U.W.
MICROBIAL
GENETICS

19 June 1st, 1958 REF: 1420

PULSATION

Orc. 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 .9 ml treatments

				Nopat	Sgt
1	D(0) pH	4.5		#	#
2		5.5		#	+
3		5.9		#	+
4		6.4		+	+
5		6.9	± .5% glucose	#	+
6		7.3		+	±
7		7.9	} some caramel		
8		8.6	formed	+	+
9		9.0		+	+

11	D(0) 10 ml		
12	D(0) thioglycolate .1% (10 ml)	+	#
13	D(0) periodate (in pH 6.9) M/100	0	0
14	" M/1000	+	#
15	" M/10000	#	+
16	D(0) sine Mg	+	+
17	W 945 expon. resusp 4x, killed by boiling few seconds	#	#
18	" " living, in Penassay	+	–
19	Penassay	+	#
20	5' control: dil 1/1000 → .05 S Gal Sm B,	0	0

4³⁰ PM. (1-19): incubation 40' – Dil 1/100, plate .05 on S Gal Sm B,

* cultures are viscous

U.W.
MICROBIAL
GENETICS

19 June 5, 1958 REF: 1420 A

	1	2	3	4	5	6	7	8	9	10
1										
2		Plate		Gal +	Lac +	T_1^r				
3		counts								
4										
5	1	310		1/50	5/50	39/50				
6	2	340		3/45	13/45	35/45				
7	3	298		5/50	8/48	30/50				
8	4	380		4/50	17/49	36/50				
9	5	310		4/50	14/48	30/50				
0	6	298		3/50	4/50	35/50				
	7	320		3/50	17/48	36/48				
1	8	280		2/50	11/45	25/45				
2	9	355		2/50	10/48	31/50				
3										
4	11	474		28/100	45/100	70/100				
5	12	365		14/100	39/100	64/100				
6	13	0								
7	14	127		0/80	3/80	35/80				
8	15	390		3/100	19/100	67/199				
9	16	370		6/50	23/50	35/50				
0	17	400		13/50	23/50	37/50				
	18	148		0/50	1/50	18/50	← interrupted			
1	19	430		6/50	15/48	33/50				
2	20	0			—					
3										
4										
5										
6										
7		Comments:								
8										
9		pH has practically no effect on transfer; some also on								
0		making mixes; no recombinants take place in these								
1		controls. Range 4.5 – 9.								
2										
3		Thioglycolate has practically no effect at 1/100								
4										
5		Iodoacetate M/1000 shows partial interruption								
6										
7		Addition of living F- cells interrupts, that of killed								
8										
9		F- cells, does not								
0										
1										
2										
3										
4										
5										
6										
7										
8										
9										
0										

U.W. MICROBIAL GENETICS

PULSATION

design 1420A

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

19 June 4, 1958. REF: 1420 B

PULSATION WITH PERIODATE

Parents: W3060, W3064

ORC cultures, 5pm, 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 1.8 ml, blend and plate on D(Sm B_1) 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 1/20 — .05 → D(Sm B_1)

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

F+ reversion of W3060 ?

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

U.W.
MICROBIAL
GENETICS

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 Pulse 2 min in penassay 1 ml each with [illegible]. held at 37°. α
β Pulse 0 " " " Dilute 1:200 each point ~~.05 ml [illegible]~~ 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 ~~[illegible]~~ D(o, m) tubes.

sample of 5 m[illegible]

all others are plated at 40' (6:10 PM)

Dilute 1:10 in chilled water.

		Dilute in	M/ Periodate	
1	α	D(m)	2000	→ time series.
2	β	"	0	
3	β	"	500	
4	β	"	1000	
5	β	"	2000	
6	β	D(o)	0	
7	β	"	500	
8	β	"	1000	
9	β	"	2000	
10	α	D(m)	0	
11	β add penassay just	D(m) + P_{10}	0	
12	β prior to cells.		500	
13	β		1000	
14	β		2000	
15 (for protection)	β thioglycollate 0.1%	+	500	
16	γ } pulse	D(o)	0	
17	Δ } pulse	D(m)	0	
18	ε Mixed at 0' refrigerated to 40' replated 1:100			
19	ε - mix post dilutions 1:100 plated			
20	" 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 (1419B). Agar will neutralize ~~[illegible]~~ periodate. ? question.

No colonies! Presumably reverted Hfr_2!

U.W.
MICROBIAL
GENETICS

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 stokes

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

U.W.
MICROBIAL
GENETICS

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

U.W.
MICROBIAL
GENETICS

19 REF: 1420 D

	Gal⁺/tot	Lac⁺/tot	T_1^+/tot
A 5	0/50	1/50	
A 15	0/50	1/50	
A 30	0/50	2/50	
B 15	0/50	0/50	
B 30	0/50	5/50	
C 5	0/50	0/50	
C 15	0/50	12/50	
C 30	0/50	12/50	

Addition of W945 cells to the cross inhibits further mating, interrupts. Compare with control C.

No accelerated Gal detected in the second mating.

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

1 2 3 4 5 6 7 8 9 10

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 & 1/10 (chilled) plated on ♀ Gal from 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 1/200 & 1/500 chilled, plated on ♀ Gal from 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 from 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

U.W.
MICROBIAL
GENETICS

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 ?								

U.W.
MICROBIAL
GENETICS

Periodate

1421A

June 1 19 58 REF:

1	2	3	4	5	6	7	8	9	10

W3062♀ × W3064♂

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

.1 ml cells + .4 ml periodate : M/200; M/2000; M/20,000 and H_2O.

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

Mate equal volumes 15' at 37°, dilute 1:100 and plate on D-13, 0.5%.

Tests by shelling

Cross	♂ treatment	♀ treatment	8P2 · Pulmi. 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, via 0.1 M, rather than H_2O.)

at M/2000, inactivation maybe differential.

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

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 ♀♀. [Viral kinetic and viability controls.] 1421B shows M/200 periodate is toxic; M/2000 is not.

If Hmyne inactive, will periodate bacteria deactivate ♂♂?

U.W.
MICROBIAL
GENETICS

Periodate

1421-B

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(a)). 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 mk o.1 ml on EMB Lac for viability.

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

U.W.
MICROBIAL
GENETICS

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.

U.W.
MICROBIAL
GENETICS

Periodate.
Effect on F⁺.

1421C

5 June 1958. REF: 1421

ORC W6, W3064 pneumassay $\xrightarrow{20\times}$ water. Chill ...

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 pneumassay glycerol, rotate 15 minutes, chill:
dilute and plate for F transfer. add sm 200 u to aliquot of 4 → 8.

	×0.1	.04
3 α	uncountable	85−, 58+
4 β.	200−, 130+	84−, 64+
8 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, ✓
β sm
(β only) add periodate to M/2000, incubate 10 minutes and
7 β plate .05 ml 26, 30 large,

20× cells. .2 + .8 — D(m) → β; D(m) periodate → α } 10 min inc., add 3064 .2 ml → .05 ml 1 α, 2 β; 1+4 pneumassay glyc. → 15 min. inc. Rot. Chill → Spin Res. D(m) in 1 vol. → 10^{-7} ml EMB lac 3 α, 4 β, 8 β sm; .05 ml D sm B, 5 α, 6 β; add periodate M/1000 (β) 1v. → inc. 10 m. → .05 ml D sm B, 7 β.

8 aliquot with sm 200 u/ml.

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

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

Viability

11 —
12 M/2000
13 M/1000
14 M/500
15 M/200
16 M/100.
17 — onto
plates prepared with
0.1 ml M/200

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

U.W.
MICROBIAL
GENETICS

19 June 5, 1958. REF: 1421 C

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.

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

U.W.
MICROBIAL
GENETICS

1421 C

June 6 19 58

REF:

	1	2	3	4	5	6	7	8	9	10
1										
2	Counts	–	on	B-0	:	1/100				
3									Penicillase conc.	
4										
5	11	:	~1000		234		192		0	
6										
7										
8	12		~1000		121		132		M/2500	
9										
0										
	13		683		32		46		M/1250	
1										
2										
3	14		128		3		3		M/625	
4										
5										
6	15		12		0		0		M/312	
7										
8										
9	16		1		0		0		M/155	
0										
1	17		~1000		183		207		penicillase after	
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										

U.W.
MICROBIAL
GENETICS

19 June 5, 1958. REF: 1421 C

PERIODATE ON F+ TRANSFER -

ORC culture of W6, ~~3000~~ 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 " —

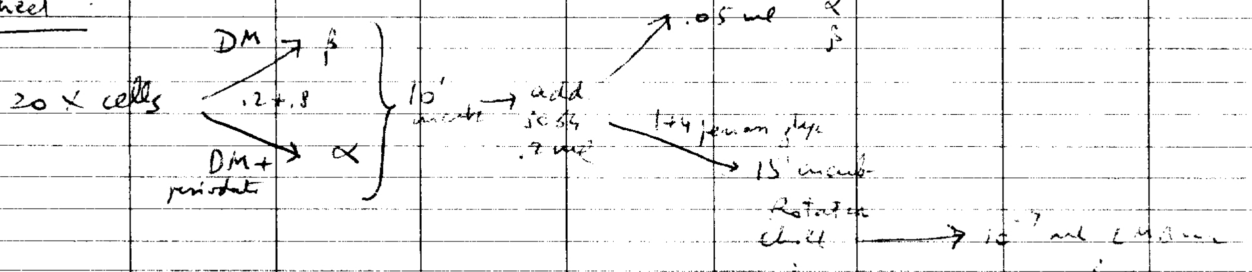
Incubate 10 minutes, chill. Add .2 ml W 3064 and ~~[illegible]~~
plate .05 ml samples on D(B₁Sm).
1 = α (Plate recombination).
2 = β -

Dilute 1+4 in prewarmed ~~penassay~~ glycerol (+ β *‡) rotate 15 minutes, chill; dilute and plate for F transfer (Dilution ½ 10⁶, 1 ½ 10⁴ 8 lac)
3 = α
4 = β.

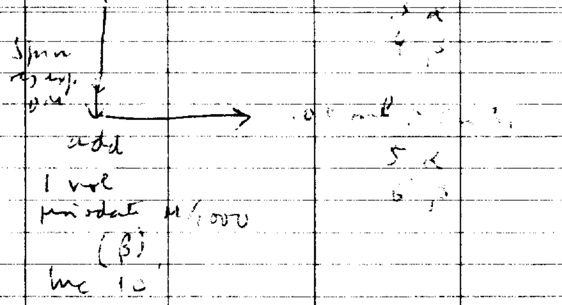
Spin and resuspend in ½ volume DM. Plate .05 ml samples on D B₁Sm
5 = α
6 = β (F+ fertility) -

β only: add periodate to M/2000, incubate 10' and
7 = β plate .05 ml on D(B₁Sm)

Flowsheet



* also, only β:
8 = β. dilute 1+4 penassay glycerol then rotate 15 minutes, chill, dilute and plate for F transfer



‡ Penglycerol 1%

.25 ml D gal B₁
7 3

U.W.
MICROBIAL
GENETICS

Periodate and other oxidant effect on Hfr.
Regeneration of receptors

1421 D

5 June 1958 — RE 1421

As 1421A. — Pretreatments of W 3060.

Incubated at 37 C unless otherwise stated

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

Mate + equal volume W3064 15 min, dilute 1:200 and plate .05 ml. (3B 1:1000)

				Counts	
Periodate	M/2000	37°	10 m.	~~7/4~~	0
"	"	at 0°C.	10 m.	~~8~~	4
"	D(m)	~~37°~~	~~20 m.~~	43	43
"	M/2000	37°	5 m.	~~2/6~~	2
Ferricyanide	M/10000	37	5 m.		36
	M/1000		10		24
	M/100	"			22
Dichromate	M/10000				15
	1000				20
	100	"			2

: [19, 37, ~45] should be x 1/5

Bubble oxygen	37	10 m.	} fairly severe foaming by hypodermic.	2
	37	30 m.		4
	0°	30 m.		9

.1 ml 20x cells + 2 ml D(m)

These 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 0.2 ml + .2 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 recontribution.
Conclude: periodate effect is sure; incomplete at 0° and < 10 minutes.
Ferricyanide has no effect on fertility at M/100. (look for viability!) (Manganate a useful indicator). Dichromate effective only at M/100! (presumably viability).
Bubbling oxygen for 10 minutes induces F⁻ phenocopy! Should also be repeated.

U.W.
MICROBIAL
GENETICS

19 June 13 1958 REF: 1421 E

DIFFERENTIAL EFFECT OF VARIOUS AGENTS ON MATING ABILITIES.

ORC W 3870, W 3064, 3pm, 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	

0.4 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 A_1 (control ♂), .5 in new tubes A_1, B_1-B_6; from tube B_1 (control ♀) .5 ml in A_1-A_6, B_1.

20' incubation then dilution 1/100, plating .05 Dfm B_1

7. Plate recombination control. Parental susp. diluted 1/100 → 1/50 dilutions mixed, .05 Dfm B_1 -

Culture not enough recombinants! Culture no.?

19 June 11, 1958 REF: 1422

RDE - inhib. of ♂♂ & mating.

ORC, Spun, resusp in 1/10 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 (Sm B_1).

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 (Sm B_1)

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 Sm B_1

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.
MICROBIAL
GENETICS

19 June 12, 1958 REF: 1423A

DELAYING ENTRY - Chloramphenicol.

Testing possible 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 B_1.

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

E.

Control of plate recombination at various concens. of cells.

E. From same mother suspension, .025 ♂ + .025 ♀ at 10 ml chilled water; → .05 in D5m B_1 = E_1

↘ 1 ml diluted serially → 2.15 chilled water = E_2-E_7

↓

Plate counts:

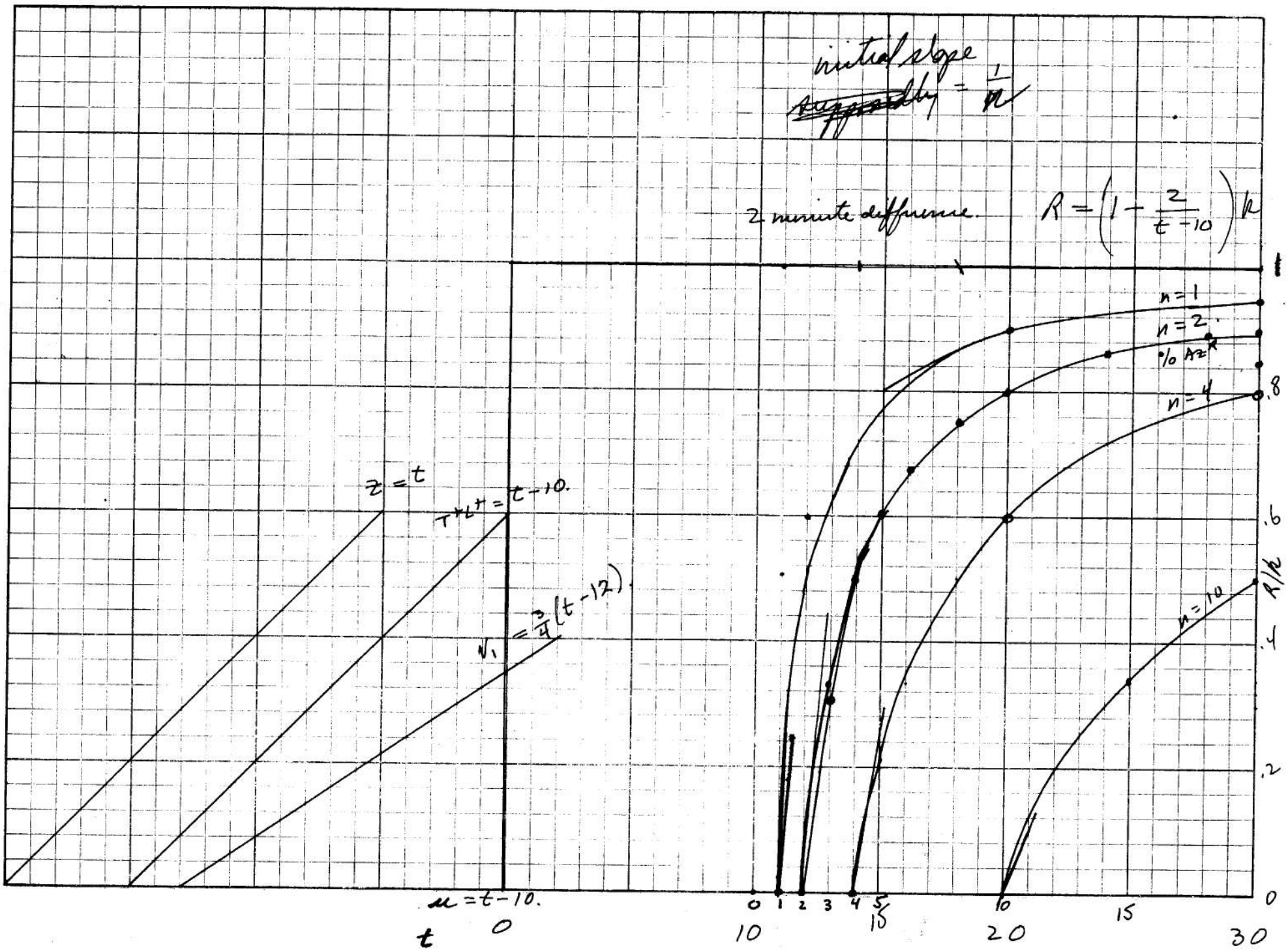
E 1	2, 9
E 2	1, 0
E 3	1, 0
E 4	0, 0
E 5	0, 0
E 6	0, 0
E 7	0, 0

	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, slow mating (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 = 1/n
2 minute difference.
$R = \left(1 - \frac{2}{t-10}\right) k$
n = 1
n = 2
% Az R
n = 4
n = 10
z = t
T + L' = t - 10.
$= \frac{3}{4}(t-12)$
N_1
u = t - 10.
t
0
10
20
30
15
0
1
2
3
4
5
10
15
.8
.6
.4
.2
0
R/k

$Z = t \cdot n_a \cdot n_f$

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

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

Note: slopes have to be multiplied by k.

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