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19 June 1st, 1958 REF: 1420

PULSATION

O.r.c. W 3060, W 3064, spun, resusp. in cold Penassay. *

2.5 ml + 2.5 ml in a big flask – 5' pulse. Mix at 37°

0.1 ml transferred to 0.9 of the following. Chill at 5'. Dispense .1 ml to .1 ml treatments

			No put	Gal
D(0)	pH 4.5		++	+
	5.5		++	+
	5.9		++	+
	6.4		++	+
	6.9	± .5 % glucose	++	+
	7.3		++	+
	7.9	} some caramel formed		
	8.6		+	+
	9.0		+	+
D(0)	10 ml			
D(0)	thioglycolate .1% (10 ml)		+	+
D(0)	periodate (in pH 6.9)	M/100	0	0
	"	M/1000	+	+
	"	M/10,000	+	+
D(0)	give Mg		+	+
W 945	expon. resusp 4x, killed by boiling few seconds		+	+
"	"	living, in Penassay	+	–
Penassay			+	+
5' control	dil 1/1000 → .05 S Gal Sm B1		0	0

4:30 PM. (1-19): incubation 40' – Dil 1/100, plat up .05 on S Gal Sm B1

* cultures are viscous

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19 June 5, 1958 REF: 1420 A

	Plate counts	Gal +	Lac +	T_1	
1	310	1/50	5/50	39/50	
2	340	3/45	13/45	35/45	
3	298	5/50	8/48	30/50	
4	380	4/50	17/49	36/50	
5	310	4/50	14/48	36/50	
6	298	3/50	14/50	35/50	
7	320	3/50	17/48	36/48	
8	280	2/50	11/45	35/45	
9	355	2/50	10/48	31/50	
11	474	28/100	45/100	70/100	
12	365	14/100	39/100	64/100	
13	0				
14	107	0/80	3/80	35/80	
15	390	3/100	19/100	67/99	
16	370	6/50	23/50	35/50	
17	400	13/50	23/50	37/50	
18	148	0/50	1/50	18/50	← interrupted
19	430	6/50	15/48	38/50	
20	0		—		

Conclusions.

pH has practically no effect on transfer; some also on mating unless no recruitment takes place in these conditions. Range 4.5 – 9.

Thioglycolate has practically no effect at 1‰

Periodate M/1000 shows partial interruption

Addition of living F-cells interrupts, that of killed F-cells does not.

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PULSATION

design
1420 A

June 1 1958

REF:

Kinetic experiments based on pulsed mating have been unsatisfactory until now on account of an unsatisfactory separation of recruitment and entry phases. In previous control experiments, extent of recruitment varied as approximately the 1/2 power of cell density (instead of expected square), so that subsequent recruitment accounted for 5-25% of total yields. This is quite unsatisfactory.

In this experiment, screen for reagents which may impede mating without preventing further entry.

Following are suggested:

1. pH range in .5 units from 4.5 to 9 Adjust D(0) by HCl or NaOH
2. tris
3. versene
4. thioglycollate
5. ethanolamine
6. dead and live S^s F^-
7. fluorouracil deoxyriboside ↓ not available today
8. periodate M/100 M/1000 M/10.000
9. D(0) ∅ Mg

Experimental design: Mate ORC W-3060 and W-3064 (washed and resuspended in cold penassay) at sat'd cell density for 5 minutes at 37°. Dilute 1:10 into indicated solutions (made up in D(0) unless indicated, and incubate to 60'. Dilute 1:100 for plating .05 ml on SGalThSm. The pulse matings should gave a baseline count of SRP including some 10% Gal+.

Toxicity will be indicated by a loss of SRP

Inhibition of entry will be indicated by failure of Gal+ among SRP

No inhibition will be indicated by marked rise in SRP

Inhibition of mating ∅ inh. of entry will be indicated by stabilization of SRP with establishment of Gal+.

Controls needed: Plate recombinants (plate mixture after dilution))
Early interruption: plate mating at 5';)
1:1000 dilution at 5'; plate at 5' 15' and 60') Dilute in D(0).....
1:10 dilution: plate at 5' 15' and 60')

Dilution only 1:10 from penassay!

Criticism: may have already been pulsed by high density. Luca's conclusions:
pH 5 to 8.5 no effect. 4.5 gradual reduction or slight 4 no effect. 2,3 not tried
D(0)-Mg no effect. periodate negative (partial interruption and null-recruitment)
6B no effect! But 6A

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19 June 4, 1958. REF: 1420 B

PULSATION WITH PERIODATE

Parents: W3060, W3064

ORC cultures, spun, susp in chilled water 20 x until used then diluted in: Penassay, 1/20,

DM (D(M) + asparagine 1‰)

DO (D(O) + " + glucose .5%)

α1 Pulse 2' in Penassay, mixing equal amounts of parents: 1 ml each in a flask, starting from chilled susp mixed while chilled. Dilute after 2' in DM periodate M/2000, 1/100, incubate and sample every 5' until 40'; dilute sample of 0.2 ml in 8 ml, blend and plate on D(Sm B1) 0.05 ml.; for 20' onwards also further 1/5 dil. plated

α10 From pulse dilute in D(M) 1/100, without periodate; sample only after 40' and plate 1/5 and 1/25 .05 → D(SmB1)

There were no colonies except in α10 (when there were 4 colonies).

F+ reversion of W3060?

Moreover while this part of the experiment showed contamination with staff, the second part of this same exp., which used the same cultures, showed no or less contamination, but was also sterile

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periodate pulse.

1420B

June 4 1958 REF:

Matings. Premix chilled matings (ORC W3060, W3064 in chilled penassay resuspended 1x. Pulse = time held at 37°. α

α Pulse 2 min in penassay 1 ml each in tube.
β Pulse 0 " " " Dilute 1:200 each parent ~~(.05 ml then mix~~ in 10 ml tubes.
γ " 2 " " D(o) .5 ml in tube
Δ " 2 " " D(m) " "
ε " (α) 0 kept chilled : for plate recombinants

add periodate to chilled D(o, m) tubes.

		Dilute in M/	Periodate	
sample of 5 m.	α	D(m)	2000	→ time series.
all others are plated at 40' (6:10 PM)	β	"	0	
	β	"	500	
	β	"	1000	
	β	"	2000	
Dilute 1:10 in chilled water.	β	D(o)	0	
	β	"	500	
	β	"	1000	
	β	"	2000	
	α	D(m)	0	
	β add penassay just prior to cells.	D(m) + P_{10}	0	
	β		500	
	β		1000	
	β		2000	
Sex protection	β thioglycollate 0.1%	+	500	
	γ } pulse	D(o)	0	
	Δ } pulse	D(m)	0	
	ε Mixed at 0° refrigerated to 40° & plated 1:1000			
	ε - mix post dilutions 1:100 plated			
	" 1:1000 plated.			

Does dilution into periodate give a pulse? Possibility that M/2000 periodate is toxic if not neutralized? Should try adding thioglycollate at various times, providing thioglycollate does neutralize. M/2000 periodate was not toxic in 15 minutes with a heavier cell suspension (1421B). Agar will neutralize ~~pro~~ periodate. $ question.

No colonies! Presumably reverted H_{fr}!

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19 June 9, 1958. REF: 1420 C.

Pulsation with periodate.

3060 (?); 3064. Sheet giving details of cultures missing.

Pulsed 2', diluted 1/100 in

P. DM + asparagin 1/1000 + glucose 1/1000 + periodate M/2500
C. " " " —
B. Penassay broth

Samples taken at 20', 30', 40', 50', 60' (or parts), diluted
1/5, and blended and plated on D Sm B1.

Plate counts. (Large colonies; also some minute colonies).

	P	C	B	
20'	3,9	7,5	12,8	911 stroked on B kc 6/11
30	1,4	10,3	8,14	
40	6,4	2,2		
50	8,5		6,9	
60	7,10	8,12	28,24	

1420 C - Lac strokes

P	20	4+, 9−
P	30	1+, 4−
P	40	1+, 9−
P	50	3+, 9−, 2 w
P	60	6+, 10−, 1 w
C	20	7+, 6−
C	30	6+, 7−
C	40	4−
C	60	9+, 11− (some w:)
B	20	6+, 14−, 1 w
B	30	10+, 15−, 1 w
B	50	7+, 9−
B	60	21+, 34−

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19 June 11, 1958. REF: 1420 D

INHIBITION & INTERRUPTION BY LIVING CELLS.

Cell suspensions 10 × (same as in exp. 1422)

A C. W 3870 0.1 ml
W 3064 0.1 ml
Broth 1.0 ml
} 10' incub → 0.1 + 0.5 ml W945 + 0.9 broth (A)
↘ 0.1 + 1.4 broth (C)

B. W 3870 0.1 ml
W 945 0.1 ml
Broth 1.0 ml
} 10' incub → 0.1 + 0.1 W3064 + 0.9 broth (B)

A, B, C : at 5', 15', 30' 0.2 ml + 1.8 chilled water.
0.05 D (from B)

Plate counts.

	A	B	C
5'	91,72	–	164,128
15'	162,182	77,85	456,410
30'	160,155	343,510	460,486

	Gal⁺/hot	Lac⁺/hot	T₁⁺/hot	
A 5	0/50	1/50		
A 15	0/50	1/50		Addition of W945 cells to the cross inhibits further mating, interrupts. Compare with control C.
A 30	0/50	2/50		
B 15	0/50	0/50		No accelerated Gal detected in the second mating.
B 30	0/50	5/50		
C 5	0/50	0/50		
C 15	0/50	12/50		
C 30	0/50	12/50		

Note. W945 is added in some excess over physiological conc. of cells. Interruption may be due to transfer to ~~oversaturated~~ ~~plugged~~ cell concentration. Retest with normal cell conc.

19 June 25, 1958 REF: 1420 E

8 INTERRUPTION BY LIVING ♀♀ FEMALES.

3 hrs. R.C. W 3064, W 3870, W 945.

Sperm, resusp. in fresh broth.

A ♂ : ♀ = 10:1 ratio.

2 ml 3870 + 0.2 ml 3064, incubated 20'

then:

AC: 0.9 ml transferred with 1 ml warmed pipette to empty, warm tube, incubated 20', diluted 1/20 & 1/100 (chilled) plated on ♀ Gal Sm B_1 (.05).

AD: 0.1 ml transferred to 0.9 ml W 945 warm suspension. and incubated 20', diluted 1/2 & 1/10 chilled. plated as above

B ♂ : ♀ = 1:1 ratio

1 ml 3870 + 1 ml 3064, incub 20'

then:

BC: 1 ml. transferred with 1 ml. warmed pipette to warm tube, incubated 20', diluted 1/100 & 1/500 chilled, plated on ♀ Gal Sm B_1 (.05).

BD: 0.1 ml transferred to 0.9 ml warmed W 945 susp. incubated 20', diluted 1/10 & 1/50 chilled, plated on ♀ Gal Sm B_1.

Purpose: to test if addition of living ♀♀ interrupts even when the whole experiment is kept at the same total conc. of cells throughout, and this concentration is within theoretical limits of

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19 June 28 1958 REF: 1420 E

1	2	3	4	5	6	7	8	9	10
Dilution 1 : too many colonies.									
Dilution 2 only counted.									
AC2		Gal + 9 ; 7		Gal-	about 1000	per plate,	two plates.		
AD2		53 ; 60			2000				
BC2		3 ; 2		"	400				
BD2		21 ; 11		"	1000				
	The effect of interruption is reversed. What happened ?								

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Periodate

1421A

June 1 1958 REF:

W3060 × W3064

Same cells as 1420. Harvest 5 ml into .5 ml water.

.1 ml cells + .4 ml periodate : M/200; M/2000; M/2,000 and H_2O.

after 10 minutes treatment at 37° add 4.5 ml penassay + 1% glycerol.

Mate equal volumes 15' at 37°, dilute 1:100 and plate on D.B. sm.

Trine by chilling

Cross	treatment ♂	♀	8P2 · Prelim. A3	Count
	0	0 *	+	149
	2000	2000	−	2
	200	200	−	0
	20	20 *	−	0
	2000	0	−	2
	200	0	−	0
	20	0	−	0
	0	2000	+	45
	0	200	÷	1
	0	20	−	0

(1-6 diluted in D(m) rather than H_2O.)

at M/2000, inactivation may be differential.

Preliminary results are very promising (for periodate as a de-sexing agent). Could the aeration phenocopy have been oxidation of surface receptors? If so should use rH poise.

See 1420 for origin of this experiment. Periodate is of course known to affect the receptors of animal cells for influenza virus. (Will flu agglutinate any bacteria?).

Try other oxidants? Ferricyanide?

Conclusion (within limit of test): sexual competence is destroyed by periodate, ♂♂ more sensitive than ♀♀. [Need kinetic and viability controls.] 1421B shows M/200 periodate is toxic; M/2000 is not.

If they are inactive, will periodated bacteria deactivate ♂♂?

June 3 19 58 REF: 1420;1421

Harvest CSH W-3060, W-3064 3x7 ml each into 1 ml water. (Keep chilled during day before beginning experiment about 7PM. 1421 suggested differential effect of periodate. This is essentially a repetition with some added controls for viability and reproducibility.

In each case, add 0.1 ml cell suspension to 0.5 ml treatment solution (in B(m)). Treat 10 mins. at 37; neutralise 5 mins. by addition of 4.5 ml penassay+1% glycerol. Mate for 15' by mixing .5 ml each suspension. Then chill, dilute 1/100 and plate 0.1 ml on B-B$_1$sm agar: duplicate plates. Also dilute 10^{-6} and plate 0.1 ml on EMB Lac for viability.

	Treatment: (periodate conc.)		Viability		SRP counts	
	♂	♀	Lac+	-		
1	-	-	28,29	60,38	27	27
2	M/10000	-	49	78	20	15
3	5000	-	32	85	7	8
4	2000	-	30	71	0	0
5	200	-	2	104	0	0
6	-	10000	107	84	25	60sm.
7	-	5000	30	62	47	23
8	-	2000	36	52	38	37
9	-	200	53	1	0	0
10	2000P	-	43	68	102	55
11	-	2000P	22	71	50 sm	23

(A few SRP counts were slightly distorted by smearing)

These counts reflect a substantial pipetting error, but not enough to weaken the experiment.

Tubes #0-11 (P) had periodate neutralised by penassay-glycerol/prior to addition of the cells for 5'

CONCLUSIONS: M/200 periodate is toxic; M/2000 and less is not.

M/2000 periodate differentially inactivates fertility of ♂♂, with no perceptible effect on fertility of ♀♀ or viability. M/5000 has a partial inhibitory effect; M/10.000 is doubtful. Must explore possibility that mild treatment of ♀♀ enhances fertility; try especially neutralised M/2000 or M/10000. This might be explained if there is a phase of periodate treatment with slows the separation of the pairs.

Also need a more careful study now of effects on interruption and on speed of effects.

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Periodate

1421-B

June 3 19 58 REF: 1420;1421

Harvest ORC W-3060, W-3064 3x7 ml each into 1 ml water. (Keep chilled during day before beginning experiment about 7PM. 1421 suggested differential effect of periodate. This is essentially a repetition with some added controls for viability and reproducibility.

In each case, add 0.1 ml cell suspension to 0.5 ml treatment solution (in D(m)). Treat 10 mins. at 37; neutralize 5 mins. by addition of 4.5 ml penassay+1% glycerol. Mate for 15' by mixing .5 ml each suspension. Then chill, dilute 1/100 and plate 0.1 ml on D-B_1sm agar: duplicate plates. Also dilute 10^{-6} and plate 0.1 ml on EMB Lac for viability.

Treatment: (periodate conc.)

	♂	♀	Viability Lac+	Viability Lac−	SRP counts	
1	−	−	28,29	60,38	27	27
2	M/10000	−	49	78	20	15
3	5000	−	32	85	7	8
4	2000	−	30	71	0	0
5	200	−	2	104	0	0
6	−	10000	107	84	25	60sm.
7	−	5000	30	62	47	23
8	−	2000	36	52	38	37
9	−	200	53	1	0	0
10	2000P	−	43	68	102	55
11	−	2000P	22	71	50sm	23

Hfr tests on Lac+
0/10 from 1
0/2 from 2.
Valid tests? cultures attenuated of Hfr.

(A few SRP counts were slightly distorted by smearing)

These counts reflect a substantial pipetting error, but not enough to weaken the experiment.

Tubes 10-11 (P) had periodate neutralized by penassay-glycerol for 5' prior to addition of the cells

CONCLUSIONS: M/200 periodate is toxic; M/2000 and less is not.

M/2000 periodate differentially inactivates fertility of ♂♂, with no perceptible effect on fertility of ♀♀ or viability. M/5000 has a partial inhibitory effect; M/10.000 is doubtful. Must explore possibility that mild treatment of ♀♀ enhances fertility; try especially neutralized M/2000 or M/10000. This might be explained if there is a phase of periodate treatment with slows the separation of the pairs.

Also need a more careful study now of effects on interruption and on speed of effects.

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Periodate.
Effects on F^+.

1421C

5 June 1958. REF: 1421

ORC W6, W3064 penassay $\xrightarrow{20x}$ water. Chill Ag.

α: .2 ml + .8 ml D(m) + asparagine + M/2000 periodate } W6
β: .2 " " D(m + " }

Incubate 10 minutes. Add .2 ml W3064 and:
Plate .05 ml samples on DB, sm.

1 α Crowded
2 β Crowded

dilute 1+4 in penassay glycerol, rotate 15 minutes, Chill:
dilute and plate for F transfer. Add sm 200u to aliquot of 4→8.

3 α ~0.1 uncountable .04 85−, 58+
4 β. 200−, 130+ 84−, 64+
5 β sm. 290−, 2+ 120−, 0+

Spin and resuspend in 1v. Plate .05 ml samples on DB, sm

5 α 18, 23 large
6 β 107 large, ✓
9 β sm

(Bonly) add periodate to M/2000, Incubate 10 minutes and
7 β plate .05 ml 26, 30 large,

20 x cells. D(m) → β .2 + .8; D(m) periodate → α } 10 min Inc., add 3064 .2 ml → 1 + 4 penassay glyc. → 15 min. Inc. Rot. Chill → Spin Res. D(m) in 1 vol. → add 1v. periodate M/1000 (β) → Inc. 10 m.

.05 ml 1 α 2 β

β aliquot with sm 200u/ml.

10^{-7} ml EMBLAC 3 α 4 β 8 β sm

.05 ml D sm B₁ 5 α 6 β.

.05 ml D sm B₁ 7 β.

Pretreat F^+ with various concentrations of periodate.
Inoculate 20x W6 1:1000 in D(m)., 1:100 in treatment tubes (10 m.)

plate .05 ml, directly and 1:100 (duplicate).
Viability

Conclusions: 1 & 2 shows that periodated F^+ still gives overwhelming yield of plate recombinants (key regeneration of receptors?)

12 —
13 M/2000
14 M/1000
15 M/500
16 M/200
17 M/100.
18 — onto
plates prespread with.
0.1 ml M/200

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19 June 5, 1958. REF: 1421 C

1	2	3	4	5	6	7	8	SECOND PART

PERIODATE OVER F+ INHERITANCE

W6, conc 20x, same as 1421 C 1st part, diluted 1/1000 —

0.1 ml to 10 ml DM + asparagine 1‰ + 0 periodate 11
M/2500 " 12
/1250 13
/625 14
/310 15
/150 16

After 10' treatment at 37°, undil → B(0)
↓
1/100 → B(0) in duplicate.

Alg 17. spread over B(0) and B(Lac) .1/50 periodate, 0.05, then 1/100 dilution of exp. 11. (untreated cells) —

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1421 C

June 6 19 58

REF:

1	2	3	4	5	6	7	8	9	10
Counts	—	on	B-O	:	1/100			Periodate conc	
11	:	~1000		234		192		0	
12		~1000		121		132		M/2500	
13		683		32		46		M/1250	
14		128		3		3		M/[illegible]	
15		12		0		0		M/3[illegible]	
16		1		0		0		M/155	
17		~1000		183		207		periodate agar	

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19 June 5, 1958. REF: 1421 C

PERIODATE ON F+ TRANSFER

ORC cultures of W6, ~~3064~~ 3064 – Spun, resusp in water, chilled (20x)

α. .2 ml of W6 + .8 ml of DM + asparagine + M/2000 periodate

β. .2 ml + .8 ml "

Incubate 10 minutes, chill. Add .2 ml W 3064 and

plate .05 ml samples on D(Bi Sm).

1 = α (Plate recombination).
2 = β –

Dilute 1+4 in prewarmed penassay glycerol, rotate 15 minutes, chill: * ‡

dilute and plate for F transfer (Dilution ½ 10⁵, [illegible] .04 [illegible])

3 = α
4 = β.

Spin and resuspend in 1x volume DM. Plate .05 ml samples on D Bi Sm

5 = α
6 = β (F+ fertility) –

β only: add periodate to M/2000, incubate 10' and

7 = β plate .05 ml on D(Bi Sm)

Flowsheet

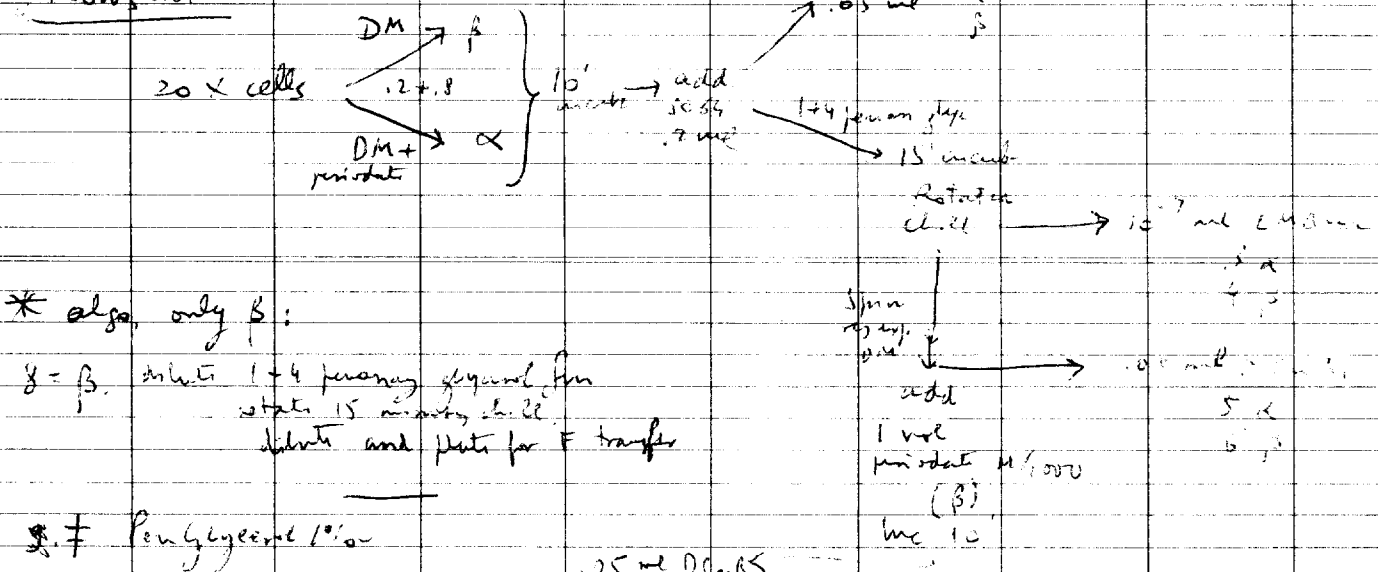
20 x cells → DM .2+.8 → β; DM + periodate → α } 10' incubate → add 3064 .2 ml → .05 ml α β

→ 1+4 penass glyc → 15' incub

Rotate chill → 10⁻⁷ ml [illegible] 3 α 4 β

Spin resusp. DM → .05 ml [illegible] 5 α 6 β

add 1 vol periodate M/1000 (β) Inc 10



* also, only β:

8 = β. dilute 1+4 penassay glycerol fm
rotate 15 minutes chill
dilute and plate for F transfer

‡ Pen Glycerol 1%

.05 ml D Sm BS

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Periodate and other oxidants
effect on H/s.
Regeneration of receptors

1421 D

5 June 1958 RE 1421

As 1421A. — Pretreatments of W 3060.

Incubated at 37 C unless other stated

20x cells diluted .1 + .4 ml into treatments, 10 m. Neutralize by penassay - glyc 5 m.

Mate + Equal volume W 3064 15 min., dilute 1:~~1000~~ 200 and plate .05 ml.
(3B 1:1000)

				Counts	
Periodate	M/2000	37	10 m.	~~7~~	0
"	"	at 0°C.	10 m.	~~5~~	4
"	D(m)	~~37°~~	~~20 m.~~	~~43~~	43
"	M/2000	37°	5 m.	~~36~~	2
Ferricyanide	M/10000	37	5 m.		36
	M/1000		10		24
	M/10000	"			22
Dichromate	M/10000				15
	1000	"			20
	10000				2
Bubble oxygen		37	10 m.		2
		37	30 m.		4
		0°	30 m.		9

; [19, 37, ~45] should be ~~[illegible]~~ 1/5

.1 ml 20x cells + 2 ml D(m) then 2.5 ml penassay glycerol to compare with others.

Regeneration: Use samples of ① after neutralization.
This is now at .1/5 = 1/50 x 20x = .4x (i.e. .4 of saturating concentration).
Dilute further 1:10 in penassay: F(t) take 0.25 ml + .75 ml of 1x ♀♀, incubate 12 m.
(test mating) dilute dilute 1:10 + plate.
0 minutes

postpone.

Control counts not very satisfactory and no control on plate recombination.
Conclude: periodate effect as before; incomplete at 0° and < 10 minutes.
Ferricyanide has no effect on fertility at M/100. (Look for viability!) (May generate a useful indicator). Dichromate effective only at M/~~100~~ 100! (presumably viability).
Bubbling oxygen for 10 minutes induces F⁻ phenocopy! Should also be repeated.

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19 June 13, 1958 REF: 1421 E

DIFFERENTIAL EFFECT OF
VARIOUS AGENTS ON MATING
ABILITIES.

ORC W 3870, W 3064, spun, conc. 20 x in chilled water.

	A	B
1. DM	1 3	1
2. Nutrient Broth + $CaCl_2$ 1‰	4 ~~4~~ 1	0 3
3. RDE + $CaCl_2$ 1‰	3 1	4 0
4. Lysozyme .5%	2 0	2 0
5. Dichromate M/1000	2 2	0 3
6. Ferricyanide M/100.	1 0	2 4
	M: 0 0	

0.4 ml of each reagent added to
A. : .1 ml of ♂ susp. 20x conc.
B. : .1 ml of ♀ " " .

Treat for 10' at 37°. Chill, add 4.5 chilled broth to every tube.

From tube A1 (control ♂), .5 in new tubes A1, B1–B6; from tube B1 (control ♀) .5 ml in A1–A6, B1.

20' incubation then dilution 1/100, plating .05 D from B1

7. Plate recombination control. Parental susp. diluted 1/100 → 1/50 dilutions mixed, .05 D from B1 –

Conclusion: not enough recombinants! Culture n.g.?

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19 June 11, 1958 REF: 1422

RDE - inhib. of ♂♂ & mating.

ORC, spun, resusp in Pen 10 x, chilled. ♂ = 3870, ♀ = W3064.

1. RDE 1 ml + 0.1 ml ♂, 10' incub. → 0.1 ml + 1 ml broth + + 0.1 ♀: 20' incub. → 1/100 dilution → .05 ml D(SmB1).

2. RDE 1 ml + 0.1 ml ♀, 10' incub → 0.1 ml + 1 ml broth + + 0.1 ♂, 20' incub. → 1/100 dil. → .05 ml D(SmB1)

3. Same as 1. but broth used instead of RDE

4. Same as 2. " " " " " -

Plate counts:

1.	201, 181	Sum	382
2.	185 164		349
3.	277 215		492
4.	171 216		387

5: 2 ml broth + 0.1 ♂ + 0.1 ♀, 25' incub → 5A, 5B

→ 5A → 0.1 in 1 ml RDE for 15' → 1/10 → .05 D SmB1

→ 5B → 0.1 in 1 ml broth " " "

About 400 colonies in every plate of the 5A, 5B series.

Conclusions:

No effect of RDE detected on mating ability of cells.

Note: no Ca^{++} added.

U.W.
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19 June 12, 1958 REF: 1423A

DELAYING ENTRY -

Chloramphenicol.

Testing for the delay of spontaneous interruption.

ORC W 3870, W 3064, spun and resusp. in water 20× concentrated. Chloramphenicol solution 1‰.

A. Penassay 1 ml + ♂ 0.1 ml + ♀ 0.1 ml + 0.05 Chloramph. (= 40 γ/ml)

B. " " " + —— (control)

C. " ♂ dil 1/50 0.1 ml + ♀ dil 1/50 0.1 ml + 0.05 chloramph.

D. " " " —— (control)

A, B: at 30', 60', 90' and 135' dilution 1/500 (0.02 in 10 ml), plating of .05 on D5m B1.

C, D: at same times dilution 1/10 (.1 in .9 water) and plating.

E.

Control of plate recombination at various concen. of cells.

E. From same mother suspension, .025 ♂ + .025 ♀ at 10 ml chilled water; → .05 in D5m B1 = E1

↘ 1 ml diluted serially → 2.15 chilled water = E2-E7
↓

Plate county:

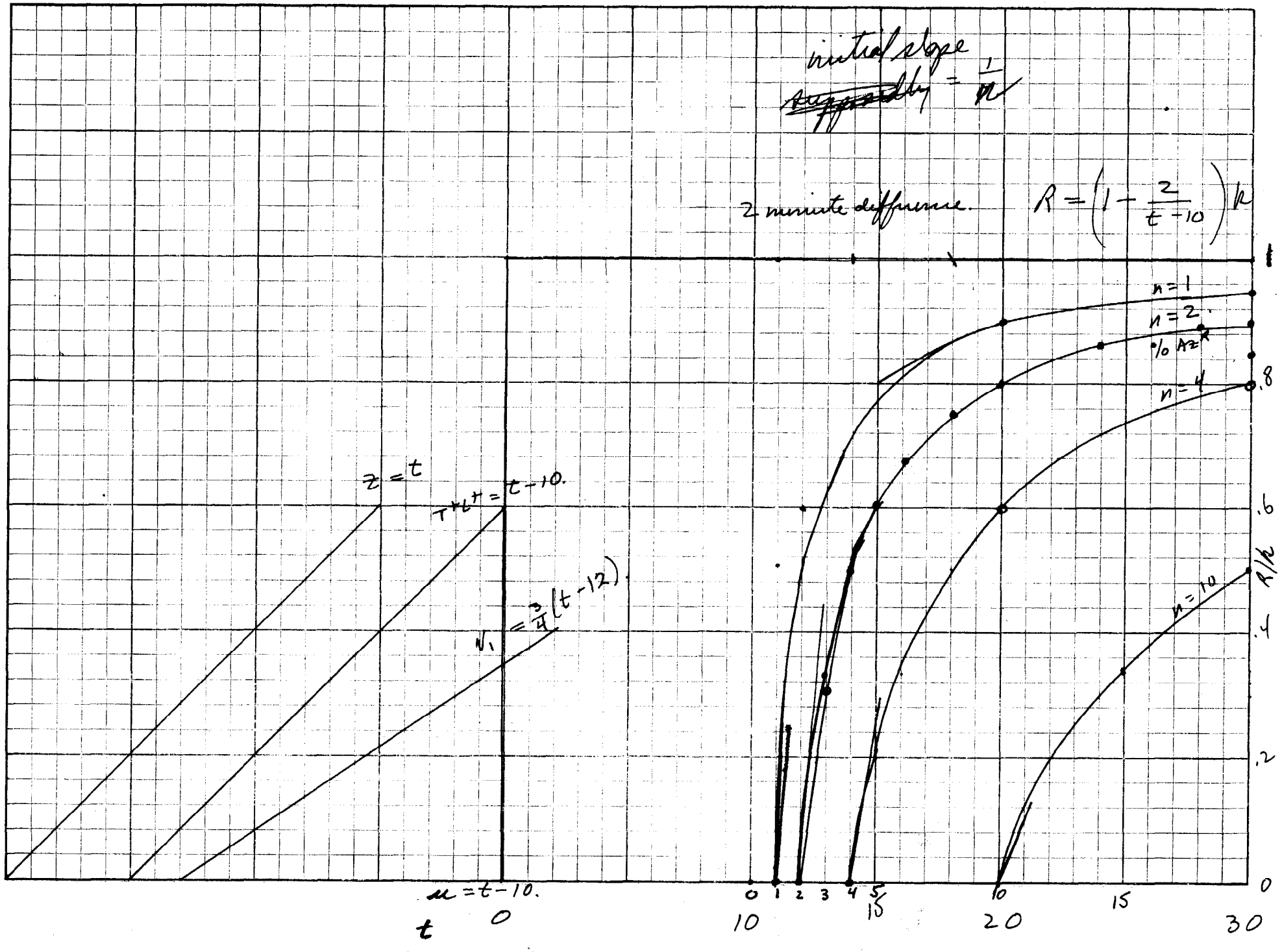
E 1	2, 9
E 2	1, 0
E 3	1, 0
E 4	0, 0
E 5	0, 0
E 6	0, 0
E 7	0, 0

	Gal	lac
A 30	0/7	2/7
A 60	0/5	1/5
A 90	0/9	0/9
A 135	0/6	0/6
B 30	0/13	0/13
B 60	0/10	1/10
B 90	1/10	2/10
B 135	1/25	2/25
C 30	0/23	7/23
C 60	1/20	11/20
C 90	1/10	5/10
C 135	0/7	4/7
D 30	2/50	16/51
D 60	4/50	19/50
D 90	2/50	22/50
D 135	9/50	22/50

Plate counts.

	30	50	90	135
A	7	5	9	6
B	13	10	10	25
C	23	20	10	7
D	65	218	320	520.

Conclusions.: A, B in unphysiological conditions, low viability (excess of cells or poisoned culture?). In C, chloramphenicol may have slight bactericidal effect; at any rate does not permit multiplication, as judged from prototroph count. Chromosome entry possibly undisturbed.

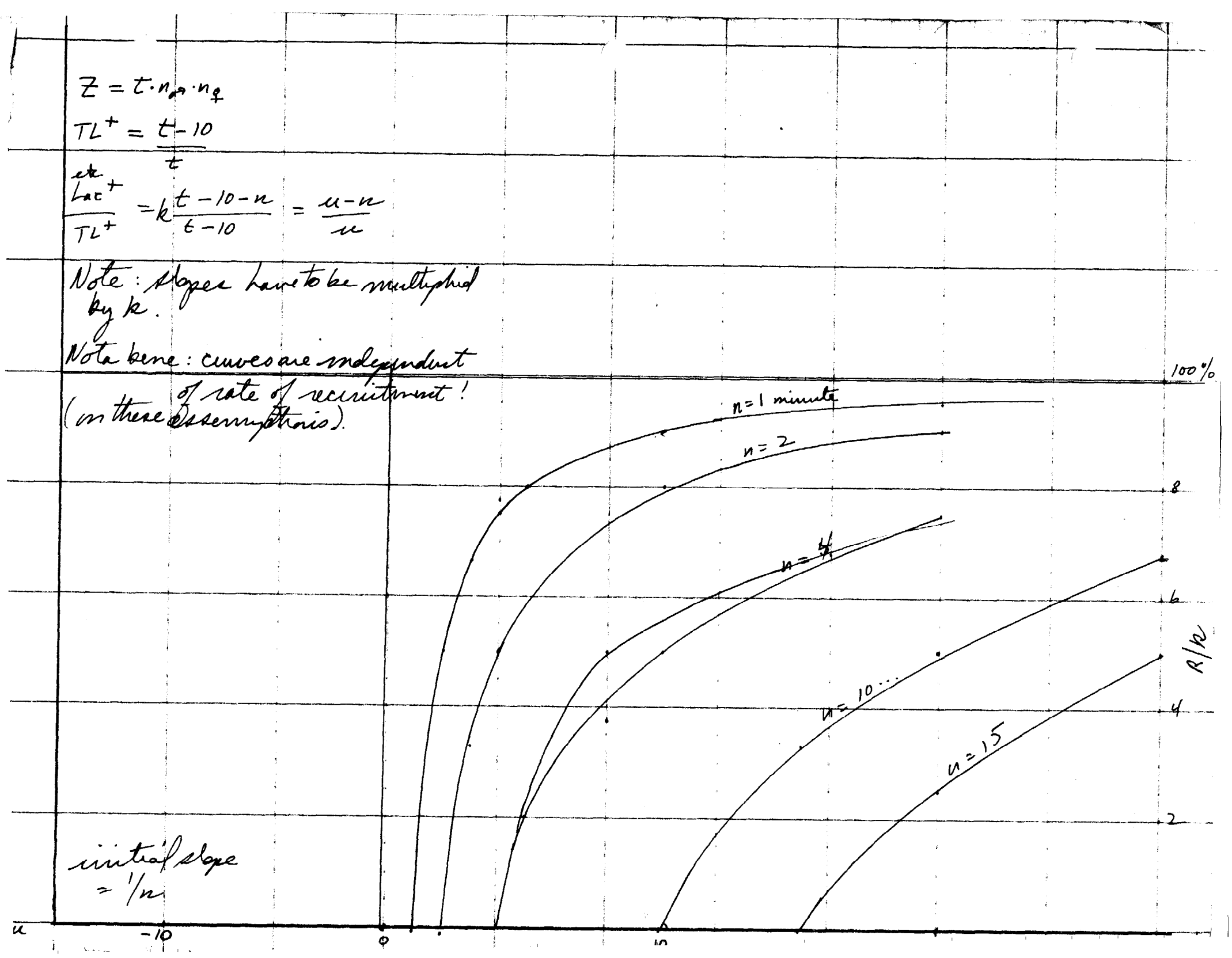


initial slope
~~supposedly~~ = 1/n
2 minute difference.
R = (1 − 2/(t − 10)) k
n = 1
n = 2
% Az^R
n = 4
n = 10
z = t
T+L+ = t − 10.
v1 = 3/4 (t − 12).
u = t − 10.
t
0
10
15
20
30
0 1 2 3 4 5
10
15
R/k
0
.2
.4
.6
.8
1

$Z = t \cdot n_a \cdot n_q$

$TL^+ = \frac{t-10}{t}$

$\frac{\text{etc. } Lac^+}{TL^+} = k\frac{t-10-n}{t-10} = \frac{u-n}{u}$

Note: slopes have to be multiplied by k.

Nota bene: curves are independent of rate of recruitment! (on these assumptions).