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1415 A

May 8 19 58 REF:

Plates from g L replicated from DO to B Gal and B Lac.

I-B, 0.05 = all Gal-
9 ~~Gal~~ Lac +, 13 Lac-

I-C, 0.05 = 2 Gal +, 28 Gal-
12 Lac +, 18 Lac-

I-D = 1 Gal +, 22 Gal-
6 Lac +, 17 Lac-

all Gal +'s are Lac +

Background overgrowth.

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REF: 1415 B 3

1	Count[2]	Gal+[3]	Lac+[4]	T_1[51]	6	7	8	9	10
A	88	2/88	15/88	51/88	}	Control			
Ao	7	1/7	2/7	4/7					
2A	92	3/92	17/92	57/92					
B	25	0/24	2/24	8/24	}	Azide			
2B	6	0/6	0/6	1/6					
C	38	1/41	9/41	26/41	}	Acridine orange.			
2C	40	1/40	2/40	20/40					
D	9	0/9	3/9	6/9	}	DNP			
2D	1	0/1	0/1	0/1					

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1415 B.3

May 9 2A 2A B

19 58

REF: (and 2B)

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	all −	−	+	all −	−	+	
2	−	−	+		−	+		−	+	
3	−	−	−		w	+		−	−	
4	−	−	−		−	−		−	−	
5	−	−	+		−	−		−	+	
6	−	−	+		−	−		−	+	
7	−	−	−		−	−		−	+	
8	−	−	−		−	+		−	−	
9	−	−	−		−	+		−	−	
0	−	−	+		−	+		−	+	
1	−	w	+		−	−		−	+	
2	+	+	−		−	+		−	+	
3	−	−	−		−	+		−	+	
4	−	−	−		−	−		−	−	
5	−	−	−		−	+		−	+	
6	−	−	−		−	−		−	+	
7	−	−	+		−	−		+ / −	+	
8	−	−	+		+	−		−	−	
9	−	−	−		+	−		+	−	
0	−	−	−		−	−		−	+	
1	−	+	−		−	−		−	−	
2	−	+	−		−	+		−	+	
3	−	−	+		−	−		−	+	
4	−	−	+		−	−		−	+	
5	−	+	−		−	−				
6	−	+ / w	+		−	−				
7	−	−	+		−	−				
8	−	−	−		+	−				
9	−	−	+		−	−				
0	−	−	+		−					
								2B		
1	−	−	+		−	−		−	+	
2	−	−	+		−	+		−	+	
3	−	−	−		+	−		−	+	
4	−	−	−		−	+		−	−	
5	−	−	−		−	+		−	+	
6	−	−	+		+	−		−	+	
7	−	−	+		−	−				
8	−	−	+		−	−				
9	−	+	−		−	+				
0	−	−	−		−					
1	−	−	−		−	+				
2	+	+	−		+	−				
3	−	−	−							
4	−	−	−							
5	+	+	−							
6	−	−	−							
7	−	+	−							
8	−	−	−							
9	−	−	−							
0		−	−							

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~~1415 B 3~~
~~MG1 2~~

A

May 9
~~April 28~~ 1958

A

AO

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	TI	GAL	LAC	TI	GAL	LAC	TI	
1	−	−	−	−	−	+	+	+	+	
2	−	−	+	−	−	−	−	−	−	
3	−	−	−	−	−	+	−	−	−	
4	−	−	+	+	+	±	−	−	+	
5	−	−	−	−	−	−	−	−	−	
6	−	−	−	−	−	−	−	+	−	
7	−	+	−	−	+	−	−	−	+	
8	−	−	−	−	−	+				
9	−	−	+	−	−	+				
0	−	−	+	−	−	+				
1	−	−	−	−	−	+				
2	−	−	+	−	−	+				
3	−	−	−	−	−	−				
4	−	−	−	−	−	+				
5	−	−	−	−	+	−				
6	−	−	+	−	−	−				
7	−	−	−	−	−	+				
8	−	−	+	−	−	−				
9	−	−	+	−	−	+				
0	−	−	+	−	+	±				
1	−	−	+	−	−	+				
2	−	+	−	−	−	−				
3	−	−	+	−	−	+				
4	−	+	−	−	−	−				
5	+	+	−	+	+	−				
6	−	−	+	−	−	+				
7	−	−	−	−	−	−				
8	−	−	−	−	−	−				
9	−	−	+	−	−	+				
0	−	−	+	−	−	+				
1	−	+	−	−	−	−				
2	−	−	+	−	+	+				
3	−	−	+	−	−	+				
4	−	−	−	−	−	+				
5	−	−	−	−	w	+				
6	−	−	+	−	−	−				
7	−	−	−	−	+	−				
8	−	−	−	−	−	−				
9	−	−	−							
0		-/w	−							
1	−	−	−							
2	−	−	−							
3	−	−	+							
4	−	−	−							
5	−	−	−							
6	−	+	−							
7	−	−	−							
8	−	+	−							
9	−	−	+							
0		−	+							

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1415 B3

C 2C D (and 2D)

May 9 1958

REF:

	1	2	3	4	5	6	7	8	9	10
	GAL	LAC	T1	GAL	LAC	T1	GAL	LAC	T1	
1	−	−	+	−	−	+	all −	−	+	
2	−	−	−	−	−	+	↓	−	−	
3	−	−	−	+	+	−		+	−	
4	−	−	−	−	−	−		+	−	
5	−	−	−	−	−	+		−	−	
6	−	−	+	−	−	±		−	+	
7	−	−	−	−	−	−		−	−	
8	−	+	−	−	−	+		+	−	
9	+	+	+	−	−	−		−	+	
0	−	−	−	−	−	+				
1	−	−	−	−	−	−				
2	−	+	−	−	−	+				
3	−	+	−	−	−	−				
4	−	−	−	−	−	+				
5	−	−	−	−	−	+		2D		
6	− /w	−	+	−	−	+				
7	−	−	+	−	−	−	−	−	+	
8	−	−	+	−	−	+				
9	−	−	+	−	−	−				
0	−	−	+	−	−	−				
1	−	−	−	−	−	+				
2	−	−	−	−	−	−				
3	−	−	+	−	w	−				
4	−	−	−	−	−	−				
5	−	−	−	−	−	−				
6	−	−	+	−	−	−				
7	−	−	−	−	−	+				
8	−	−	+	−	−	+				
9	−	+	−	−	−	+				
0	−	−	−	−	+	−				
1	−	w/−	−	−	−	−				
2	−	−	−	−	−	+				
3	−	+	−	−	−	−				
4	−	+	−	−	−	−				
5	−	−	+	−	−	+				
6	−	−	−	−	−	+				
7	−	−	+	−	−	−				
8	−	−	−	−	−	+				
9	−	+/−	−	−	−	+				
0	−	−	+	−	−	−				
	−	−	−							
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

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Azide effects on mating

1415A

5 May 1958 REF:

Ultimate purpose: compare azide effects with Az^r ♂ v ♀.

Below: A = —; B = Azide 100r/ml C = Acridine orange 60r/ml D = DNP $\frac{M}{1000}$
[Note: agglutination of ♂♂ in C.] Make up 2 x in BGA.

Design: Effects on mating (during pulse) I; Effects on entry (after pulse) II; Reversibility III.

I Pretreat each parent before and during mixing; dilute, incubate + plate.

II Dilute untreated pulse into inhibitor solns. Incubate.

III Redilute these and incubate further.

Pulse	1 min.	Dilute & Incubate	
Treated	→	BGA 1 hour.	Plate: I
Untreated	12 m. →	treatments 1 hour (A, B, C, D chilled)	→ Plate: II; Dilute into BGA → Incubate 1 hour. Plate IV
	Plate III.		

Reversibility of inhibited entry is tested after 12 minutes.

8 PM ff. W3060, W3064 refreshed from ORC 5 tubes each harvested into 0.5 ml (100x) in BGA. Chilled until used. Timing by chilling in ice water.

Mr. But Meyer present

I Add 0.1 ml each parent + .1 ml inhibitor (2X). Mix and incubate 1 minute (pulse)
Dilute 1/10,000 in warm BGA + plate at 60 m. (10^{55} PM). : I: A, B, C, D.

II. Tube A at t_1 (1:100) and dispense 1 ml to each of 5 tubes. Chill at t_{12}. (Plate III
AO - keep chilled. A, B, C, D add equal vol. inhibitors and incubate 10^{20} - 11^{15}

III ↓ ~~Plate after~~ 1:50 dilution at 11^{15} < plate II; Incubate 1 hour + plate III.

Note: water bath went to 43° at 11 PM.

Comments: IA - 0 prototrophs. II and III seems all 0!

IB { 25 prototrophs; 38; 36

Faulty IA? Or do the "inhibitors" encourage mating? If they do not inhibit mating they do appear to inhibit entry and according to B reversibly.

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Azide at. on entry.

1415 B.

6 May 1958

REF:

Repeat 1415 in view of 43° incident. Change designation to mean entry of T2.

Use same cells as yesterday (chilled at 100X).

Pulse (1:1:2ml fresh BOA) 7 minutes at 37°. Chill and dilute 1:100 in BOA

disperse 1 ml volumes of dilutions. Add 1 ml of inhibitor. AO = chilled A (not inc.

Incubate 30 minutes. (Plate semis at at 1:50 dilu.) Dilute all samples 1:100 in

warm BOA and incubate 60 mn. Plate 0.1 ml.

.05 ml samples plated.

1 Plate A only at t_0

2 Plate AO, A, B, C, D at t = 50.

3 Plate AOABCD at t = 110

4

Needs reincubation, counting, and full scoring. Goal as T_1

semis 2 shows inhibition of entry

3 its reversibility.

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1415-6

28 May 1958. O = 5^{45} PM

REF:

all but 3060 - inoc. from stants 11AM:

	1	2	3	4	5	6	7	8	9	10
	♂			♀						
1	W3060	Az^S		W3064	Az^R					
2										
3	W3947	Az^R		W3935	Az^R					
4										
5	A = ♂S	♀ S								
6										
7	B = ♂S	♀ R								
8										
9	C = ♂R	♀ S								
0										
	D = ♂R	♀ R.								

Tubes: 1.0 ml each. add .03 ml azide.
1 water at 13'
2 azide -0'
3 azide 5'
4 azide 12'

Harvest dilute exp. cultures to chilled pinassay. Mix chilled 2x concentrates (at about 3x10^8/ml). Time by warming in temperature bath.

At 40' chill + blend. (after mixing, before warming). Add 4 ml chilled water. Plate .05 ml and .05 of 1:10 dilns.

5 kept chilled. Plate for plate recombinants. (at 230 minutes dil. .2 ml in .8 ml water and plate .05 ml). Relay on plating for interruption.

	1	2	3	4	5
A	10^4	0	<100	200	0
B	"	0	<100	850	2
C	"	1	200	[illegible]	0
D	"	6	2.0+	[illegible]	1

29 May 1958 REF: -6

-6 suggests that concentration of ~~...~~ azide used here was too high for activity even of Az^R parents. Try column 2 of yesterday's expt with range of concentrations added at t = 0. Time by chilling. ORC cultures resuspended in penassay and chilled. 2.5 ml each mixed for four crosses. Same as 1415-6.

Note: use of ORC cultures today. Various concentrations of azide, added to .9 ml each cross while chilled. Make up $(\sqrt{10})\%$ × $10^{-\frac{n}{2}}$ sums.

i.e. ~~3.16%~~ ~~1%~~ .316% (1) .1% (2) .032% (3) .01% (4) 0 (5) final conc.

from 10X these conc. stock solns. (A1-2-3-5 are halved in volume).

Diluted 1/1000 at 45' incubation and plate .05 ml on Dsm B. (No blending)

0 = 640 PM

But A5, B5 << C, D.

Counts. 3PM 31 May

conclude W3060 n.u.g.

decreasing azide →

	1	2	3	4	5
A SS	0	0	7	51	38
B SR	0	1	6	18	44
C RS	0	1	43	108	~~...~~ ~400
D RR	1	10	41	118	318

↑ effective concentration ↑ threshold

No indication of a differential effect of azide. ~~...~~ S×S ~~...~~ shows same yield reduction as R×R. Need to reconsider plan

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19 May 3rd 1958 REF: 1416

DNA - LEAKAGE.

W3060, W3064 rotated to saturation or almost ($2\frac{1}{2}$ hrs)
9 cultures each.

Suspend in BGA 15x after one washing.

NBC : not blended controls
BC : blended controls
M : mixture, blended

Sample at times indicated, 0.5 ml into 3.5 ml of chilled DW to be spun.

(M): 1.5 ♂ + 1.5 ♀ ml ; incub 37° for 10' → blended → incub. →
↓ time 0 ↓ 20' ↓ 40' ↓ 60

(BC) 1.1 ml ♂, blend → incub 37°
↓ time 0 ↓ time 60'

Same for ♀
1.1 ml ♂ + 1.1 ♀, both blended before : → incub.
↓ time 0 ↓ 20' ↓ 40' ↓ 60

(NBC) Same as BC, but unblended suspensions.

All blendings: Virtis 30"

Notes. Caldwell & Hinshelwood give amount of DNA in Bact lactis aerog as: 2 mg DNA-P per 10^{12} cells.
P-content of DNA is 9%. Hence 220 γ per 10^{10} cells.
Sensitivity of methods: 259 mμ : $\varepsilon(P) = 6650$.
MW of a nucleotide: 354.
5 γ/ml should give E = 0.10.
Ceriotti: 2.5-15 μg/ml.

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19

REF: 1415

Tube Numbers:

1 BC, ♂ Time 0
2 " ♀ "
3 " ♂+♀ "
4 NBC ♂ "
5 " ♀ "
6 " ♂+♀ "
7 M after blending
8 BC ♂ + ♀ time 20
9 NBC " " "
10 M Time 20' after blending.] mixed by mistake.
11 BC ♂ + ♀ Time 40'.
12 NBC " " " "
13 M Time 40'
14 BC Time 60' ♂ + ♀ (0.2 instead of 0.5).
15 BC " ♂ ♀
16 BC " ♀ ♂.
17 NBC " ♂ + ♀
18 NBC " ♀ ♀
19 NBC " ♀ ♂
20 M Time 60'

At the end after dilution: cooled, centrifuged twice, stored at -20° until read in the spectrophotometer.

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REF: 1416

	1	2	3	4	5	6	7	8	9	10
1		Difference in E between 260 & 440 -								
2										
3			BGA = -13 - Correction applied to all							
4										
5										
6										
7			BC Blended matings		NBC Non blended matings					
8										
9		Time								
0										
	♂	0'	11		19					
1		60'	39		23					
2							Blended Mating			
3	♀	0'	16		16					
4		60'	28		-					
5										
6										
7	♂+♀	0'	11		4		-			
8		20'	13		-		-			
9		40'	11		12		43			
0		60'	18		20		25			
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										
1										
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6										
7										
8										
9										
0										

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Slit 0.1

19

REF:

	1 220	2 230	3 240	4 250	5 260	6 270	7 280	8 290	9 300	10
BGA	[illegible]	.059	.042	.035	.029	.025	.023	.018	.015	
1	.320	.175	.117	.118	.115	.098	.063	.045	.034	
2	560	.262	.162	.162	.165	.138	.092	.058	.040	
3			194	187	192	163	122	.092	.062	
4			132	142	141	120	87	35	.044	
5										
6			.180	170	.171	147	105	73	.058	
7										
8	480	270	175	175	175	148	098	65	45	
9+10	500	275	170	170	170	150	98	65	45	
11	520	315	220	215	218	185	118	78	55	
12		235	143	138	142	120	88	45	30	
13		250	138	150	165	134	85	35	20	
14		215	50	55	55	45	39	20	05	
15										
15		238	132	142	147	110	65			
16		168	88-90	105-108	115-116	85-88	45			
17		242	148	150	155	125	85			
18	20	172	130	140	142	120	77			
19	18	235	155	155	165	140	94			
9				No. 13		No. 16				
0	Tube No 13		λ							
			230	240						
1	Slit .05		232	200						
2			4	180						
3			6	155						
4			8	154						
5			240	140						
6			2	142						
7			4	145						
8			6	148						
9			8	152						
0			250	152						
			2	160						
1			4	162						
2			6	162						
3			8	163						
4			260	170						
5			2	166						
6			4	167						
7			6	158						
8			8	143						
9			270	138						
0			2	122						
			4	110						
1			6	110						
2			8	90						
3			280	72						
4										
5										
6										
7										
8										
9										
0										

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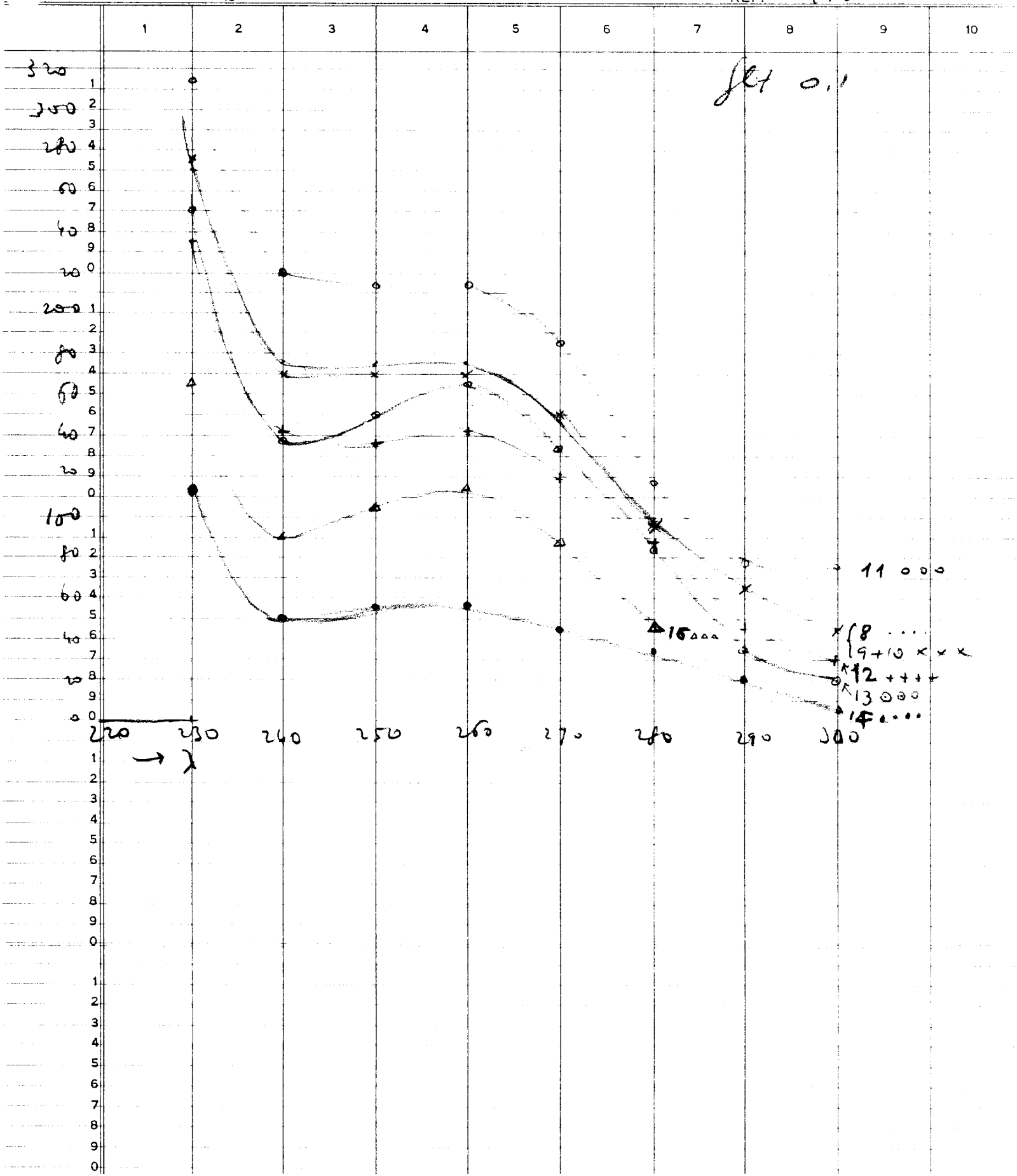
REF: 1416



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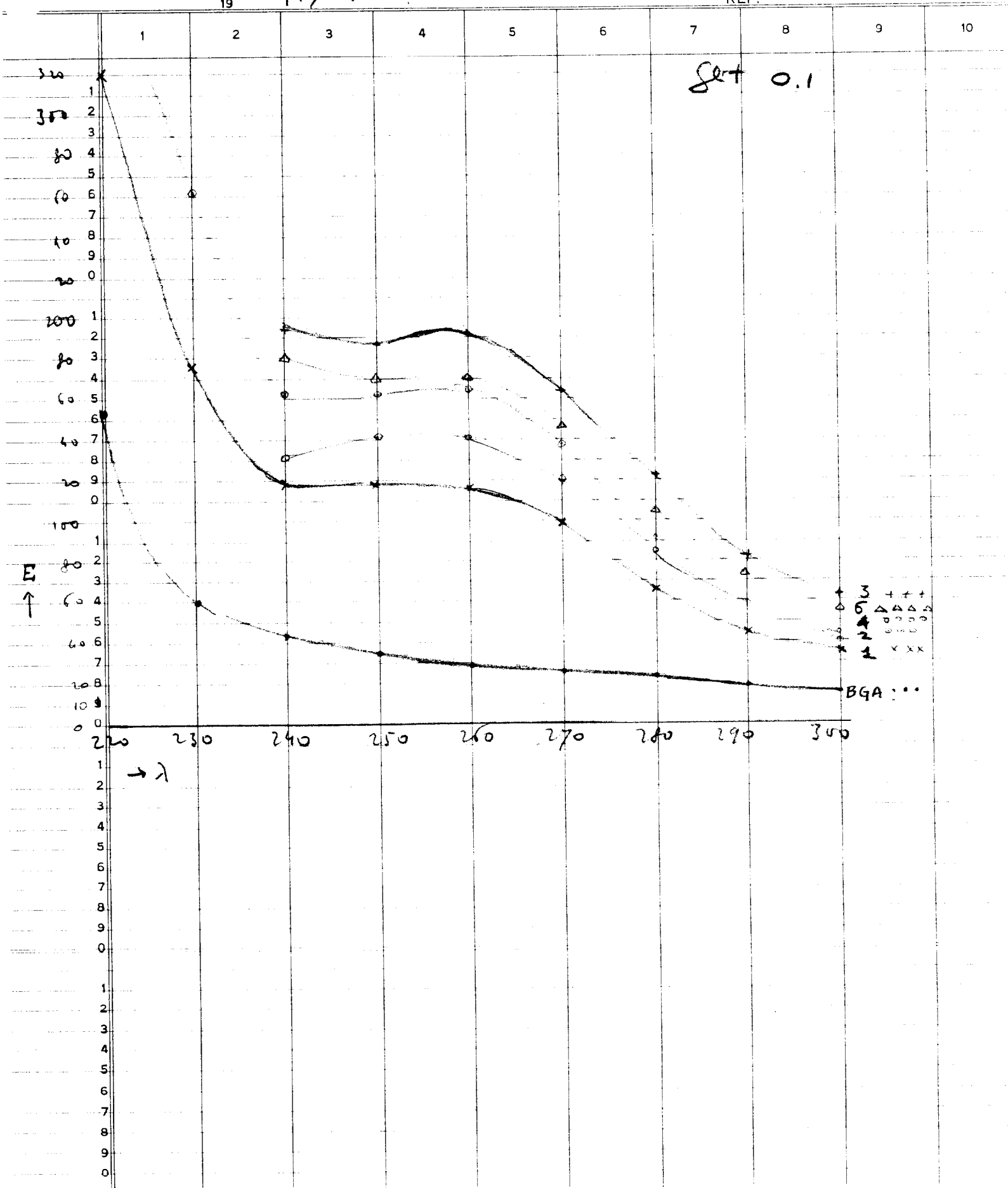
19 May 7, 1958 REF: 1415.



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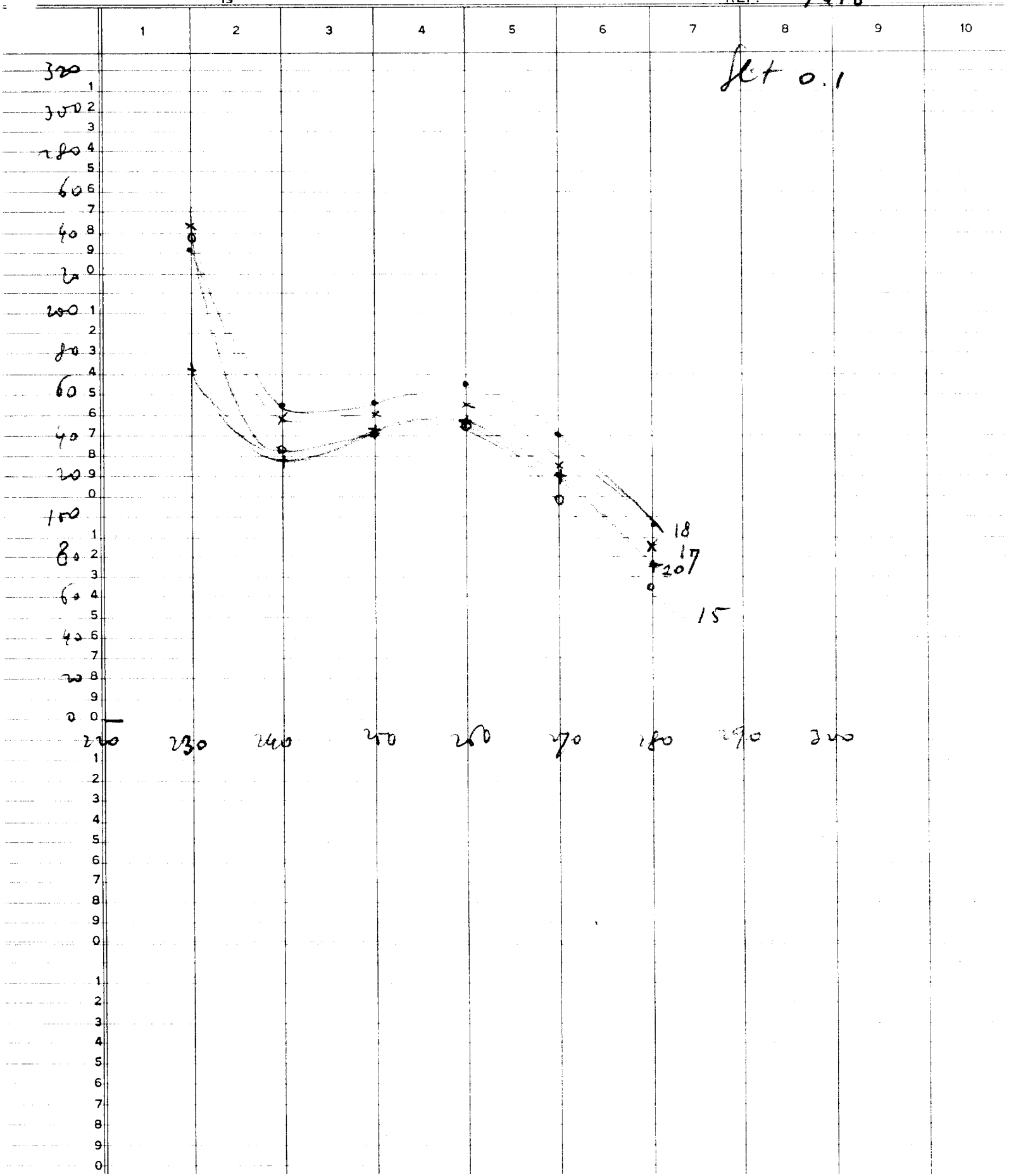
REF: 1616



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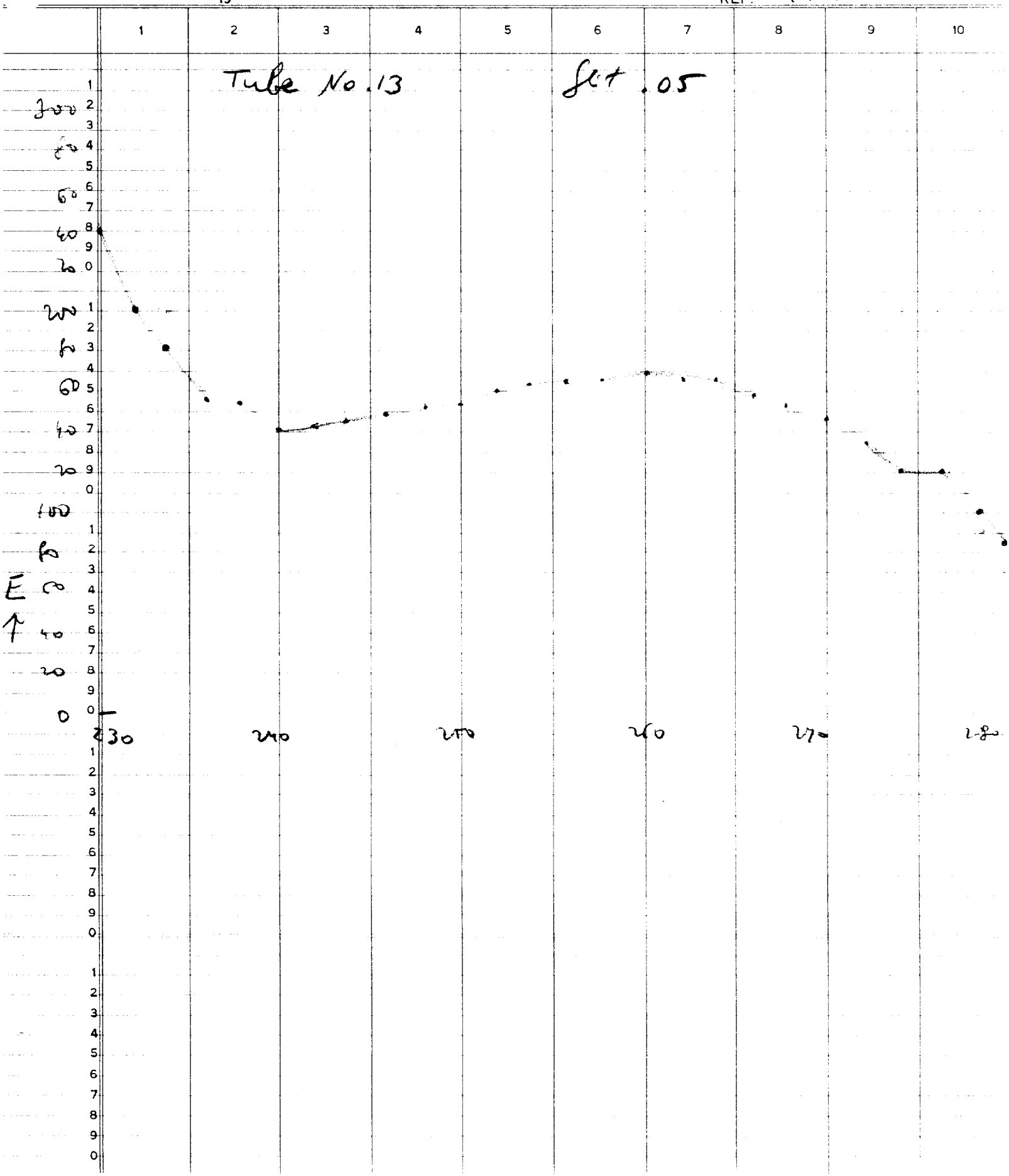
REF: 1416



1958, May 8th REF: 1417.

W1895. exp. culture *, 1 ml + 7.5 Penassay, 2½ h rotation
W3064 24h cult. same.

Spun, resuspended in Penassay 0.2 ml per tube. (50 x conc)

♂ 0.1 ml + ♀ 1 ml. ♀ pulse in water bath.
Dilution 1/1000 in broth.

At 10', 20', 30', 60', ~~90'~~, 60' blend and plate 0.05 of undil., ~~1/10~~, ~~1/100~~ on B Lac Sm, and of ~~1/10~~ on undil D(B,St) and D(0).

Plate recombination control: C
Control of recombination in diluted mixture: D

D: dilute W1895 & W3064 conc. susp. 1/1000 in broth, mix 1:10, incubate 60'. plate undil, ~~1/10~~ B Lac Sm, and ~~1/10~~ on B,St, D(0)

C: same: plate from 1/1000 dilutions W3064, 0.05 ml and 0.01 ml of 1/2000 dil of W1895. on B Lac Sm, D(♂+B,), D(0)

* 3 parallel cultures kept in frig for reuse.

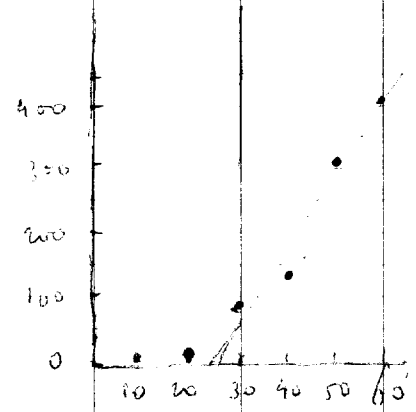
Photometry of 1/100 dil. in water of conc. suspensions:
♂ 68%
♀ 75%
} λ : 650 mμ

Plate counts.

B Lac Sm : growth entirely negative.

D(0) : all plates including C and D have approximately the same number of recombinants : about 1000 _ All plate recombination _

	min St B_1
C	10
D	~800
10'	12
20'	19
30'	86
40'	138
50'	320
60'	410 .



Pulse useless with Hfr_1 ?
May be due to use of Penassay for pulsing.
However there is a high recombination rate in D
Time pattern likely to be ~~of~~ indicative of zygote recruitment rather than of anything else.

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19 REF: 1-717/2

W 3936 (1895 Hfr, Hz^R) 1 ml + 7.5 ml Pen 1^h rotation
W 3064 " "

Spun, resuspended in fresh Penassay at double conc.
Mixture: 1 ml ♂ + 9 ml ♀ in water bath
Sampled: .5 every 10' diluted in chilled DW 4.5 ml.
and then further dilutions and platings on the
following media: (.05)-
3) D-0 B_1 threonine leucine methionine Sm
4) " B_1 methionine Sm
5) " Methionine Sm
6) MLac - threonine leucine B_1 meth. Proline, Sm.

Dilution	0'	10'	20'	30'	40'	50'	60'
A 1/10	4,5	3,6,4,5	4,5	5	5		
B 1/100	3,6,4,5	4,5	3,6,4,5	3,6,4,5	4,5	5	
C 1/1000	3,6,4,5	3,6,4,5	3,6.	3,6,4	3,6,4	3,6,4,5	4,5
D 1/10.000	3,6	(3,6) ↑ by mistake			3,6	3,6,4	3,6

40' is actually 43'-

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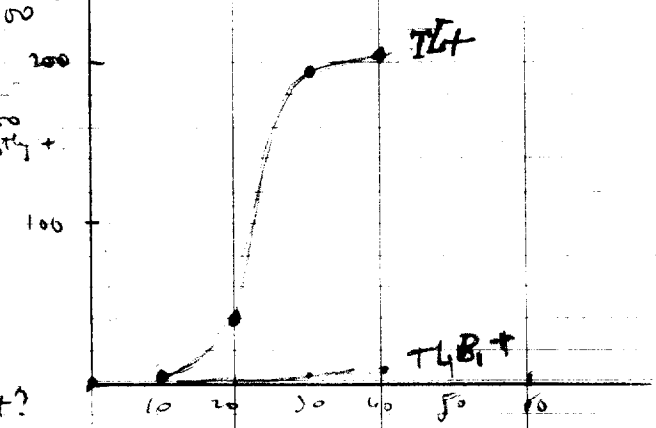
1417B

19 5/23 REF: 1417/2

Time	Dilution	3 D(MTLB, sm)	4 D(MB, sm) [TL+]	5 D(M sm) TLB,+	6 (growth only after 48 hrs) MLac (TLB, MP sm)
		Medium	4 glucose growth		
0'	A		0	0	
	B	∞	0	0	20, some +, plus background
	C	~6000	0	0	~2000 mostly +
	D	~700			
10'	A	∞	34	0	24 +, many neg papillae, plus background
	B		2	1	
	C	∞	0	0	~3000 mostly −
	D	~3000			227 mostly +
20'	A		394	2	
	B	∞	34	0	~100 neg small & background.
	C	∞			~2000 mostly +
30'	A			3	
	B		198	0	25 + background
	C	∞	20		~2000, some +
40'	A			7	
	B		201	0	
	C	∞	8		~120 + and −
	D	1500			600 all +
50'	B			0	
	C	∞	7	0	~5000, some +
	D	2500	1		~600
60'	C		24	0	
	D	2000			~200 mostly +



Note on medium 6.

On most plates there is a segregation into Lac+ & Lac−. Due to gal+?

[illegible] the low transfer of [illegible] due to [illegible] and [illegible] of [illegible]

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Hfr,

W2150 × W3064 ; chloramphenicol

1417C

26 June 1958.

REF:

Timing of Xyl, Mtl among $T\Sigma^1 S^0$ recombinants. (also among $Lac^+ S^+$?).

Skaar reported a zero incidence of $Thal^+$ etc. in both crosses. These might be very late markers, and as such might be most influenced by the inhibition of replication.

Try timing and effect of 20 r/ml chloramphenicol on the Xyl ratio, etc.

W2150 : W3064 chilled; .1 : .1 : 5 ml. ~~Plate~~ Dilute 1:100 and plate .05 ml D sm B_1.
20 × ORC

at times indicated. t = 00, 0, ~~15~~ 16, ~~20~~ 22, 30, 40, 50, 60, 90, 90C [chloramphenicol from beginning].
undil.

Counts (Bc. 2 days; at room temp to 29 June).

P29.

t mins	(SRP - D sm B_1)	B	Gal sm B_1	M	Lac	Gal	Mal	Xyl
00	~10^4				50% ?	0	½?	13 ?
0	1							
16	32				33%	0	1?	0
22	65				66%	0	0	2
30	351							
40	~ 500				50%	0	0	0
60	10^3	0		0	66%	0	0	3
90	10^3	0		0	66%	0	1	8
60C	10^3			0	66%	0	1	2
90C	10^3 -			0	50%	0	0	0

note variability of early entry!

16 indicates $T\Sigma$.

90C < 90 but too many to count. repit., and 1/10 dilns

What is Xyl ratio, etc.

July 1. – To refrigerator to await scoring for late markers.

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19 May 19, 1958. REF: 1418

TRANSFORMATION

Inserting DNA by making holes with F+ or Hfr and partial osmotic shock of recipient protoplasted ♀.

DNA: source W 3064, protoplasts, concentrated to 10^9/ml and shocked in DW. Kept at -20° until used; thawed, 4 vols. of alcohol added, filaments removed by glass rod to .5% saline. Partial redissolution, cell walls not entirely eliminated. Blending to resuspend.

Recipient: W 1827 (= W6 F-). Exponential, → protoplasted 2½ hrs, spun, concentrated from 10 tubes into 5 ml (= 20x)

Hole makers: W6 expon conc. 15x (to 0.7)
W 1895. expon. " (to 0.7).

(A). W6 + W 1827 ♂: 0.6 + 0.4. 3' waterbath, then add, under blending, 2 ml of DNA prep. - incubate 60', then plate .05 on B Gal Sm, S Mal (for lack of D(0))

(B) same with W 1895, but 10' waterbath

(C) same with 0.6 lambda prep., 5' waterbath

(D) Control of 3064: .05 on B Gal Sm, S Mal

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19 May 24th, 1958. REF: 1419

DNP resistance.

Broth cultures drops W 3060, W 3064 spread on Pheassay agar with

		W 3060	W 3064
DNP M/20	1 ml	–	—
"	.2 ml	growth	growth + ~50 papillae
"	.04 ml	growth	growth.

W 3064 papillae, isolated on ~~Pen~~ B Lac and on DNP M/20 1 ml plate – No growth on the latter. Streaks on B Lac replicated on .5, .4, .3 ml of DNP · growth of almost all. Slants made.

May 26th

.2 ; .38 ; .4 ; .5 ; .7 ml of M/20 DNP per plate and spread (broth drop of W 3060, W 3064) again

		.2	.3	.4	.5	.7 ml of DNP
48 hrs	3060	growth	scanty growth papillae	~40 small colonies	5 colonies	no growth
	3064	growth and darkening of medium, some papillae	irregular growth.		1 colony	"

After ~~one~~ more days of growth at room temperature, there are about 100 resistant colonies in W 3060 on .4 and 21 in .5; 1 colony in W 3064 on .4 and 3 colonies on .5.

Colonies from W 3060 on .5 streaked on B(Lac) for further testing.

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19 May 31st, 1958 REF: 1419

	1	2	3	4	5	6	7	8	9	10
			Testing DNP. resistance.							
			.2	.3	.4	.5	.7			
1	# 3060		+	±	−	−	−			
2	3064		+	∓	−	−	∓			
3	3064	DNP/1	+	+	−	−	−			
4		/2	+	+ (*)	−	−	−			
5		/3	+	+	∓	−	−			
6		/4	+	+	−	−	−			
7		/5	+	+	−	−	−			
8	3947 = W 3050 Az^R		+	∓	−	−	−			

(*) determines a slight brown coloration of the medium.
(formation of intraanilin ?)