37-X 666 / serum and clones.

1245

DATE: APR 12 1955 REF:

A. Misc. tests on serum, diluents.
Water in Sutter tap, this lab distilled, or enzyme institute double distilled appears to be suitable diluent (contra "spent broth") to limit growth. Broth 2.1C-20 cells retained motility, did not flatten unduly and growth was limited. (Unavoidable contamination with broth of isolation.)

B. 9, 21 ⊕ resp. were completely inhibited by 1: anti-b, 2: anti-i serum. Overnight in B1 (b) large clone of small cells, no motility except a single wriggling cell (planted out) A12 A.G. In B2, noted clone at center as above but at periphery, net and cords of long cells and filaments (some in serum?) This was also noted later in i-serum.

C 12/2B pupm. Free degas 12^{20} Spot ① to 2^{20}
DCG picked (13) $2^{50}-3^{30}$ Inc. to 5^{35} and plate in MGA 60%. Inc. 5^{35} PM - 8^{30} AM (15 hours).

35 (34 clones + 1 pure swarms)
9 blanks. (probably picked late) - held to 5/31/55 for photo and counting.

Serum inhibition of trails

1246.

DATE: APR 13 1955 REF:

1	2	3	4	5	6	7	8	9	10

A. Serum effect microscopic. Both trails isolated from soil (.25 ml)

B. FA 11 (A) × SW666; FA 32 (B) × SW666 and put in 1:100 i, b.

sevums in broth. b seums inactivated both very quickly ≠ i after 30–90 seconds (usually).

Then tried plating. (by c. 345). Harvest c. 350/ml A; 500/ml B

(Next middle broken + numbers in B are doubtful.

A– .2 ml samples (est. count is 50 cells/plate). in MGA 60 + (1) 0 Plates . somewhat messy but profuse T + S.

(6) b 1:100 3 Inh. swarms; not all singles.
(5) b 1:100 5 swarms (3 Inh.); 75 singles; No trails.
(4) i 1:100 Spread contam. But no trails
(3) i 1:1000 6 large 3 small swarms (b, i ?) } No trails

why two kinds here ? b

B .1 ml samples (do.) (1–6).

1 Swarms too messy; don't do
2 (MGA) [5 swarms, 56 1's; 7 short trails]; [45; 77 1's; 10 T short]
3 No T or S.
4 No T or S.
5 3 Inh. S.; No T
6 2 sw (1 spread contam ?) No T.

1:1000 is adequate to inhibit trails!

serum inhibits b trails!

abandon i × b system for this study

DATE: APR 13 1955 REF:

37 - X 666

D. SW967 plate in MGA, MGA60 for (minor tails — SPM. in <24 hours but too crowded.

E ~~Re~~ ditto ~~check~~ SW666 MGA60 only — at 2 days, no minor tails, same differing colonies as ●

F 1237A+ for flares — incubate SPM - 48hr. MGA only note singles, not pure?

G control SW673 "

H { 1 MGA
2 MGA 60 } → excessive proportion of swarms. (? age of preparation ?. Do not use.

APR 14 1955 Platings of c. 50 tinters only in MGA60 + .01 ml seeums. all show swarms.

	tails
a	+
b	−
c	+
d	+
~~e~~	−
k	+
r	+
12	+
B-O	±

a, c, and would be quite good for further test.

(c. 5 30 PM)

D replate P 14 A 15: excellent development of minor tails in 60% MGA; initial only in MGA but will probably show later.

12 ~~9~~ DIA from plates. — Refrigerated 10 A.M. 5/15. 60% MGA. Reincubate sister plates

Needs to be done in my own expts.

APR 15 1955
13

	Today	
Serum effects	✓	
Swarm sibs		*
flares	✓	
Terminals	start	*
E M.		
SW 967 / HA 10	✓ ✓	
Paired subclones for multiplicity of "T" (Use i T ?)		*
Isolations in water ?		
Viability pH 4		
Viscosity pH.		
Trails in 40+ ✓	(viability of subclones multi, step inactivation)	

APR 15 1955

Notes: Phase Var - TH2 ph2 monogh?

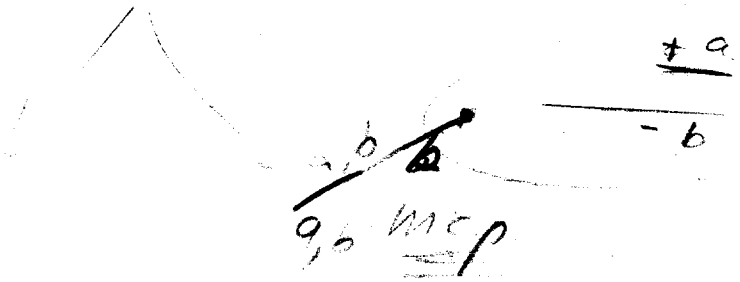
Mention to Bruce 6/i — of. burgndof.

Inc. Tax

Fla^{+} $H.^{a}$

↓

Fla^{-} $H.^{b}$



Serum inhibitors a → x

1248

DATE: APR 14 1955 REF:

1 APR 15 1955	3	4	5	6	7	8	9	10

14: n.g.

4/15: Frese degps, FA76a (S. miami 9) → SW666 11AM.
FA37 → x " "

Preliminaries
SW1250, 1252

not tabulated but results indicate that a does not inhibit b trails, part with a/b trails. b inhibits all trails ξ at 1:100 as well as 1:100

Reverse is # 4'

4131

3 cells isolated

1131 ? ~~8 cells picked~~. ~~not thus expt~~.

~~1) progeny to n_3~~

Flat: H_1^i ($8^+ : 12^-$)

1) progeny to n_3. 1/8 gave motile on transfer, found to be mixed +/−. − ~~H_1^i~~ H_1^b. ∴ Segregation at n_{43} !

2) n_{13} : all −

3) n_{13} : all −

1132 2 cells. followed duality to about n_3:

1) showed Ichem to n_3 ; n_{13}^-

2) 2 sibs both motile, catmated to n_3 ~~n_8~~ n_3, n_8 both. n_{13}^-.

11~3

1134 (C3): → 3/22., each then catmated

Precis 1132

4/15/55

1131) 1) 1 polyene to n_3. 1/8 ~~full~~ gave swarm, mixed $F1a^+H_1^i$ $F1a^-H_1^b$. Segr & hy.

2) 2 cells → n_{13}^-.

1132) 1) 1 chain ~~$n_1 \to n_3 < n_{13}$~~ $n_0 - n_3 < n_{13}$ (n_0 = (Initial + burin))

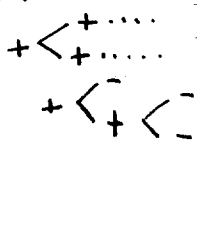
2) 2 chains $(n_0 > n_1 - n_8 < n_{21})(n_0 > n_1 - n_8 < n_{21})$

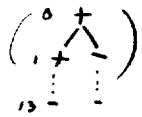
1133) 1) Swarm : pure (1/4 inviable; 3/4 → H_1^i) but late coal.

1) $n_0 > n_1 - n_1 > n_{13}$. (2 interm.)

1) $n_0 - n_1 > n_1$.

1) $n_0 - n_1 > n_{13}$.





initial only ↑

1134) E3) $n_4 > n_5 \lessgtr^{n_{15}}_{n_{15}} n_{19} > n_{19}$. (3 interm.)

D4) $n_1 > n_4$. 1 interm — n_4 term.

E5) (SW) (T+S) from 1 cell. At n_5 —— n_{17}. chain (F1) F2 = nm. E11 = swarm in remainder

C6)$_1$ ~~n_0~~ $n_0 - n_{23} > n_{33}$.

C4)$_5$ $n_4 > n_5$ ····· $n_5 > n_{10}\left[\begin{matrix}\text{5 isolates}\\ n_{10} > n_{18}\end{matrix}\right]$. 16,50 interned.

$n_{16} > n_{26}$

A3)$_1$ $n_1 - n_3 - n_8$? not tested.

B5)$_{10}$ $n_{13} > n_{15}$ ~~$n_{15} > n_{20}$~~ $n_{19} > n_{26}$

$n_{15} > n_{18}$ ⑥ n_{28}. n_{18}. ~~n_{24}.~~

Branch ~~①~~. Branch ② ③ Branch ~~④~~

Rockis 4/15/55

1134 A5 / 7 { $n_2 > n_{12} - n_{12} > n_{28}$ (2; 3 sol.)

+7? $n_2 > n_{12}$ < $n_{12} < n_{19}$; $n_{12} > n_{19}$; ... n_{40}.

(100 ⊕?)

(14 reisolations, 6 days [refr. overn.])

~~A4 { $n_1^1 > n_{14} - n_{16}$.~~
~~$n_1^1 > n_{14} - n_{19}$.~~

$A4_2$ $n_1^1 > n_5$ < n_{17}. ; n_{20}.

1138 most isol died. 1 clone saved (B4) also had trouble drying.

B4 -F72 [illegible]

$n_4 > n_{14} - n_{42}$.

n_5^2 [illegible]

$n_6 > n_{14}$ < $n_{17} > n_{27}$; $n_{18} > n_{23}$; $n_{31} > n_{39}$; n_{27}.

$n_6^{3+} > n_{16}$ —
19
22 > 27
17 > 30
32.
31.
32.
19 > 32
19 > 29
33 > 38
16
19 > 32
19 > 24
44.

∴ branching indeed
not sooner than n_9
not later than n_{16}.

(case to illustrate)

n4 + 13

n27 + n15 n45

1138 add chain after n16

n

B21

/

A21 3 C23 lost

4 C33. < 5

22 2 C24.

23 1. > 13

24 3 C25 13 A32. > 1

25 2 D25 13 A33. 72.

B22 3, 13 A31.

23 3. > 13

24 3. > 10

25 4 E23 13 B34. < 5

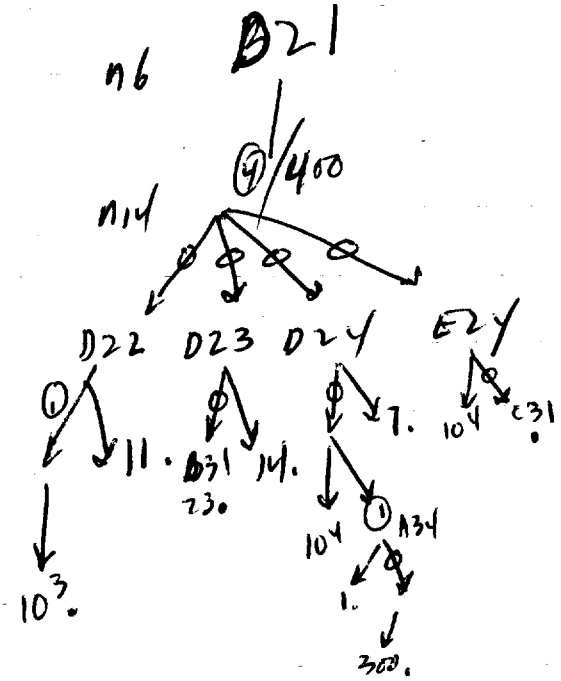
C21 . > 4

22 3. > 13

E21 13 B32. > 5

22 13 B33 2, 8 E31 1, 1, 3,

< 6.



∴ total chain lengths

n16

A21	19.	
A22	22.	> 27
A23	17	> 30
A24	32	
A25	31	
B22	32	
23	19	> 32
24	19	> 29
25	33	> 38
C21	16.	
22	19.	> 32
E21	19	> 24
22	44.	

$H_1{}^a \rightarrow X$ 1252

DATE: APR 20 1955 REF:

FA93 (sw940 4,5,12 a:-) — X sw666 . 11AM-12:15 — 12:40 pm.
FA37 " a

A) 93 — X 2 35' fuse dyes. 5PM Harvest ~ .5 x 10^3/10 ml.

B) Same dilute 502/ml. = 502/1ml .2 ml samples.
B5 = 1ml /6.

C) 37 — X (sw1237) 402/.4/ml
1. 0.1ml M&A60
2 ± "
3 .001 a
4 .001 b

plate ~ 32° PM
5/31 The results here are not tabulated but can be done.

APR 20 1955 T Repeated. T.

B	1	—	+	C	1 2 3 —	++ >90%
	3	a	+ (reduced in % + extent)		3 a	++ large tails no ends.
* some small late fronts.	4	b	—		4 b.	—* . — dried out before any shots
	5	b	— (Recheck carefully — seems			

F 12/58

Conclusions: effect of anti-a serum is specific, as it works on $H_1{}^a \rightarrow X$ but not on $H_1{}^b \rightarrow X$. The effect is, however, not complete and may be difficult to measure. Hold plates to photograph. It is possible that early chains are b and later are a or vice versa.

Save C123 B1B3 for related photos 5/31.

5/31

from 5 plates of B5 and 2B4 looks for any trails or suspicions.

on 1B4 plate only, 3 susp. trails? or clusters.

B4/a ∞

b ∞

c ∞

These may have had early ~~B~~ a phenotype with little enough b to swim in presence of anti b.
Plates to DCG to attempt resolation of these trails.

6/4. DCG found (on replating isolates)

a: gave fuzz 1's and many clusters (ca 1000's) (after 3 hour incubation 37° then overnight at 23°. MCA) photographed.

b: pure 1's

c: mostly swarms – attempt to isolate any 1's (doubtless contain)

post partition of clones

1257

DATE: APR 20 1955 APR 21 1955 REF:

1	2	3	4	5	6	7	8	9	10

Re 1257 pupn.

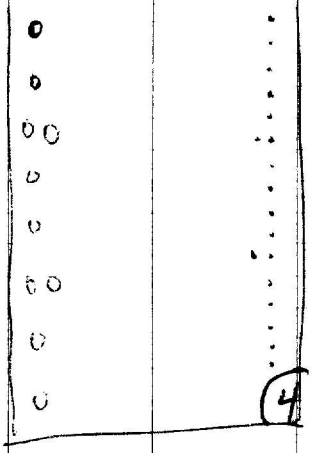
305 fuse dupn

APR 21 1955

10 1252 pupn.

fuse dupn 145

D C B A



2¹⁰ - 2³⁵

spot 56 (1)

30 A 1 -15

D 1 -11.

Examine 5:30 Most active without being handled

40 { A 2, 3, 4, 5, 7 B1 B7 B9 C 1 2 3 4 8 14

{ D 2, 5. Check for circulation & swarm, trail.

5¹⁰ P 22 - Pick seven clones + plate samples

50 est 10⁴/ml / 10 ml = 10³/ml plate .02 ml .1 ml samples in MCA

activity is soothing

DATE: APR 22 1955 REF:

	1	2	3	(+)	(−)	6	7	8	9	10
A	1 (+) wf			—	0					
	2 (1) (1) wf			3	4					
	3 (1+) (1) F	v. active		10	4					
	4 (1)			10	4					
	5 (11) F			4	4					
	(1)			1 0 .	4	Research for (+) by trap:			0	
	11.			11 —	4					
	10 wf 1.	vs		0 .	4		"		0	
	F + . 1. 1.			—	0					
	11.	dirty		6	4					
	14 11.			14	4					
	15 11.			1	4					
B	1 (11) + .	v.a.		0 .	3 pd					
	wf 1.			4 —	4					
				18	0					
	25 ++ 1..			1 0 .	4					
	T T + .			12	3+					
	1 T .			(1)	4					
	(1) 1.			3+	4					
	(1) +			2	4					
	1. dirty			1 0	0					
	wf T			2 8	4					
	1 (1) not			1	4					
	T (1) slow			3 —	4					
	29 wf			c. 30	4					
C	1 (1)			swarm	20% - 3+:					
	c2 (1)			—	0					
	(1)			4 —	4					
	1.			—	0					
	wf T T			1	0					
	(1)			0 .	(200) 3					
	T 1.			20	4					
	10 + . dirty			1	4					
	c11 + 1..			50% sw	4					
	12 + (1)			2	3+					
	13 (1) wf			—	0		4/23.			
	15 (1) 1...			3	3+					
	18 (1)	"D1" →		40+	(500)		Proved to be swarm now		(10%)	4.
D	1			1	3+					10
	2 1.			sparse	<1	Inv. wf				
	3 1.			3	4					
	4 1.			0 .	3 typ.					
	5 1. (1)			8	4					
	1. T			swarm? 100	3		✓	10% met		
	1 T			sw 50%	4		✓			
	+ .			8	4					
	+ .			10	0					
	11 wf 1.			—	0					

(over)

Save swarms

D1
D7
D8
C2
C11

① from samples ducting to assay (plated specific, over)

{ in slabs for whole clone
in MCA tubes for pooled motile
pool Pla⁻ cols. from MCA over to non-mot.

Replate

D1 - see ufus

D8 - too crowded to count swarms ca. 50%

to recover components stocks for later colony tests.

37 ~~xxxx~~ --x SW666
undivided clones

April 21, 1955

56 (1) isolates, grown to 2^{13} and summarily examined for motiles. Counts are underestimates. No tech losses

NG ~~12~~ 13 Swarms 4 No motiles 6 less than 9 22 10 or more 11

Maximum extimate: 40+. 5 clones were harvested for replating of the intermediate chains.

No.	Est motiles	harv. + nm.		mot left behind	Plate
B3	18	20		4	120 1's ~~many~~; 2 vs T. 80%
B12	28	22	4	10)	11 + 1 vs T 1 cluster
C9	20	28	6	5	1 tail, def. multiple but compact 28 1's.
C1	30	37			4 v.s. tails 000. 31 1's
D1	40	45	10	10)	34 swarms + 13 nm. No tails.

* Replate Residue 8 sw; 92 cols. (own)

Estimates on clones with many chains are therefore moderately low. Some of the zeros may have had a motile but this was looked for. However, these drops were not searched with a trap owing to shortness of time.

In addition, 4 drops had apparent swarms, but it was difficult to estimate incidence of non-motile elements. Therefore these were blind-picked and plated immediately. (picked to 10 ml, est. 60-70% recovery; plate .02 and .2 ml samples)(This will help evaluate estimate of clone size as 2^{13}.)

Swarm	% mot.	Found	clone size $\log_2$	Plate
C2	20	10%	11	.02 4 sw; 43 1's .2 33 sw - not counted
C11	50	20%	13	.02 4 sw 29 1's .2 5 sw - ~250 1's
D6	100?	—	10	[No swarms or 1's plated D6...]
D8	50+	10%	4	.02 3 1's .2 3 sw; ~~35 1's~~

swarms or 1's plated but not counted.

Repeats

The data may be grouped as follows:

mots.	clones	12+ snl
inv	A1 9 11 B2 11 15 C3 5 6 14 D 10 11 ; D2 D5	12+ snl
0	A10 15 B5 C8 D4 A7	6
1	A6 14 B4 7 10 12 C7 10 D2	9
2	B9 C12	2
3	A2 B8 14 C14 D2	5
4	A5 B1 C4	3
m6	A12	1
8	D6 D9	2
"10"	A3 A4 D10	3
11-12	A8 B6	2
14 18 20	B12 B3 C9	3
20+	B12 C1 C9 ~~B5~~ see above	~~4~~ 3

C15-D1 conf. in tally but not pltg.

Initial active:

sw D1 D7 D8 C2 C11

D/swarms: Semi 45/55? Flat were removed from DI before plating, the count on residue of 8 swarms: 92 colonies (in $\frac{.02\ ml}{10\ ml}$) is not fair estimate but the ratio must still have $\approx 10\%$. Late segregation?

April 21, 1955. Zur faire Tails.

(1). Serum inhibitions. of anti-a, b // b → x b
a → x b
i → x b.

(a) Smi a does not inhibit b → x b, probably specific.
Is b effect specific? Would need a $F1a_1^- H_1^{\pi}$, e.g.
(Input c S. Heidelberg initials?).

(b) Should also be tried on intermediates as early tails might all tend to be H_1^b and agglutinated.

(2). Late branching? Pedigrees + platings of $\begin{matrix} n_{1,0} \\ n_{1,3} \end{matrix}$ isolates

(3). E-branching < platings of initial sibs
any large tails in sibs to swarms?

(4). Use all segregates H_1^b (a) tail ends
(b) swarm sibs — esp. of $H_1^b H_n^{\pi}$.
(c) look for b-resistant tails.

Today: (A) Replate a/. and of a/i → x b.
(B) start (2).

Tonight Dennis notes — summary for (4).

Does b (minnesota) serum also inhibit H,[a] Fla[+] → H,[b] trails. 1252

using Fla,[−] H,[non b]?

DATE: APR 2 2 1955 REF: 1250

"b"

New Pups FA10 → SW666 10[10]–12[15]–12[40] Mich.

Free tags 240. Harvest 445 ~800/wk.

Inc. c. 5[30] – 10[50]

A. FA10 → SW666 (.1 ml) B a → . (.2 ml) ~100 cells/plate

9A23: trails

		A b → b	B. a → b.
1. —	—	++	++. 10 swarms, non concentrated
2. b minnesota (Edwards)	1/100 b	—	— no trails
3. "	1/1000 b	—	— no trails
4. a	1/100 a	++	± } much the same.
5. a	1/1000 a	++	± } appearance now ≥ MGA 4 dilutions.

B pups. may be late, segregated.

∴ b, minnesota also inhibits completely. Reaction may be specific for → H,[b] but this cannot be verified unless a Fla[−] H,[non b] can be isolated. (result of 1250B5 plating?)

Search for b trailers. 1252A2 ⑤ ② 1252B2 ④ each show 1 marginal/pl. (same not diff.)

C 2 ○○ 1 ○○○○ } no marked swarms.

3 ○○

1250B5 4 plates

SW1216.

5/31 ~~from 1250B1, B5 pools carefully.~~

Pools of 1252A, B 5/31

APR 22 1955
APR 23 1955

Tetrazolium- formazan chains preliminary

P22. Prepare stained cultures. Add 1 ml overnight culture to 7 ml broth + TZ .005% .05% Incubate c. 3-4 hours. Also (A) add TZ (1/200 .5%) to 1 ml culture directly.

Best method of preparation appears to be growth for short interval with TZ. Probably only older nongrowing cells willstain.

Refr. to 1 P 23. Test isol. to agar, small liq. drops. Main traouble with agar is confusion from dirt even under oil. Probably better in fluid with a nonmotile culture.

1PM isol. 1) mot. W-2344 to A1

4 PM 3 more to A2

4PM 6 stained W-2802 to small drops near situs C. These were terminally marked. (Z = formazan granule)
Hunt for rare medial marked= 7,9

2 W-1177,(dividing) 9 is terminal, 10 is medial.

of 10 clones found, 3/10 still located "Z". Fate limited - see protocol.

APR 25 1955 Try out agar block methods to immobilize ... Mostly a failure owing to dirty agar — hard to refind individual cells. Try methods instead. (cf. test 1-2 yrs ago). Disadvantage of oil chamber is solubility of released Z in oil.

But pul. observations above bear out earlier concept

(1) ⬭ → ⬭⬭ as regular mode of division

(2) most Z unipolar

(3) Z dies out.

DATE: APR 2 5 1955 REF:

Standard system now: SW-940 (FA 93; H_1^a) 22 —x SW 666.
37°

A. Overnight SW666. 1ml + 1 ml .01% TZ broth. Stained 9:00-10:30. Wash and add FA 93 to pellet. Inc 11 AM - 1 PM. (Cf. B); sediment pellet for harvesting motile init.

A= cells prestained; 2hours+ phage/

B. 1:1 + FA93 9:30-11AM. Add = vol. TZ broth. Incubate till stained (1PM). Sediment to harvest pellet. (3i/2 hrs. + phage).

B= cells poststained.

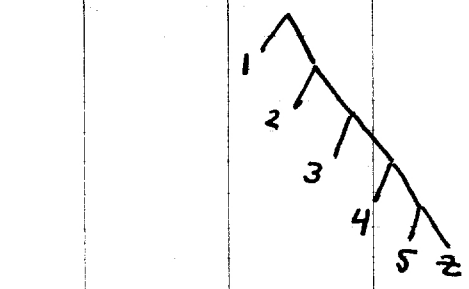
Found: many motile initials in each, but almost no motile A were stained (overstained?) About 2% of motile B were labelled; c. 50% of parent population.

Summary: 28 isolates from B, 2 from A. 5 clones inviable. initially., only one Z (granule) chain died later. Z chains were followed for 4 to 6 fissions. 1 clone gave a swarm (c. 50% motile) = 31B/

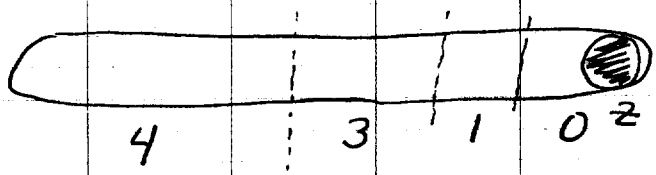
E = preponderance of motiles (>10)

If clones are summarized: 8 clones showed E. This appeared as follows:

n	⊕ found	Random expectation
1	4	4
2	3	2
3+	1	{1
Z	0	{1

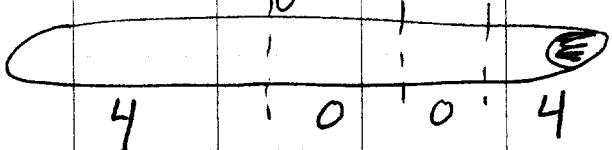


or, if cell is



The result agrees with random expectation, but possibility of a negative correlation of Z (after n3 or n4) should be tested.

Original notion if E were polar was expectation of:



if Z had random chance of masking the E or ~E pole initially. However, Z may either inhibit motility if it masks the E pole, or correlation may be one of selection (in terms of age correlation). Further study is needed of Z-masked chains, in 2 senses. (over)

Also see data on plating of intermediate ⊕ and variation in number of ⊕ among clones; partition among subclones.

APR 25 1955

1254

DATE: 8 P.M.

does this recur at 6/56

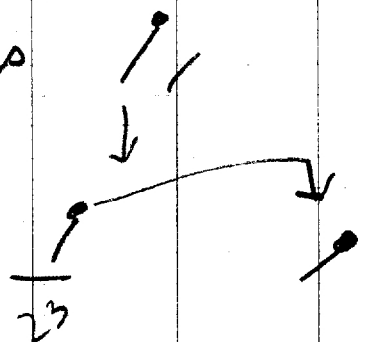
REF:

	1	app.	3	total	5	6	7	8	9	10
1.	5 4 4 3 4 z	0 shr. w. lam. no trans.			4 4/1. 4 3					
10	8 4 4 5 (23) 4 4	0			4 4 5 5 5 4 4	½.	0			
15	1 8.	0	(all 4 not)			bies 4/16 not.		c. 0/8	d 4/4	
18	2 n.	only not in a.	2 not in b.	bies 3/8	4'					
19	New rod formed twice 9PM									
20										
21	7	0			4					
22	5 2		b. hes 0/32		4		(1+, 1-)			
23	5 2				5	0/2	. 6.			
24	8		3/8							
25	16 zuf.				4					
26	4				4					
27	8 z									
28	8 z uf.									
29	1	0.								
30	4	0			6					
31	1 2 3		6.		3					
32.	4	0			6 (1 var. ? already)					

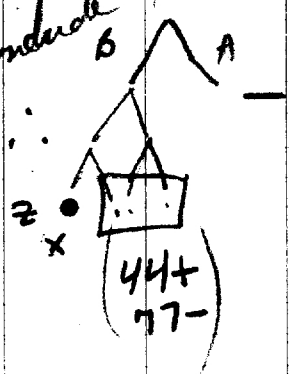
Reexamine at 8PM and isolate residual z from pid. # of var z. to eight not dep.

St. RT.

E.G. 31 was



conclude 6/56

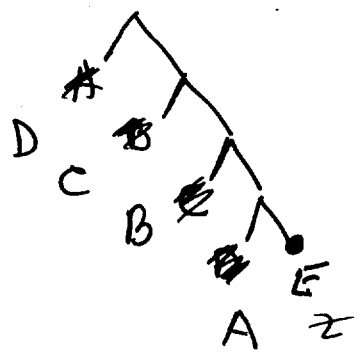


Reconstruction:

ingroup, rightmost is Z chain cell.

the others are successively later sibs of it.

e.g.



1254

DATE: APR 26 1955 10[30] AM. REF: 21

	A		B		C		D		E	F	7	8	+	9	n	10
1	0	4	4	4	(25)	4	0	—		29	22 → 3[00] PM.		2		4	2 died
2	0	3	1	4	1	4	0	4		2					4	
3	0	—	2	4						—					0	
4	2	4	(15)	4	1	4	0	4		18	too close to pools.		1		4	
5	0	4	1	4	4	4				5					3	*
6	1		3	4	(12)					16			2		2	*
7		—								—					0	*
8	0		3		0		0		0	3					6	*
9	1	4	1	3	0	3	0	4		2					4	
10	0		(30)	3	0					23	23 → tube for plating		1		2	*
	1		(18)	4	0		2		1	22	18 →		1		5	
	0		1		0		1		0	3					6	*
	0		3	3	0	3	0	3	0	3					5	
	1		(38)	4	1	4	2	4	•	37	28 →		1		4	
15	4	4	3	4	2	4	1	4		50	43 →		3-4		2	
	0	—								—					6	
	2	4	3	4	0	4	1	4		6					5	
18	(18)	4	2	4	6	4				26	17 →	6 →			2	
19	1.	100,	(3).												1	
21	1		0		1		0			2					4	
	0		4		0		0			0					4	
	1		0	3	(23)	4	0		0	23	21 →		2		6	*
	2		2		0	2+	0			4					5	
25	3		1							4						
	1		0	3	0	3	0	4		1	4 →				4	
	(42)		1		2		2			47	41 →		2 w/ 2 cl		0	x
	1		5		0					6			0		0	x
30	0		2		0	3	0		0	2	0				5	
	0	4	SW	3	0	•				SW no trails	Pickup later - prune ?				4	
32																
	×	↑	×	↑	×	↑	×	↑		total clumps in whole clone.						
35																*
																Unresolved granule

+ is clumps in which persistent motiles appeared.

n = number of passages for which 2 was followed.

Save + Recover (1 in 1 ml) the clone same

Flagellobius in 31B swarm. Plating of sample from 44 swarms: 77 colonies, no trails.

(not necessarily isolated!) .01 ml.

50

1/56

↑ must be log$_{10}$ Fla$^-$ in clone ⊗ must be Fla$^+$

TZ, methocel; divided clones

1255

DATE: APR 2 8 1955 REF:

Preliminary expts 4/18 showed that 2% Methocel 4000 immobilized cells so they would stay together after fission and form necklaces. Use to test destruction of Z in a chain, and to isolate after mz or my. Make up Methocel in Penassay. The methocel completely immobilizes the bacteria. ?What concentration allows Fla^+ to swim?

93 → Sea 666 8:30 – 10 AM, add = vol TZ .01% to 12:05. Spin down and refr.

Abandoned.

B) Apr 29. 2 PM stained W-1177 (c. 2 hrs. mixture overnight + TZ broth). Plate out in methocel broth on c.g., over oil. Also isolate a few definite anpmalies.

Conc. Most cells stain unipolar. Rare (5% bipolar, subpolar). Most chains show terminal granule. Onc. cells lyse in random position.

Some exceptions with interstilial granules— probably from subpolar cells.

Need: observations at first division of subpolar and bipolar cells.
should also spot a fair number of unipolar cpntrols.

2 chans

1255

DATE: APR 2 8 1955 REF:

(+)	1	2	3	4	5	6	7	8	9	10
A1 A	to A2A									
B		1¼ – to L.								
C		111 1 to XU		A4A						
D		11	R							
B1 A		i								
10 B			0							
C										
D										
C1 A										
B										
20 C										
D										
A2 A	moves.									
B										
C										
30 D										
A 3 A										
B2 A										
B										
C										
40 D										
B3 A										
B										
C										
D										
50 C2 A										
B										
C	moves.									

Exp. n.g. too much shrinkage specimen fluid added to Methanol disappeared instantly present. Let go – at bottom.
c. 5P27

DATE: REF:

		(−)	C.2 38M spotted	2	3	4	5	6	7	8	9	10
E1	A	(+)	—									
	B		—									
	C		—									
	D		—									
E2	A		—									
	B		—									
	C		—									
	D		—									
E3	A		—									
	B		—									
	C		—									
	D		—									
30												
40												
50												