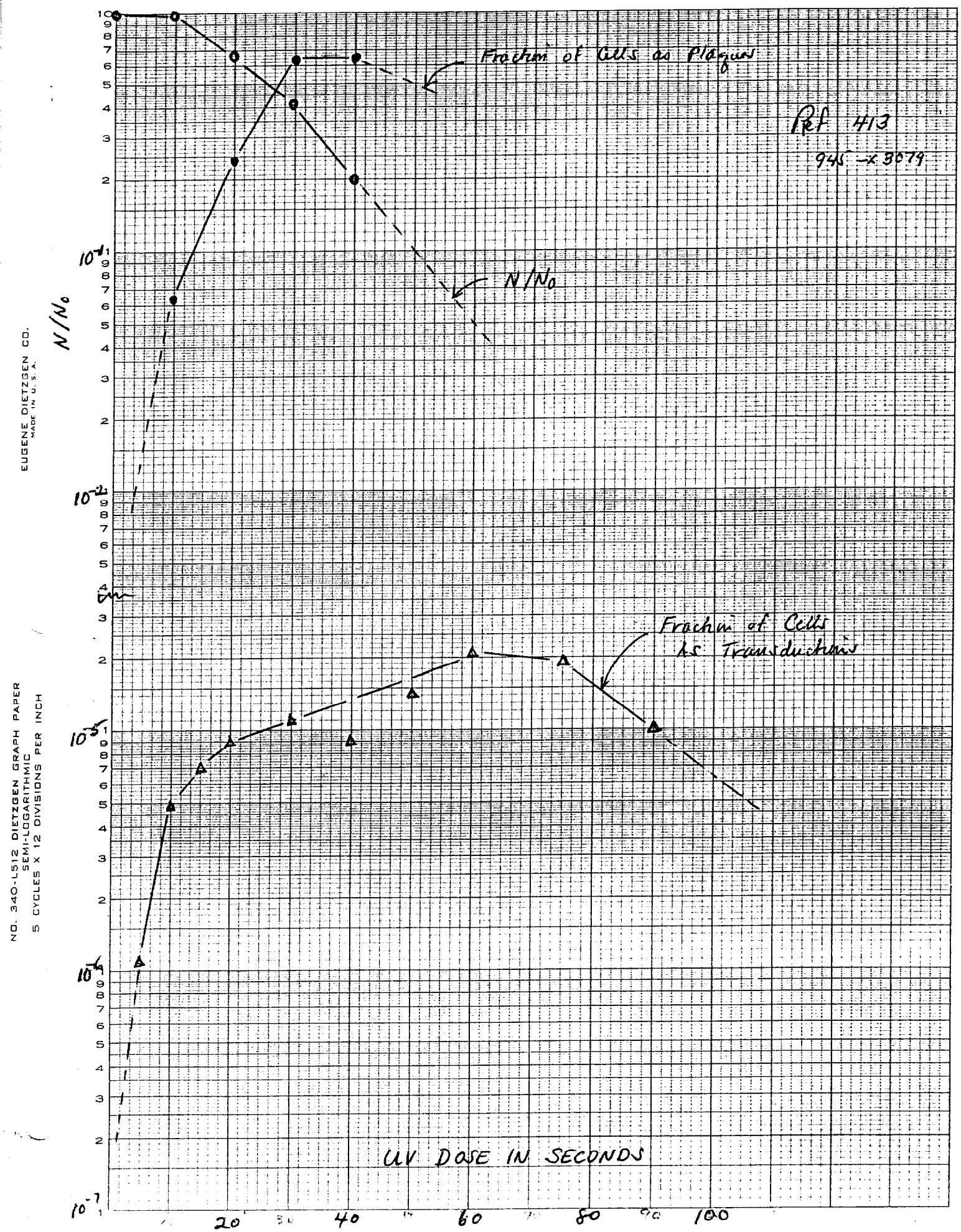
2.0×10^8

Dose	Plaques	Cells	Total cells + plaque	N/N₀	Plaques/ml	Fraction (cells) as Plaques
0	0	198 } 200	198	1.0	0	0
10	13	207	220	1.0	1.3×10^7	0.064
20	49	136	185	0.66	4.9×10^7	0.24
30	127	86	213	0.42	1.3×10^8	0.64
40	130 many agar lumps	42	172	0.20	1.3×10^8	0.64

2. Transduction induction — bi 1-2 of OD = .60

2×10^8

Dose	Papillae	Δ	Transd/ml	Fraction (cells) yielding transd.
0	23	0	0	0
5	45	22	2.2×10^2	1.1×10^{-6}
10	110	97	4.7×10^2	4.9×10^{-6}
15	161	138	1.4×10^3	7.0×10^{-6}
20	190	167	1.8×10^3	9.0×10^{-6}
30	247	224	2.2×10^3	1.1×10^{-5}
40	208	185	1.9×10^3	9.0×10^{-6}
50	292	269	2.7×10^3	1.4×10^{-5}
60	444	421	4.2×10^3	2.1×10^{-5}
75	411	388	3.9×10^3	1.9×10^{-5}
90	235	212	2.1×10^3	1.0×10^{-5}


Fraction of Cells as Plaques
Ref 413
945 -x 3079
N/N₀
Fraction of Cells as Transductions
UV DOSE IN SECONDS
20
40
60
80
100
EUGENE DIETZGEN CO.
MADE IN U.S.A.
NO. 340-L512 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 12 DIVISIONS PER INCH

Isolation of Lp^R from 2307

1. Overnight culture - diluted and plated to give ca 100 colonies per plate
2. 9 plates - two counted
   1. 97 colonies } all colonies gal -
   2. 109 colonies
3. replicated to B gal plate spread with 3079, UV'd 20 seconds 50 cm, etc.
4. On the nine plates 3 colonies found not to give a lysogenic reaction (2 on one plate, one on another
5. The colonies were streaked on λ, 3079 after purification found λ^R and non lysogenic
6. Lysates made (not as conc. as usual) - after 4 hours spotted on 3079 on B gal

W2307 Lp^R = W3172

1. ca. 20 small plaques
2. ca. 17 " "
3. ca. 9 " " /45/3 = 15 x est. 0.01 = 15000 for dil = 10 = 1.5 x 10^4

About this time also 2345 treated in the manner above.

1. 1 colony of ca. 300 (on 3 plates) found non lysogenic
2. on subsequent test (1) found Lp^S

W2345 spont. Lp^S = W3173

(2) Hfr with 945 after 3 hr growth in Pen and plated 1-100 on M gal, ind. on S gal

(3) tested against HFT 2⁻, 1⁻, + by 2⁻, 0 by 1⁻

(4) gave no phage when treated as above in lysate test

945 - Are the ~~LFT~~ cells giving HFT lambda on LFT⁺ cultures in mutants? Ca 2×10^3 945 colonies per plate replicated to 3079 spread plate, B gal and UV'd 20 sec. Incubated 3 days gal + looked for. Many observed, suggestive that some are UV induced reversions of 945 (as was the case previously with 1210) 15 plates in all representing ca 40,000 colonies. Areas on the 945 plate ~~located from~~ corresponding to the Gal + on 3079 located and picked and retested by sporting on 3080 and preadsorbing - This to find a clone of 945 cells giving HFT lambda. Gal + area picked from 3079 plate to confirm transduction. 12 945 areas picked per plate.

Results

1. first 5 945 areas = 60 total found LFT. None of the Gal + areas on 3079 corresponding to these showed seg. clones.
2. to distinguish between gal + are to reversion of 945 plates replicated to B gal SM, since 3079 is S^R. From the Gal + on Gal + SM reference made to (1) the original plates to obtain reference to original clone (2) the UV ind plates to see

to 413A

DATE: 3/27/56 REF: 413A

if HFT+ clone induced by UV.

Z series = original 945 plate - un UV'd
A " = 3079 + 945 UV'd
S " A series replicated to B gal SM

3. The SM Gal plates show Galt, indicating transductions or reversions of the doubly -- (or something more complicated).
Examples ~~[illegible]~~

	Galt			Galt	
Z3	19		Z12	7	
A3	24		A12	33	
S3	11 4/6		S12	18	3/6 started gave mosaics 1st striking
Z5	9		Z4	11	
A5	ca 100		A4	40	
S5	58	3/6 gave mosaics 1st stock	S4	18	3/6, etc.

Theoretically Z + S = A, however many many Galt are papillae and may not replicate well

4. From Z3, Z12, Z5, Z4, Z6

(A). 7 areas corresponding to the areas of transduction on 3079 (as determined by replication to SM) picked and spotted on B gal. Grown to spot size (24 hrs); then from these spots ~~[illegible]~~ growth picked, tested for HFT via UV on 3080.

(B) Results of the 35 areas picked and tested, none found HFT+ after 48 hours.

Repeat this experiment adding HFT+ 1 to obtain lower limits of resolution of the method. Suggestion is that all producing HFT λ are not closely related.

5/2/56
Preparing for Kalchar experiments

Obs that 1, 6, 7 all are defective for ethzyme II (P Gal transferase?)
Prepare gal2-, gal4- stocks also 2734 (= non transferable gal-)

1. Because 3092 easily reversible, a new stock made by HFT5 - x 3110
A. a seg clone obtained.

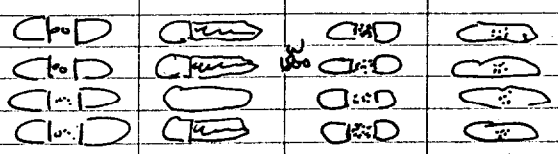
2. ~~2743~~ 2734 crossed with 1895 - growth in both, plated M gal-
A gal - colony picked purified once, streaked on D(0)
and tested against HFT(+)

A. 6/6 colonies grew D(0) (labelled 413A-1) = W3142
B 6/6 colonies not transf. to + by HFT(+) - all resist. to λ.

DATE: 4/20/56 REF: 414

1. Recheck ~ 269-1 lysate.

	269-1	λ^2	Rpt 269-1 10^{-2}	10^{-4}
3079				
3080				
945				
(#3) 343-8				



↑ Resistant colonies pick

very tiny plaques

2. Examination of the resist. of 1.
After two purification streaks – 2 from each culture

		269-1 (1-100)	Growth	λ	λ-2	
3079	(1)	R	normal	S	S	414-1
	(2)	R	"	S	S	~~[illegible]~~
3080	(1)	R ?	"	R	S	-2
	(2)	R	"	R	S	—
945	(1)	R	slight mucoid	R	R	mucoid – a contaminant?
	(2)	S	normal	—	—	
343-8	(1)	R	"	S	S	-3
	(2)	R	"	S (partial)	S	-4

Stocks

These tests in B gal – all cultures Gal –

Stocks discarded 3/9/59

341-9 Lysate 4/23/56 – To show that fragment size is constant – titers of 2⁻ × 1⁻4⁻, 4⁻, 1⁻ are the same

Assay

(lit 10^4 → 0.05 ml surface plating)

① Preliminary – /3080 (1-4⁻) 101 transd.
/3102 (1⁻) 4 transd, ca 500 plaques.
/3104 (4⁻) 4 transd, ca 500 plaques

② Rpt. same dilution
/3094 5 transd.
/3091 8 transd
/3080 96 transd.

~~307-1A Mutant of 307-1C Velvet overnight culture~~

DATE: 5/1/56 REF: ~~[illegible]~~ 415

307-1C Segregation. 1⁻ — X 7⁻ see 308A, 307

1. Overnight culture from a single colony B gal.

2. dil $10^2 - 10^4 - 10^6 - 10^8$ → 0.05 ml samples spotted on B gal, 6 per plate. (8 plates)
Incubated 4.0 hours, 0.05 ml - 0.1 ml 1% ~~[illegible]~~ added and the ~~[illegible]~~ area respread.

$\frac{10}{100 \times 2} = 0.05$ $e^{-.05} = 0.951$ $e^{-1.1} = 0.338$

3. Parent susp. ~~[illegible]~~ ml

Calculation

	Gal+	Gal-pop	Gal-	Total		
3 samples dil. 5?	1. 10	209	10	229	230/100 x 2 =	2.3/2 = 1.1 cells/sample
Got a "+" cell	2. 10	212	9	231		

		Gal+	Gal-pop	Gal-	Total cells			
3 samples dil. 4? Got a (+) cell	4 Samples Plate 1, 1.	220	0	0	220			
	2.	0	222	0	222			
16 sample Got no cells.	3.	0	6	0	6	?		
	4.	0	211	1	212			
	5.	58	ca 100	ca 100	—			
	6.	0	9	0	9	?		
	Plate 2 1.	0	265	1	266			
	2.	0	0	0	0	NG		
	3.	0	185	0	185		C.C.	
	4.	0	209	0	209	0.602r	N log N	a
	5.	3	309	3	312 ←	1.8	7.78×10^2	2.3×10^{-3}
	6.	0	2	0	2	?		
	Plate 3 1.	2	192	0	192	1.2	4.38×10^2	3.6×10^{-3}
	2.	0	309	1	310			
	3.	2	164	0	164			
	4.	0	148	0	148			
	5.	0	17	119	136			
	6.	0	381	0	381			
	Plate 4 1.	65	116	2	183			
	2.	0	0	0	0	NG		
	3.	0	272	3	275			
	4.	47	1	0	48			
	5.	0	183	0	183			
	6.	0	0	0	0	NG		
	Plate 5 1.	3	252	4	259			
	2.	0	233	0	233			
	3.	0	77	0	77			
	4.	0	0	0	0	NG		
	5.	0	260	105	365			
	6.	0	21	0	21			
	Plate 6 1.	0	158	3	162			
	2.	0	38	0	38			
	3.	0	245	1	246			
	4.	0	129	0	129			
	5.	0	468	1	469			
	6.	0	223	10	233			

DATE: 5/1/56 REF: 415A

1	2	3	4	5	6	7	8	9	10

Continuation —

	Gal+	Gal-pep	Gal-	Total	
Plate 7 1.	0	0	0	0	NG
2.	0	153	0	153	
3.	0	68	0	68	
4.	1	ca 50?		ca 800	
5.	0	450	1	451	
6.	125 cont	25	0	150	
Plate 8 1.	0	223	1	224	
2.	0	0	0	0	NG
3.	0	72	0	~~257~~ 72	
4.	0	–	ca 100	467 –	
5.	0	272	5	277	
6.	0	223	2	225	

This hit. is R/s (307-1C)

For comparison with Lp+ heterogeneous are 389 for 307-1A

II. Single Gal- seg obtained from as many clones as possible – 17 obtained from 17 clone.
Tested / HFT 6- / HFT 7-

6- Lp^s = 8
7- Lp^s = 8
7- Lp^R/s = 1 = 415A-1

3057 – in search of Lp^R

1. ca 2000 colonies tested by replication / 2730 seg (Gal2-) – None found non lys

DATE: 5/3/56 REF: 416

(413)

Redo a 945 experiment to see if "mutants" cells are responsible for + tand activity in LFT cultures.

1. Technique - nearly confluent colony formation of 945, to which was added a few HFT+ 1- cells as a control - replicated to 3079 (Bgal and UV'd. Incubated 2 days and replicated to Bgal, Bgal SM (Gal2-prototrophic) on

AMEND

This plate then replicated to M Gal

1. 113
2. 118
231

2. Aerated 945 diluted $10^2 - 10^4 \rightarrow$ 2 ml + 9 ml (1.0 ml) $\rightarrow$ 0.1 ml / 10 plates

$10^6 \rightarrow 0.05$ ml

Example of Gal+ after 2 days

UV'd plate 300
Bgal replica 300
Bgal SM 10
Mgal replica 300

Overnight 389-1 $10^2 - 10^4 - 10^6$

0.05 ml = 108
65
173

Replicates / 2230 seq UV'd

25 col LFT
11 col LFT

173 x 1/12

2. This contains 231 x 2/9 x 100/10 = 4620/9 = 512 945 cells

Original plate (non UV'd) ca 10

This experiment abandoned - Replications to Bgal SM showed ca. 10 Gal+ pure irradiated plate which presumably are transductions of 945 → 3079. The irradiated plates (and Bgal replica of them) showed at ~~[illegible]~~ about 200-300 Gal+ clones - of these replication to M Gal showed at least 200 were prototrophic and derived presumably

W2872 (1210 x 21λ)

in search of HFT 8-

1. 17 seq tested against ~~[illegible]~~ 3094 - all LFT but 8- appears to have a selective advantage of 4- as it grows through Gal2- background.

2. 21 seq tested / 3094 - all LFT
15 seq tested / 3094 - all LFT

W7580 x W2854

no add 14
0.1 ml lyr 54 (poor lysate)

1. 13 seq tested - 12 Gal2-, 1 Gal2- Gal2-

DATE: 5/11/56 REF: 417

Segregation in 323-2
as in previous expts

overnight cult 10^2 10^4 10^6 10^8 → 0.05 ml samples B gal - respread 4 hours

↓

assay 0.1 ml

	+	−	pop −	Total
1.	18	30	49	
2.	22	24	116	

Respread Clones	Colonies +	−	pop −
#'s 7–11		no growth	
12.	21	0	0
13.	0	0	22
14.	0	33	0
15.	0	0	92
16.	0	0	30
17.	0	0	7
18.	0	1	0
19.	0	13	2
20.	0	0	42
21.	0	0	24
22.	0	0	81
23.	0	0	3
24.	0	0	16
25.	0	6	0
26.	0	0	4
27.	3	4	37
28.	20	0	22
29.	0	0	28
30.	0	0	43
31.	0	8	12
32.	0	0	18

DATE: 5/13/56 REF: 418

1	2	3	4	5	6	7	8	9	10

Segregation from W2874 ($2^-3^- \times 7^-$) 2^-7^- heterogenote - It is full Gal+, does it still contain both Gal - alleles?

1.

/2-	/7-	Number
+	o	14
o	+	4

} both alleles present but segregation pattern reversed

2. 14/14 2^- tested for HFT+ /3091 all LFT

4/4 7^- tested /3092 all LFT

At this time W2308 X — HFT 2^- } to compare reciprocal cases
W2915 X — HFT 7^-

made in the "standard" way 0.1 ml of 1:100 dil overnight culture + 0.4 ml HFT lysate - diluted to obtain ca 100-200 colonies / 13 gal plate (1/50 < 1/50) Results not recorded but ca 200 colonies/plate, >50% show contact with phage (partial lysis)

FOR SEG. ANALYSIS SEE 420

W2308 - 2 ~~apparent~~ transduction clone apparent $\boxed{418\text{-}1, 418\text{-}2}$

W2915 - 1 transd. clone obtained - not as "+" as the above $\boxed{418\text{-}3}$

A. The initial plates of this experiment replicated to M Gal + 3091 and irradiated with UV 60 seconds - This, to see if any homogenotic clone formed by transduction (are transductions thus far have been selected on the basis of plus phenotype, in the case of (-) recipients (-) or mosaic phenotype in "+" recipients. No evidence of HFT (by gal+ clone on M Gal) indicating no Gal_2^- homogenote formed.
The extent of the test: ca. 500 gal_2^- colonies (W2915) } Planned to replicate
ca 300 gal_7^- colonies

to Gal_2^- indicator frustrated by reversion of recipients

5/14/56

1210 X — 308-5 }
8^- X — 1-6^- } for stock for reversion

no add - 0
0.1 ml 308-5 84 (small) ① 15/15 found 8^- 15 tested /3091 and found LFT
lysate (original, perhaps 2 years old) ② 7/7 found 8^- 7 tested /3091 M Gal found LFT

1210 X — 344-6
8^- X — 6-7^- ① 19/19 found 8^- 18 tested /3091 and found LFT
no add - 0 ② 11/11 found 8^- 10 tested /3091 M Gal - and found LFT
(old lysate) 0.1 ml 153 (small) ③ 16/16 " "
344-6

2580 X — 346-4

no add - 0 ① 20 seq tested 19 Gal_2^- } See 347
0.1 ml 1 Gal_6^-
346-4 64 ② 10 seq tested

DATE: 5/16/56 REF: 419

1. In the experiments to see if HFT+ cells are distributed as "mutants" in LFT clones (such as on 416) many galt clones possibly transductions, reversions or something else observed in the UV irradiated mixture of amor and revenant. The number appears too high. Both cultures used in these later experiments were F- and normal crossing is prohibited. Are heterocaryons being formed.

2. Experimental -

a. To avoid lambda transduction Lp's cultures used.

W2915 F TLB1 Gal2 - Lac1 Ara - Xyl - Mtl - Pr - Lp's

W3079 F - M - Gal1 - Gal4 - Lac1 - V1R SR Lp's

		Papillae	
W2915	UV	9	which of these UV'd not known,
W2915	[illegible]	11	plates not labelled
W3079	UV	0	which of these UV'd not known -
W3079		0	both labelled as such

W2915 } UV Two classes of papillae large = ca 30 — Galt on restreak
W3079 } small = ca 500 — most of these just faintly + on Bgal on restreak.

b. test some of the papillae for markers.

① 6 small papillae tested on Bara - 1 galt, ara -
4 galt+w, ara -
1 galt+w, ara+w

6 large papillae tested on Bara 6 galt, ara -

3551 X — K12 (poor lysate) made 5/14/56
no odd = 0
0 lw = 19
1. 15 seq [illegible] found 1 - 4 -

346.4 X — K12
no odd = 0 (some small) this culture very rough.
0 lw = 19
1. 21 seq found 4 - 6 -

DATE: 5/23/56 REF: 420

1	2	3	4	5	6	7	8	9	10
Seq analysis of		418-1	2⁻ × 7⁻				This clone λ^R/s		
	number	/2⁻	/7⁻						
	16	+	0	= 7⁻	all λ^s				
	2	0	+	= 2⁻	both λ^R, probably λ^R/λ^s				
Seq analysis		418-3	7⁻ × 2⁻						
	Number	/2⁻	/7⁻						
	4	+	0	= 7⁻	all λ^R, probably λ^R/s				
	8	0	+	= 2⁻	all λ^s				
	1	0	0	= 2⁻7⁻ ?	appears λ^s				
2880X—2851		2⁻ × 1⁻4⁻		— transd					
	/1⁻	/2⁻	/4⁻		no add	9			
	19 +	0	+	= Gal₂⁻	0.1 ml 2851	50			
	2 0	+	+	= Gal₁⁻					
Heterogenote for Photographic Illustration.									
	Lysate of	K-12							
	Cuts.	no add	0.1 ml						
	3091	0	45						
	3092	4	64						
	3094	0	63						
	3096	2	80						
	3097	0	44						
	2857	0	88						
	2854	0	55						

DATE: 5/30/56 REF: 421

1	2	3	4	5	6	7	8	9	10

Segregation from some heterozygotes to check them before use in photography

2580 X — 308-5

Number	/1-	/2-	/6-	Genotype
16	+	0	+	Gal_2-
2	0	+	0	Gal_6-Gal_1-

2580 X — 344-6

Number	/6-	/2-	/7-	Genotype
19	+	0	+	Gal_2-
1	0	+	0	Gal_6-Gal_7-