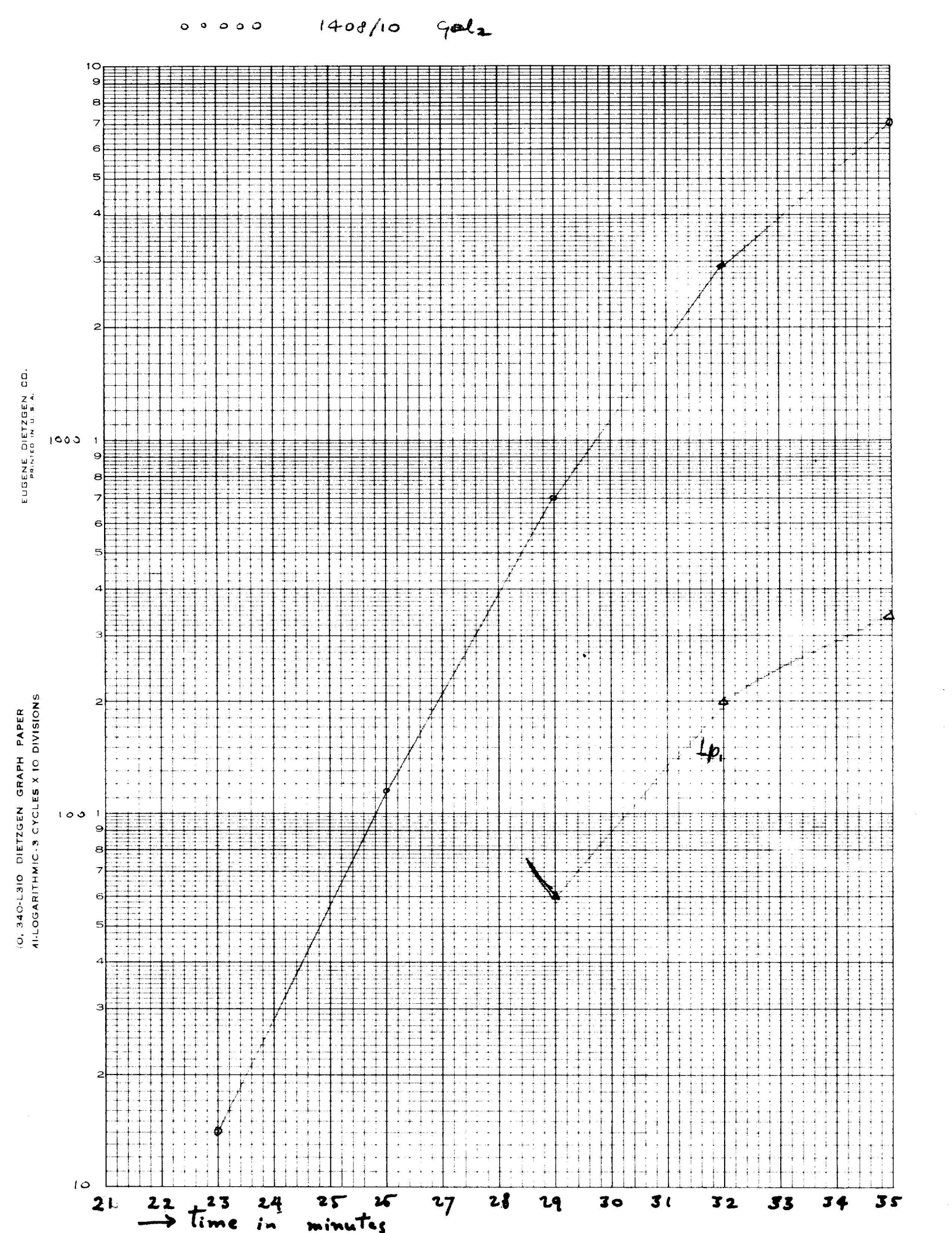
00 000 1408/10 gal2
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
10
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
→ time in minutes
Δp1
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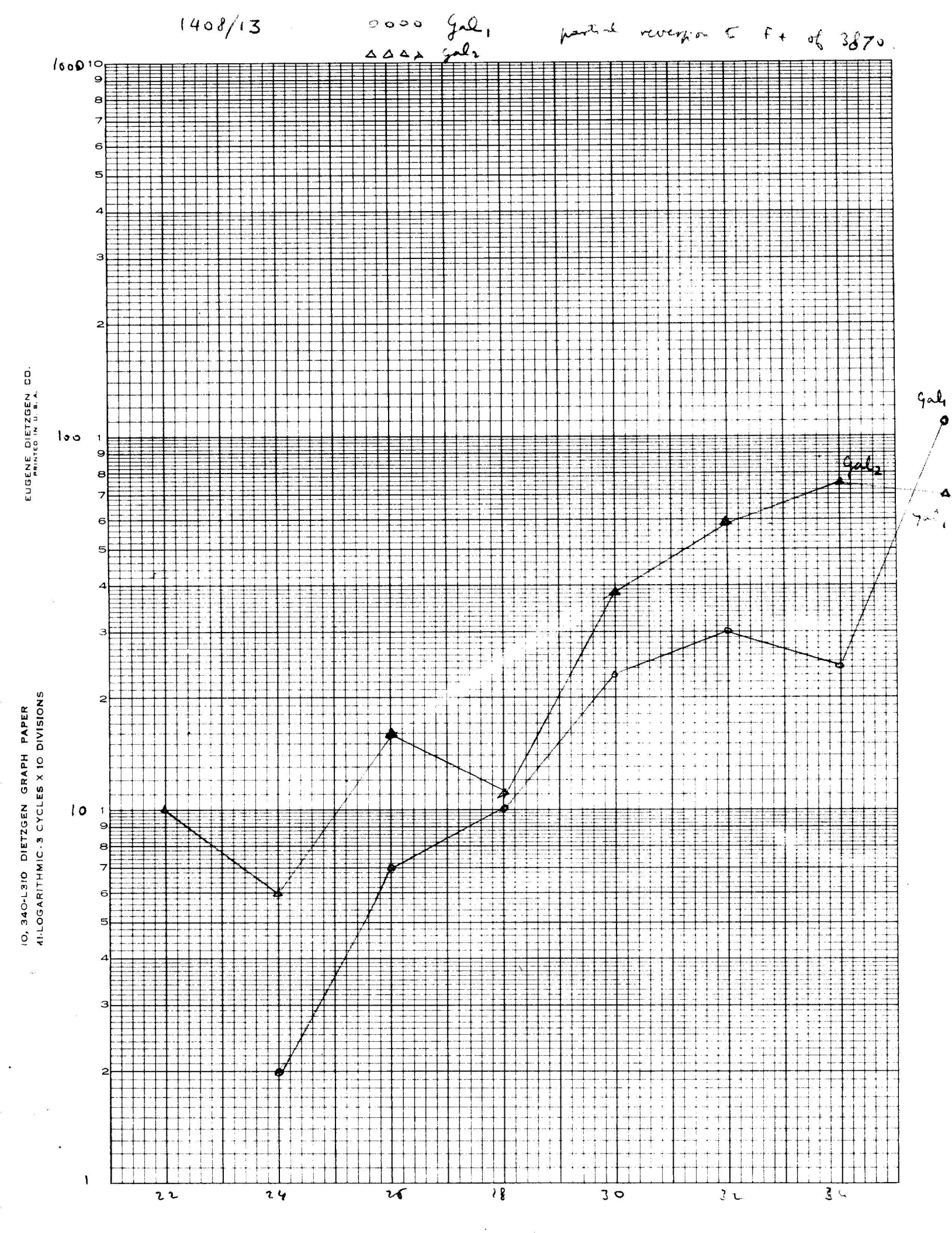
1408/13
○○○○ Gal1
△△△△ Gal2
partial reversion F F+ of 3870
Gal1
Gal2
22
24
26
28
30
32
34
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1412

B1 B2 and C C

May 1 19 58

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	−	−	+		− ↑.05	+	−	−	+	
9	−	−	−		− .1	+	−	−	+	
0	−	−	−		− ↓	+	−	+	+	
1	−	−	−		−	−	−	−	+	
2	−	−	+		−	−	−	−	+	
3	−	−	+		−	+	−	−	−	
4	−	− ↑.05	+		−	+	−	−	−	
5	−	− 0.1 ↓	+		−	+	−	−	+	
6	−	−	+		−	−	−	−	−	
7	−	−	+		−	−	−	−	+	
8	−	−	+		−	−	−	−	−	
9	−	−	+		−	+	−	−	+	
0	−	−	+		−	+	+	+	+	
1	−	−	+		−	−	−	−	+	
2	−	−	−		−	+	−	−	+	
3	−	−	−		−	−	−	−	+	
4	−	−	−		+	−	−	−	−	
5	−	−	−		−	+	−	−	−	
6	−	−	−		−	−	−	−	−	
7	−	−	−		−	+	−	−	−	
8	−	−	−		−	+	−	−	−	
9	−	+	−		−	−	−	−	+	
0	−	−	+		−	+	−	−	−	
1	−	−	±		−	−	−	−	+	
2	−	−	+		−	+	−	−	+	
3	−	−	+		−	−	−	−	−	
4	−	−	−		−	+	−	−	+	
5	−	−	+		−	−	−	−	+	
6	−	−	−		+	−	−	−	+	
7	−	−	−		−	+	−	−	−	
8	−	+	−		− ↑.05	+	−	−	+	
9	−	−	+		− .1 ↓	+	−	W	+	
0	−	−	−		−	−	−	−	+	
1	−	−	+		−	−	−	−	+	
2	−	−	+		−	+	−	−	−	
3	−/w	−	+		−	−				
4	−	−	+		−	−				
5	−	−	+		−	+				
6	−	−	+		−	+				
7	−	−	±		−	−				
8	−	−			−	+				
9					−	+				
0					−	−				

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19

REF: 1408/14

A: Total count (binocular)

B: C. from 10 3× fields (× empirical factor 14)
exp. factor 21).

Time	Gal4		Gal7		Gal8	
	A	B	A	B	A	B
0						
22	0		8		0	
24	0		11		6	
26	57		37		55	
28	210	(9)	195		128	(11)
30	471	(28)	326	(21)	224	(28)
32	630	45	420	30	427	(27)
34	994	71	630	45	1190	85
36	1246	89	1610	115	1176	84
38	1064	76	2310	165	1666	119
40	1890	135	1974	141	3080	220
50	7334	524	6496	464	8596	614
60	6930	495	6748	482	3430	245

( ) between brackets: values used for calculation of empirical factor; total plate count given in A

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19 June 18, 1957. REF: 1408/15.

Hfr Timing of Gal4, Gal2, Gal3.

Exactly as 1408/14, but:

1) ♀ parents were Gal4, Gal2, Gal3, and they were seeded in this order.

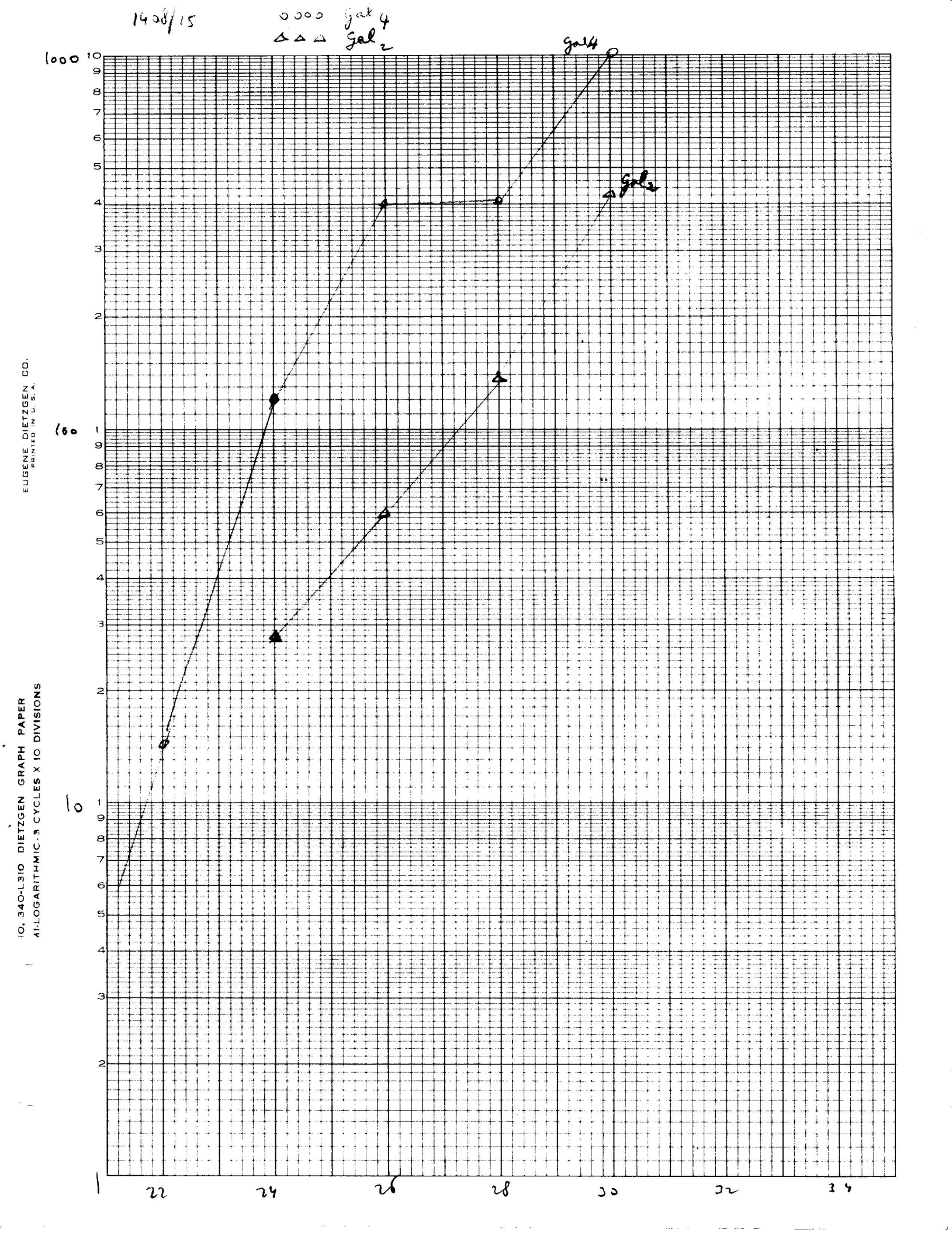
2) ♀ 3 was grown after Gal4 and Gal2 had been harvested, as a substitute for Gal4 which was supposed to be tested today and was found to be ♂. ♀ 3 was found to be slightly less concentrated than the other two females and three tubes were collected into two, they reaching 1.5 × conc. for this ♀.

3) Mating mixtures: 5 ml ♂ + 12 ml ♀.

Plate counts. (48 hrs) (72 hrs)

Time	Gal4	Gal3		Gal2
0	(35), 7	2, 0	13,	0, 0
15	7, 1	0, 3	11,	0, 0
18	2, 9	3.	27, 27	0, 1
20	3, 8	1, 0	23, 15	5, 2
22	18, 13	2, 3	18,	1, 10
24	138, 112	1, 3	16, 17	32, 24
26	451, 348	1, 1	8, 17	68, 51
28	410, 448	0, 1	11, 17	160, 110
30	t.m.t.b.c.	3, 12	13, 27	420,
35	↓	28, 25	38, 34	
40		55, 69	60,	
50		205, 198		
60		391, 356		

Note: Gal3, all large colonies; in addition, few (<20 on plate) small ones and 1 or 2 extremely small.
Gal2, Gal4: all colonies small, as in former exptl.


1408/15
○○○○ Gal 4
△△△ Gal 2
Gal4
Gal2
1000
100
10
1
22
24
26
28
30
32
34
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19 June 20, 1950. REF: 1408/16

Hfr$_2$ timing of Gal$_{5,2,1,3}$.

Source of expts 13, 14, 15.

Mating mixtures: ♀ 4 ml + ♂ 12 ml.

Order of seeding flasks: Gal$_5$, Gal$_2$, Gal$_1$, Gal$_3$.

Times: 0', 18', 22', 24', 26', 28', 30', 32', 34', 40'.
120'

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1408/16

19 REF:

	1	2	3	4	5	6	7	8	9	10
		3991 Gal 1		3992 Gal 2		3993 Gal 3		3995 Gal 22		
4	0	1		4		2		2		
6	18	2		16		20,		3		
8	20	3,6	14	28, 14	63	17, 13	45	2, 0	3	
0	~~24~~	31, 30, 32	93	61, 91, 67	219	15, 14, 14	43	24, 40, 69	133	
1	22	23, 9, 30	62	22, 59, 54,	135	13, 12, 21	46	7, 17, 12,	36	
3	26	198, 174, 177	549	212, 252, 235	699	16, 21,	55	97, 69, 137	303	
5	28	193, 231, 101	525	313, 296, 264	873	19, 34, 18	71	227, 260, 818	731.	
7	30					11, 18, 13				
9	32					26, 41				
	34					54, 23				
2	40					191,				
	parent	1		0		17		1		
6	parent	light								

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19 June 22, 1958. REF: 1408/17

Hfr_2 timing of $Gal_{1,2,8}$

Same as expts 13, 14, 15, 16.

Mating mixtures: ♂ 4 ml + ♀ 12 ml.

Order of seeding flasks: Gal_8, Gal_1, Gal_2.

Times 0, 18, 20 (22), 24, 26, 28, 30, 32, 35'.

Note: Gal_1 at 22' is actually 23'.

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19 June 23, 1958. REF: 1408/18

Hfr$_2$ timing of Gal$_2$, Gal$_4$, Gal$_6$, Gal$_9$

Same as expts 13, → 17.

Mating mixtures: 15 ml ♀ + 5 ml ♂,

Sampling at times: 0', 20', 22', 24', 26', 28', 30';
for Gal$_9$ also 35', 40', 50'.

Order of seeding: 2, 4, 6, 9

Note: Suspension of Gal$_9$ is granular, and slightly less conc. than others in spite of using 4 tubes mixing to 24 ml. (4/3 conc.)

Time 22' of Gal$_2$ seems very thin, possibly amount measured out of flask was spilled in one tube?

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19 June 25, 1958 REF: 1408/19

Hfr$_2$ timing of Gal$_2$, Gal$_5$, Gal$_7$, Gal$_8$.

Same as expt 13-18. 1^{h}10' rotation. Gal$_5$ slightly less turbid than others.

Mating mixtures: 5 ml ♂ + 15 ml ♀.

Sampling at times: 0', 20', 22', 24', 26', 28', 30'.

Order of seeding 2, 5, 7, 8.

7 2/8 and 24/2 may have been exchanged at plating?

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19 June 27 REF: 1408/20

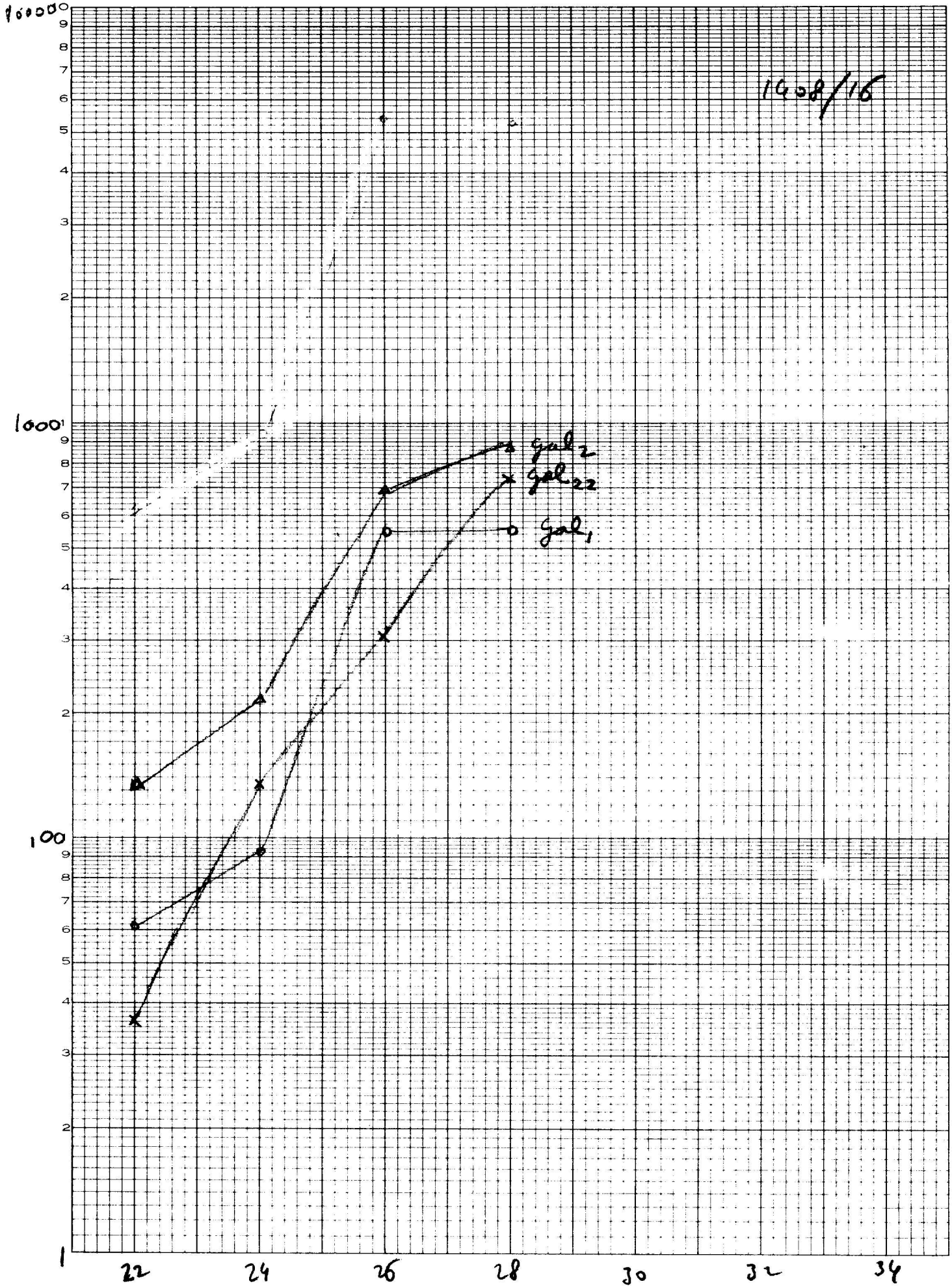
Hfr$_2$ Timing of Gal, Try.

ORC cultures of W 3870, W 3908, W 4066/1 (Try-S^{R}Gal-).
Refreshed, 1 ml + 7.5 ml for 1^{hr} 2pcm, resusp. in 2 ml
(4 x conc). Crosses: 3870 x 3908 ; 3870 x 4066 - UV Gal$_x$ W4076
I. indol T = tryptophan
Mating mixtures, in flasks: 20 ml ♂ + 6 ml ♀ = 1:3.
Samples: 0.2 ml + 1.8 chilled H_2O and blended at every time: series (A)
if plated directly (.05 ml), if further diluted: series (B) (C)
Schedule:

Time	D(SmB$_1$) & S Gal SmB$_1$		M Gal SmB$_1$ Try	
♀ Control	A	B	A	B & C
0	1/10		1/10	
10'				
20'				
22				
24				
26			26'	1/20 = A 1+1
28			28'	1/40 1+3
30			30'	1/80 1+7
33			33'	1/160 .5+9.5
36				
39		1/20 = A 1+1		
42		1/40 1+3	42'	1/500 .2+9.8
45		1/100 1+9		
48		1/200 .5+9.5		
51		1/400 .25+9.75	51'	1/1000 .1+9.9
54		1/800 .125+9.9		
57		1/1000 .1+9.9		
60'		1/1000 .1+9.9	60'	1/1000 .1+9.9
0/60 ♯		—		

0/60 : Control of plate recombination with parents kept in waterbath throughout the exp. (60').

Note: 51' killed. 33' was 40" late.



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340-L310 DIETZGEN GRAPH PAPER
4 LOGARITHMIC 3 CYCLES X 10 DIVISIONS

DATE: 4.16.54. REF: 1409-I.

ENZYMES ON PROTOPLASTS.

1 and 2 ml of 3060 + 10 ml L_2 + 10^4 U/Penicillin
0.5 and 1 ml of 3064 + "
Centrifuged, resuspended in half the amount of L_2.
Mixed in equal volumes with:

A RN-ase 2 mg/ml in L_2
B Chymotrypsin "
C Lysozyme "
D DN-ase "

N : = control + L_2 medium,
F Versene 4% 0.25 ml added, + 0.25 ml L_2 + 0.25 ml ♂
G Lysozyme 0.25 ml + Versene 0.25 + ♂ 0.25.

After 15-30': Protoplast counts in Petroff chamber. (millions/ml).
(20 small squares: sum, multiply x 10^6).

N	A	B	C	D	F	G
63	70	60	54	81	82	59.

After 2 hours:

44	27*	41	44	42	63	not done.

* many empties.

Conclusions:
Only RNA-ase seems to affect, and only to a moderate degree, the protoplasts count.

This is test of lysis of protoplasts by total count.

DATE: 4/18/58. REF: 1409-2

ACTION OF ENZYMES ON MATING ♂♂ × ♀.

3060 ♂ (2 ml + 10 ml L_2, 2'30' incub.), centrifuged, concentrated 10× in L_2 + 3064 ♀ conc. 30× in L_2.

0.3 + 0.3 ml in waterbath for 20', then 0.1 ml. added to 1 ml of:

A Chymotrypsin 1 mg/ml in L_2
B Lysozyme, "
C RN-ase "
D DN-ase
L control, L_2 medium.

40' further incubation after addition of enzyme, then

~~0,02 ml~~ 0.1 + 10.0 DW, → { 0.1 ml / 0.01 ml } on mix St B,

Protoplast suspensions: same as those used for exp. 1405-5 before diluting 5×.

Plate counts:

	A	B	C	D	L
0.1	too many, ~~ca~~	∞	∞	∞	∞
0.01	113	43	62	74	93
Gal+	0/48	2/43	0/47	0/42	1/50
Lac+	7/48	5/43	5/47	1/42	6/50
T_1'	19/48	18/43	31/47	16/42	23/50

1410

DATE: 4/18/58 — REF: 1410

INTERRUPTION AND DIPLOIDS.

2323, 2735, ~~ove~~ overnight rotated cultures, mixed in equal amounts, pulse of 8', then diluted 1/200 in warm broth, further incubation: 20'; 40'; 60' in water bath. After such times:

0.1 → min B_1 { Resplated 1': Stac NC! ; 2': Stac, Stac B_1 (P21)

1/10 0.1 → "

1/10 0.01 → "

→ B Lac.

N.B. Numbers meaningless on account of smearing and poor scores on Lac. Colonies can.

	20' [A]	40' [B]	60' [C]	
D(B_1) total	288	412	181	($\frac{1}{10}$ .1 ml).
Stac B_1	9…29	—	52…20	Lac+
Stal B_1	34…42	—	38…42	… diploid-synthetic

Lac + 1 sel + checked 4/22

2(2)

interrupted only by plating.

DATE:

REF: 1410

1	2	3	4	5	6	7	8	9	10

Irradiation of JO60 to obtain Ara-

6 B Ara plates irradiated with 9 and 10 secs.
3 putative Ara- mutants found
streaked to B Ara

DATE: 22/4 REF: 1410/2

Experiment 1410/1 repeated.

Platings of $\frac{1}{10}$ dil in DW of 0.1 ; 0.02 on min B_1
0.1 on S Lac B_1, S Gal B_1

Note: Is plating interruption sufficient with 2323 ?
Frequency of Gal+ seems unaffected by plating time.
in exp 1410-1.
S Gal milling for 40'.

Counts:			
D(B_1)	20'	40'	60'
$\frac{1}{10}$ 0.1	26	111	35
0.02	2	4	6
S Lac B_1	10 Lac+		

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19 30th April 1958. REF: 1410/3

1217 × 3064. ORC.

3× conc. in saline / .05 on plate of min B1.

3,5 hr.

1217 control: 0 colonies.

Colonies streaked on EMB lac.

DATE: 4/21/58 REF: 1411

COLCHICINE - Effect on mating

Solution of colchicine 1% in distilled water, non sterile, stored at -20°.

3060, 3064 overnight rotated cult :

0.1 ml to 0.8 ml Penassay + 0.1 H_2O (Control)*

" " + 0.1 colchicine solution 1%.

1h rotation -

CO : mixed equal amounts of colchicined broths, + 0.1 ml. colchicine/ml.

I : mixed equal amounts of (Control)* broths, 0.1 ml colchicine water added per ml.

C : mixed equal amounts of (Control)* broths 0.1 ml water added per ml.

10' incubation in water bath, then dilution 1/100 in broth, incubation 20', then: 1/10 DW → 0.05 minSt B1

↓

1/10 DW → same

↓

1/100 DW → 0.05 B lac.

	CROSSES 1/10	1/100	B Lac Lac+	Lac-
CO	152	6	6	17
I	69	7	6	14
C	146	14	6	15

Conclusions : there is perhaps a small decrease in No of prototrophs adding colchicine to the mating mixture (but not adding it in advance to the cultures : adaptive enzyme destroying colchicine ?) -

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Freezing mating cultures

1412

April 26 1958

REF: 1401-2.

ORC = overnight shaken cultures.

W3060 (overnight) 3X 10ml → 3
W3064S rotated 30X 10ml → 0.3 ml. in fresh prewarmed } .2 .2 mixed.

Prewarmed cultures mixed in a 125 ml flask. After 60 seconds added 19.6 ml prewarmed broth for 1:20 dilution. Mix gently in flask.

[A2] Add 1 ml samples to prechilled tubes in CO_2-acetone bath.

[A1] Dilute 1 + 3 ml 20% glycerol and freeze. = glycerol-freeze.

Dilute 1:250 in broth, incubate 15 minutes. Chill in ice bath.

[B] sample 1+3 ml glycerol & freeze.

Blend 30 seconds.

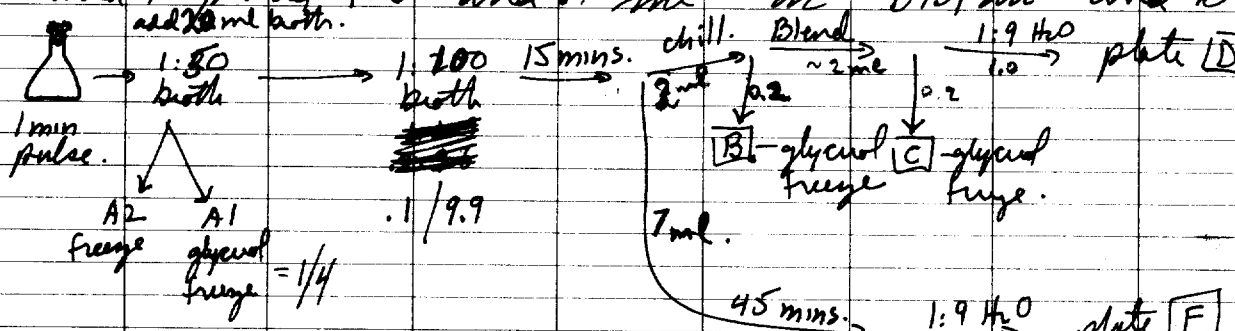
[C] glycerol-freeze

Dilute 1/9 in water and plate [D].

Incubate 45 minutes further

[E] glycerol freeze [F] dilute 1/9 in water and plate.

D and F plated .05 and .1 ml on DB, sm and B bac.



frozen tubes stored kept at -20 not on frozen CO_2.

addendum: Drosophila eggs and larvae in agar/10% glycerol. Chill in ice water & freeze quickly in CO_2 acetone. — proved unviable.

See Luca's notes: he finds that freezing interrupts pairs; yields of recombinants are about 50% of non-frozen controls. Technique is possible subject to some possible bias; may be good way to interrupt (contra first diluting in chilled saline.)

(over)

3060 x 3064.

(1412)

5 1 ml samples.

1 ml. A.

fuge 1 sample.

Pulse 1 minute. 1:20 ~~dilution~~ dilution = 20 ml.

incubate 1:~~200~~ dilution

→ 15 minutes

fuge 1 sample – 1 ml. B

~~dilute 1:10~~

~~blend~~ dilute 1:10 → fuge 1 sample C →

plate 1 sample D → } count

incubate 45 m. → fuge 1 sample E → }

plate 1 sample F → } count

---

1. – F. interruption by fusing as Tl^+ count.

B – F interruption by bac ratio.

~~B~~ –

C – D viability of zygotes.

C – E interruption by bac ratio

B, CE vs D, F viability of zygotes as TL count.

C, E vs DF viability of input.

storage; methods of thawing

FREEZE

DATE: 4/26/58, REF: 1412

3060 o.r.c. conc. 3 × broth 0.2 ml
3064 30 × 0.2 ml } in 50 ml flasks warm

After 1' pulse add 19.6 ml broth from test tube. (1/50 dil)

sample for further dilution (0.1 ml + 9.9 prewarmed broth)

(1/5000)

(A1) glycerol freeze 0.2 + 0.6 glycerol 20%.

(A2) freez

15' incubation

chill in icebath 2 ml

incubate further 45'

1+9 H2O

(B) glycerol freeze 0.2+0.6

Blend

1+9 H2O

(E) glycerol freeze 0.2+0.6

(F) plate

(C) glycerol freeze 0.2+0.6

(D) plate

Immediate plating: D, F on twin ft B, 0.05 & 0.1

counts:

		0.1	0.05
	D	12,13	7,9
	F	45,30	16,25

DATE: REF: 1412

C, E plated on 4/28/58 after thawing in water bath without further dilution (they are 2.5 × more concentrated than comparable tubes, D, F). Plate on mixt B_1 of 0.1 and 0.05 ml.

B thawed in water bath on 4/28/58, divided into B_1 & B_2

B_1 : incubated 45', plate 0.1 & 0.05 on mixt B_1

B_2 : plated at once " "

Plate counts :

	B_1	B_2	C	E
0.1	19, 15	2, 11*	26, 27	30, 47
0.05	6, 8	4, 2	8, 9	31, 12

Comparison between C & D : C, total 70 col. ÷ 2.5 = 28

D, total 41

survival $\frac{28}{41}$ = 67%.

E & F : E total 120 col ÷ 2.5 = 48

F 116 col

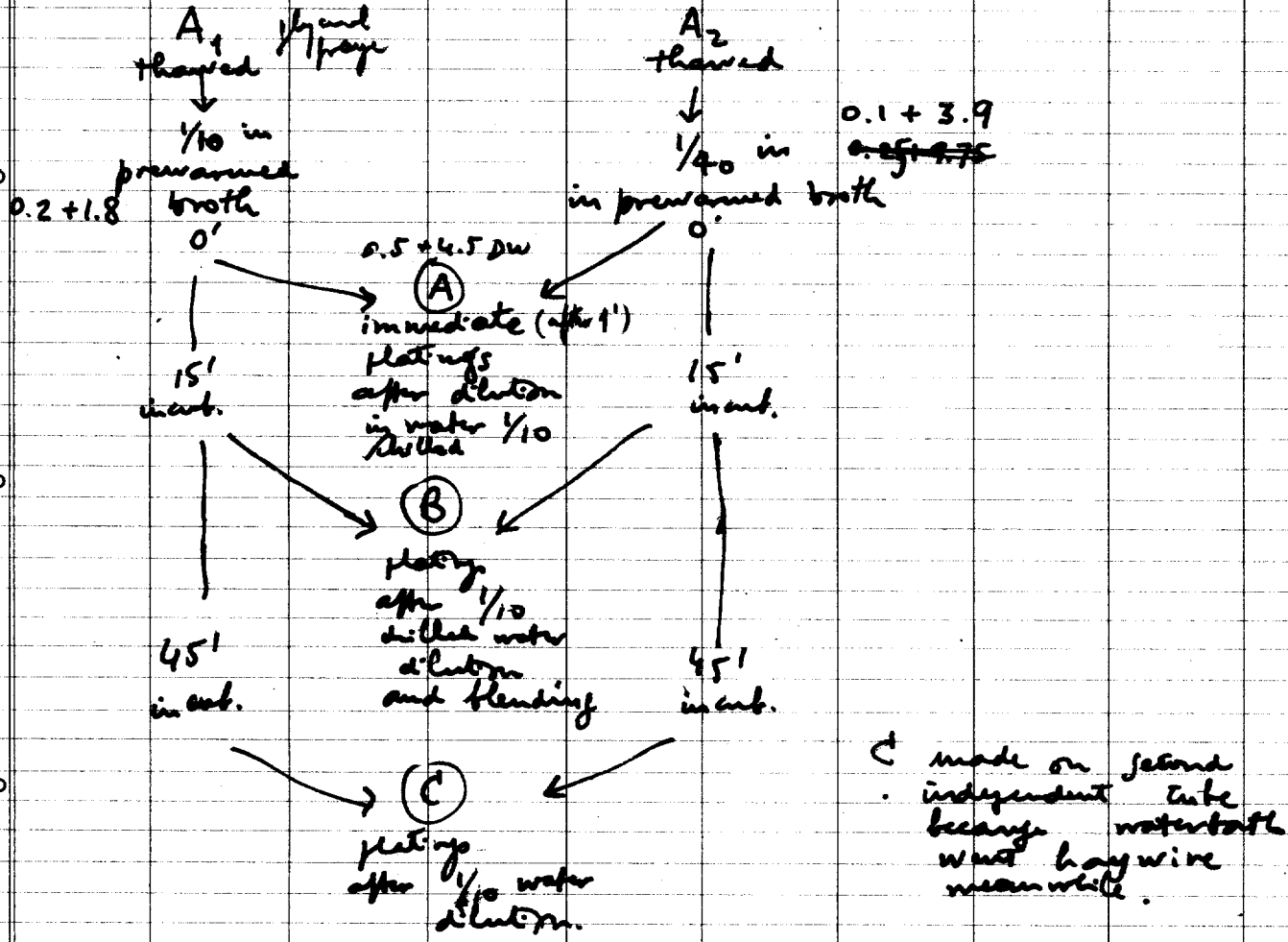
survival $\frac{48}{116}$ = 40%.

B_1 & C B_1 48 col.

C 70 col.

DATE: 4/29/58 REF: 1412

A_1 and A_2 tested for ability to resume mating.



c made on second independent tube because waterbath went haywire meanwhile.

Platings : .1 + .05 ml on minSt B_1.

Plate recombination controls.

Frozen parents (see exp 1412/2) :

3060 [0.1 ml + 9.9 ml broth] → [0.5 + 9.5 ml] → 1/10 DW
30 x conc. from expon. culture, considered 20% of saturation.

3064 [0.1 ml + 9.9 ml broth] → [.5 + 9.5 ml] → 1/10 DW
30 x conc saturated culture.

From dilutions in water: plates with 0.05 + 0.05
0.01 + 0.01