Experiments on motile lines

1131 – 1151	Feb 15, 1954 – April 14, 1954
1212 – 1262	Jan 11 – June 2 1955
1272 (Leifson)	Sep 8 1955.

2/15/54.

Mix FA12 (SW623) + SW666 + cal vol. broth About 10^{30} AM –

3^{30} etc. Search for motile cells in enc. droplets ~~Rob~~ Rare
Fla+ seen (none in FA22 →x). Pick and set to allow
clones as convenient.

Clone CD. Motile, but became unmotile on transfer
Ca 5:15 First division : separate At 7:30 ~~one~~ both daughters
had divided, shortly thereafter, before separation, second division in one line.
c. 410, after separation other line also divided, less regularly.
Slowest fission was D3 – C1 Add fluid

A16. Examined for motility. Thus most descendants are unmotile,
but D3 would have formed a swarm.

D5 D4 ① ② ③

D5 C2 D3 C1 D4 D2 C4 C3 (4)
– – + – – – – – → – / → +

Other isolates

• C5. Initially M+, then M–. Initial growth poor ⟶ NM. Lp³
• B5 Remained motile for some time unless stuck ⟶ NM. (~~E~~ Lp+)
E: 1 drop had many motile: E 2, 3, 4, 5 ⟶ all M E1 = mess ("NM").
(late). 1 clone? [E1, E4 Lp+, others Lp³.]
A: Controls: Lp+ 2, Lp+ 3, 4 ⟶ NM (~~x~~) (4 n.g.) 5 = deposit ⟶ NM. (Lp*)
Blank controls OK.
Pick for homogeneity test and serotyping.

Fla (anmotagan) <u>666</u>

A 2	–	+
A 3	–	–
A 5	–	+
B 5	–	+
C 1	–	–
C 2	–	–
C 3	–	–
C 4	–	–
C 5	–	–
D 2	–	–
(D 3)		–
D 4	–	–
D 5	–	–
E 1	+	+
2		–
3		–
4		+
5		–

all gone? T
just result would
be valid? etc.

~~A~~. Test $H_1^?$ of Fla_1^- cosegregants of clone 1131CD. Inoc motility tubes c D5, etc. and FA12. [Should have used 9!]. Look for $Fla^- H_1^i$ crossovers
~~D. + FA9 (as should be used!)~~ of course FA 9 → each gave us Fla^+

B. Test lysogenicity character on SW 666.

	Fla	H_1	Ly_{22}
C1	−		−
C2	−		−
C3	−		−
C4	−		−
D2	−		−
D3	+	i	−
D4	−		−
D5	−		−

C. Test single colonies from D3 for motility (inoc. droplets under oil)
12 Fla^- : 8 Fla^+ Save 1, 2, 3, 4
(1, 2, 5, 6, 8, 10, 12 13 16 17 20) (3, 4, 7, 11 14, 15, 18, 19)

∴ Conclude that final segregation occurred at (or since) the 4th fission. Phage apparently not penetrant (as noted frequently in this system).

B5, C5 were evidently track formers.

1	−	H − ✓
2		
3	+	i + b + ?
4	+	i + b + ?

✓ Alude finds same result + will check further. All are 1, 2...

2/19 D FA 10 → 1, 2. presently b.

A

2/16.

See 1131. 12^{25} to 3:50 PM before spotting drops.
2 cells initially motile and at 1st transfer.

○ = phen. motile

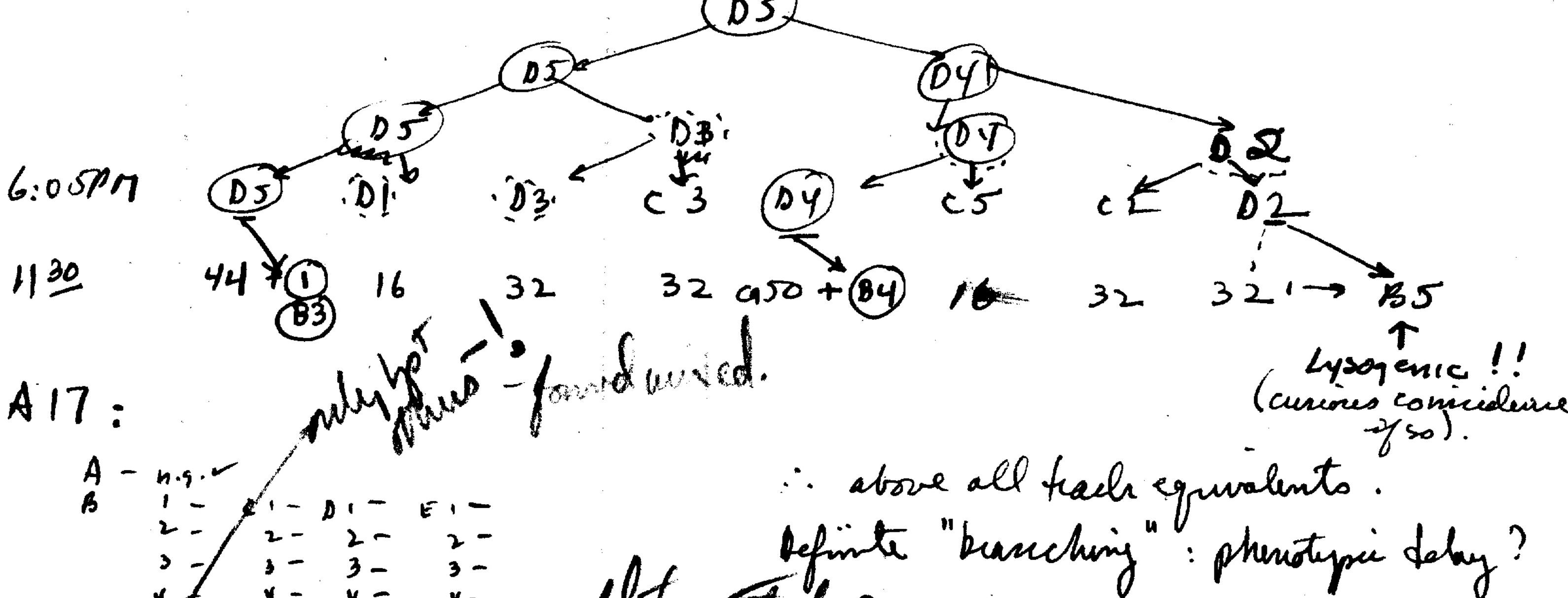
(E5) → (E5), E4
(E5) → (E1), E2
E4 → E3, E4
At 6^{15} PM → (E1) → B1, E1; E2 → B2, E2; E3 → E3, C1; E4 → E4, E4

at 11^{30} A17:

B1	E1	B2	E2	E3	C1	E4	E4
32	32	32	32	32	32	32	32

∴ at room temperature about 1 fission per hour. Had raised desk lamp to warm periodically.

+ 8 generations of trails in each branch.



Added fluid ca 11^{45} PM. Picks all drops (no visible Fla$^+$) A17 to nutrient agar (c needle).

[In future might be better to follow such trails more closely by chilling overnight ~~I~~ or try to find even late residual + by plating in soft agar!].

Fla+ → x

Febr. 17, 54

FA12 → x SW666. At room temperature, but 11:30 AM – 3:30(+) PM. probably allows too long. Rather large drops provide quite easy selection of Fla+ from large populations, Fla− falling to interface. See dictated record for details of manipulation technique.

Separate originals (E5), (D5), (E3), (C5), (B5), (E2), (E1), (B2)

No pedigrees on —.

O motile when last seen or until fission + separation — all.

E5 < C1 O, [3] < E4 + H_1^i, [2] < D3 + H_1^i, E5 + H_1

E5: (E5) (D3) (E4) (C1) — x, i, i, o [they split off early, ?]

D5 → D5 < D4 —, D5 —

C5 → C4 ±[2], C5 → C3 —, C5 —

B5 → B4±, B5 —

A18: E3 —, C5 —, B5 —, E2 —, E1 + (?), B2 —

[Plan somewhat disorganized. Probably best to collect a number of motiles early and not separate clones or else isolate a few for full clonal analysis.]

B 2, 3, 4, 5

C 2, 3, 4

D 3, 4

E 2, 3

Lp22 ... not necessarily pure ... all are +

Test and cols. — Streak before test Fla+ for purity

E1 10: all −, 12: 1+, 11−. {Alcoh says b for mess.

E4 7 all + i

E5 6 all − (b) i on pipette tube test

D3 5 all + i A.S. is checking

E5 pedigree suggests mixed clones!

But finds 1133E1 Fla+ = Lp+, Fla− = Lp−

E4 ....|000 E5

E5 0000|000000 D3

2/21/54.

Feb. 18, 1954.

FA12 — x SW666. 1 hour 37°, Refrigerate.

(a). 2/18. Day 1. FIRST ISOLATIONS 4:45 – 5:25. In this interval also, A5, B5, had divided. All cells actively motile. But note that the two early divisions both gave 1 lethal!

These isolations included:

A5
A4
A3
B5
C5
C3] from 1
C4] drop.
D4
E5.

(b) 2/18. At 10PM, – 10:45 cells had given clones of 2–25 individuals, usually with 1 motile. This was separated as indicated. Leave at R.T.

2/19 (c) 9:30 – 12N. Reexamine drops, trans to second group as indicated. R.T.

2/19 (d) Brief Exam. 5PM. Refrigerate.

(e) Reexamine A20. Where 1 motile cell already bruises, transplant to another drop in same site. Incubate at 37° from ca 12N – 4PM, then R.T. (already too long!) Refrigerate ca 6PM – 10PM. (or at 9PM?)

But this gave very large clones, perhaps also impaired motility. Some died out. It is therefore uncertain whether this is natural termination or whether 37° played some part.

(f) Reexamine P20. Refrigerate for reexamination of those clones that are not too large, and for subsequent plating.

Note: where (1) due doubt ... of lab ...

C3

check notes on time of isol. of D1-5.

(C3)

n5

22 (3/1+)

D3 10^4

10^4

D1 10^3+(1) xx

D2 10^3+(1) xx

D5 10^3+

n15

O3

55+(0)

n21

xx

∴ limited early replication only.

O3: N = 4 + 10 + 6 = (20)

D4.

(D4)

a

11 · 1+

F3 x.

xx

E5.

but → swarms

(E5)

a

25 ab.6+

F2 10^4

10^3 c. 50% mot! ca 10 cells

F1 10^4. 1+ xx

sw.

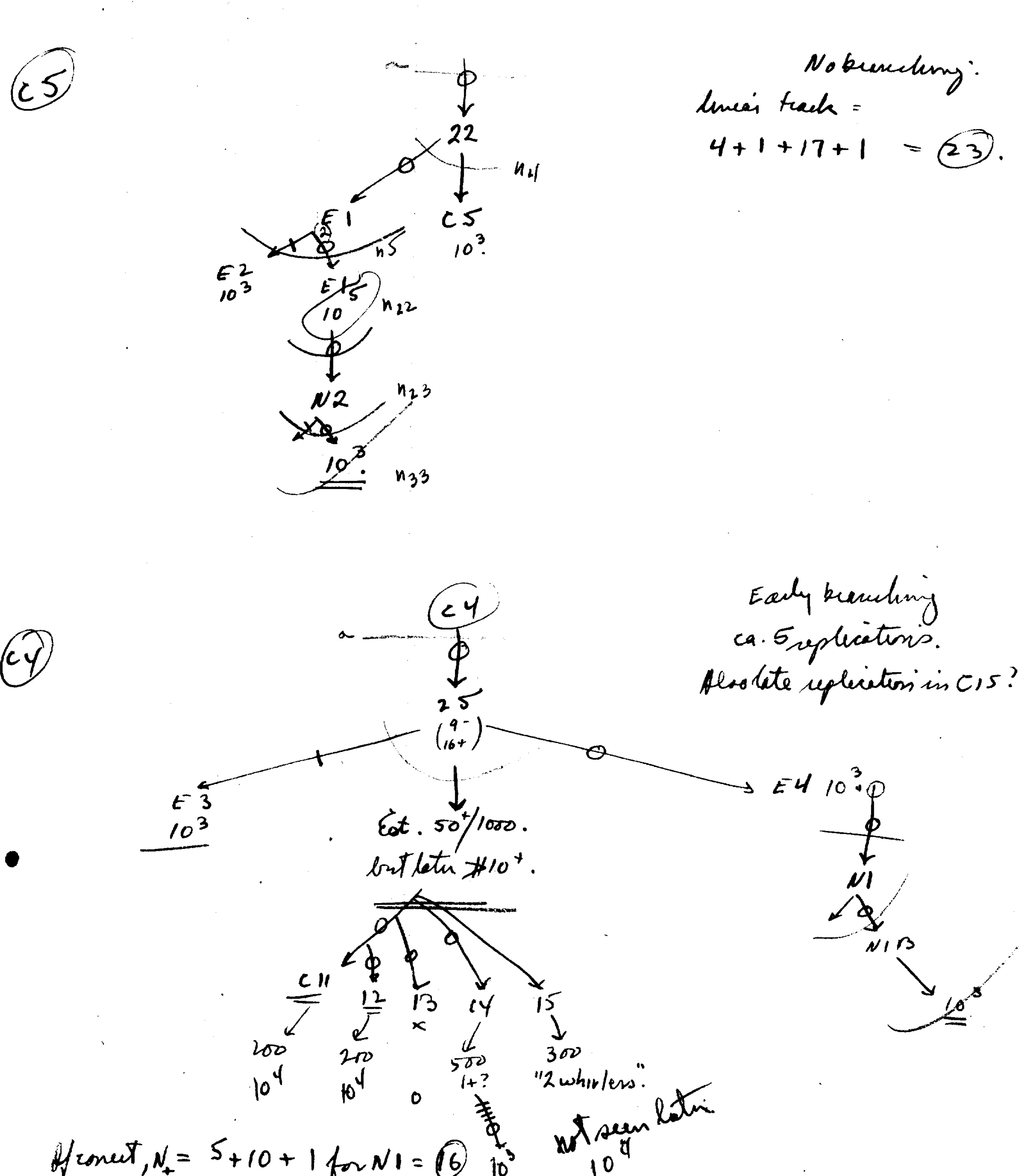
M2 x.

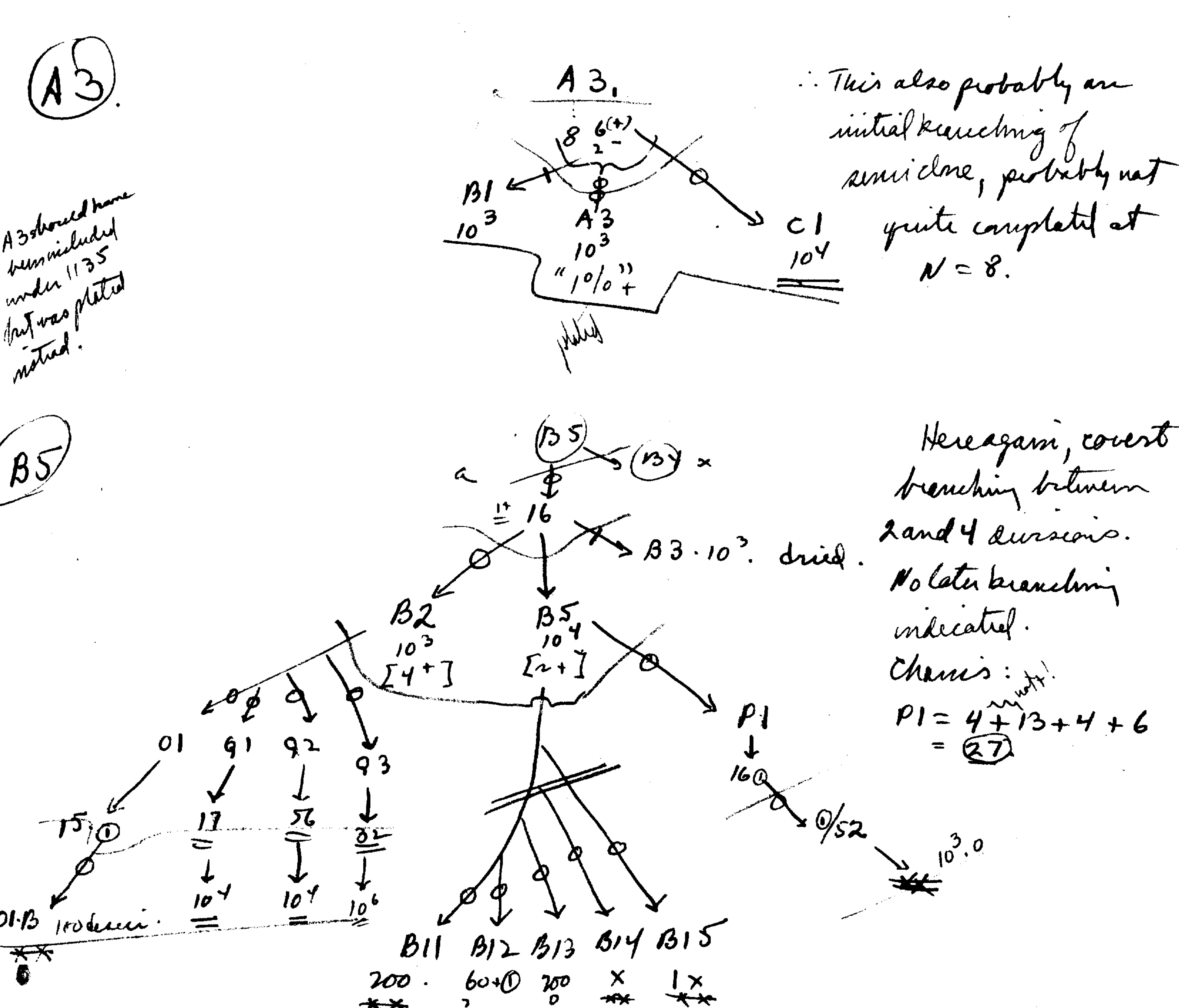
E 11 12 13 14 15

all o o

not yet exam...

~~possibly~~ T+S. from 1 cell!


c5
22
n4
E1
C5
10³.
n5
E2
10³
E15
10
n22
N2
n23
10³.
n33
No branching:
linear track =
4+1+17+1 = 23.
c4
c4
25
(9− 16+)
E3
10³
Est. 50+/1000.
but later #10+.
E4 10³
N1
N1B
10³
Early branching
ca. 5 replications.
Absolute replications in C15?
C11
12
13
14
15
200
10⁴
200
10⁴
0
500
1+?
300
"2 whirlers".
10³
not seen later
10⁴
If correct, N+ = 5+10+1 for N1 = 16
C15: 5+10+9 = 24



I was fooled by such clones as B5 thinking that numerous + [illegible] stabilization of a [illegible] clone.

Total N for All... →

is $4 \times 10^4 \times 70 \times 100 \times 4 \times 2 \times 6144$

$\cong 32 \times 70 \times 6144 \times 10^6 = 10^{13} = 2^{40}$.

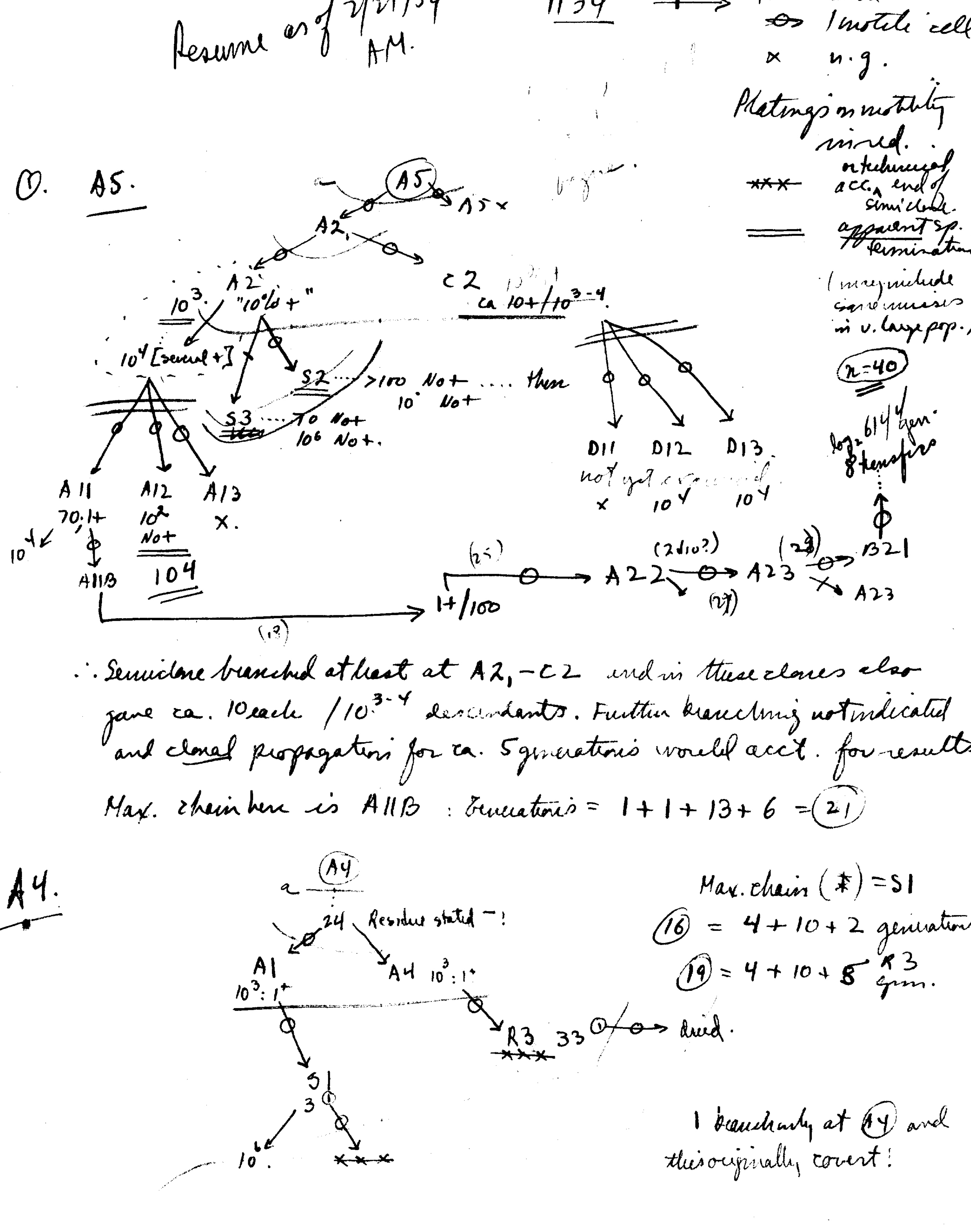
$= 10^3 \times 10^4 \times 10^6$

Total transfers actually: 16.

Semimobile sites: 8 actually followed; estimate ca 20 total.

Latest replications: ★ $n = 12$.

Should follow earlier divisions more closely.



Resume as of 2/21/54 AM.
1134
1 non motile
1 motile cell
n.g.
Platings on motility mixed.
on technical acc. end of semiclone.
apparent sp. termination
(may include some misses in v. large pop.)
n=40
log2 614 gen.
8 transfers
①. A5.
A5
A5x
A2,
A2'
C2
10^3.
"10%+"
ca 10+/10^3-4.
10^4 [several +]
S2 >100 No+ .... then
10 No+
S3 10 No+
10^6 No+.
D11 D12 D13.
not yet examined
x 10^4 10^4
A11 A12 A13
70;1+
10^2 No+
x.
10^4
A11B
104
(25)
1+/100
A22
(2d10?)
(27)
A23
(28)
B21
A23
(13)
∴ Semiclone branched at least at A2, -C2 and in these clones also gave ca. 10 each /10^3-4 descendants. Further branching not indicated and clonal propagation for ca. 5 generations would acct. for results.
Max. chain here is A11B : Generations = 1+1+13+6 = (21)
A4.
a
A4
24 Residue stated -!
A1
10^3: 1+
A4 10^3: 1+
R3 33
dried.
S1
3
10^6.
Max. chain (*) = S1
(16) = 4 + 10 + 2 generations
(19) = 4 + 10 + 5 R3 gens.
1 branching at (A4) and this originally covert!

1134n
platings

Reexamined P23.

		Lp
S	1	−
	2	+
	3	·
R	3	
Q	3	+
	2	+
	1	+
P	1	+
O	3	
	1	
N	2	+
	1	+
M	2	
A	11 → A22	+
	12	+
	13 x	
B	11	+
	12	+
	13	+
	14 x	
	15 x	
C	11	+
	12	+
	13 x	
	14	+
	15	+
D	11 x	
	12	+
	13	+
E	11 0	
	12 0	
	13 0	
	14 0	
	15 0	

leave to Dot (DCG) for Lp tests.

2/20/54.

Recorded as 1135

At 6P20, inoculate motility tubes with residues of A-F: in toto.

E5 plated directly, and washings in 1ml, now 0.1ml, 0.01ml, and on agar p

	Growth	Tracks	Swarm	Lp22 (DCG) 2/24
A1	✓			+
A2	✓			+
B1	✓			+
2	✓			±
3	✓			−
4				
5	✓			+
C 1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
D 1	✓			+
2	✓			+
3	✓			+
4	✓			+
5	✓			+
E 1	✓			+
2	✓			−
3	✓			+
4	✓			+
5	✓		✓ .1 ✓ .01	.1ml: − + phage .01ml
F 1	✓			−
2	✓			− colonies 10

No tracks seen (colonies)
Uncertainty of eff. of transfer
or whether motile cells
remained, as well as
efficiency of detection.
Try single motile cells
to agar.

Note numerous contamin
colonies in E5! (Use
anti-22 serum?).

February 20, 1954. see 1134

Data of 1134 received P19, and evidence of multiple semi clones noted. ~~Reis~~ Reisolate motile cells from indicated multiple semiclones about 3:15P20. ~~At 9P20~~, Incubate at 37°! At 9P20, Examine.

Although taken at ō stage 1134 c, these will be treated separately under heading 1135.

Some examined at 9P20 c̄ discouraging results. Refrigerate overnight for later study. (No certain + semiclones. See note 1134 e.

D, E examined 11A23. D11 0 D2,3 10^4 no + seen.
E. all 0

A23

A 11B 1+/100 ~~HO~~ → A22 → (12) —0→ A23 (2) —0→ B21 (28) 4:30P23. → B21; A23 ↘ A23; 5:15 → B23 → B24 (9PM) → B25 (5° overnight) to 9:30AM.

Δn: (7) (2) (1) (3)

✓

11A24. 0

A.M. A31 ← ; 12:15 (1) A32 —0→ A33 —0→ A34 —0→ A35 (4)

Mmod visit!

3:35 6PM 8:10PM; 12

(2) (2)

warmed by lamp!

Refr.

9:05 AM–12N R.T.

diaphasofiber certain, and 1 snake only (nonmotile). In course of day at RT gave 36 progeny after slow start, but none motile.

DATE: 2/23/58. REF:

FA12 → x SW666
5 planted.

A
1 swarm
Ø surface growth only (one or 2 loci).
4
Do not save

Presumably few cells give trails from surface plating. Try pour plate.

B. 2.

Feb. 26, 1954.

FA9(SW666) → × SW967 11-12^{30} 37° Refr. Collect F_{1} ? 3:30-4:20

4:25 PM : A1-5, B1-5, C1, C5. (A1-2, B2-C5 prob. sibs).

By 5:45 PM most above had divided once, some gave 2 motile, some 1. Refr. 5:45 PM - 9:20 A 27. Follow clones at room temperature throughout the day., separating further. (Refrigerate overnight). Most clones no longer had discernible motility. Followed in separated subclones during 2/28. Overnight P28 - A21, set out at room temperature, but the larger clones likewise were pure —. (Compare 1134-35)

This suggests that SW967 engenders many v. short sensitive clones or else conditions here used are unfavorable.

DATE: March 1, 1954. REF:

O: 60A (SW967) → × SW666 12¹⁵ – 1³⁵.
Studied to 7:45 no Fla⁺. / >10⁶ [illegible]

FA12 → × SW666 3¹⁵ – 4¹⁵ 37°. No Fla⁺ seen at this time / >>10⁶
5:30 – 6 PM, several easily isolated.
× Transfer to single drops at 6 PM refrigerate overnight
(A1-2-3-4-5 B1-2-3). A1-2 are prob. sibs; B1-2 ?Fla⁺
after transfer.

A2. Remi. under lamp 9:40 – 10:55. Rexamine.
overheated! T.O.

3/2/54. 5 – 6 PM. From earlier mixture, refrigerated (ca 2 hours intermittent incubation previous). Isolate 12 cells. (some divided before being put away).

	a.		12:30 PG.
A1	× ①		×d.
A2	①		×d.
3	. . ① [illegible]		×d.
4	①① . .		×d. (1 group only)
5	① . . .		×d.
B1	lysed		
2	. .		3.
3	→ 5. to D1 →		3+, 7- lysed
4	large clone. By 5+ PM had formed 15 tetrapairs (total A11 – F15)		ca 16. each almost d.
5	. . . .		
C1	①		1.
2	1.		d.
3	1.	+ ~~ . .	. ca 8. each, some lysed
4	1.		1
5	1.		ca 20. some lysed.

(all pairs ±)

T.O.

B3 → D1
a = 2:10 PM 3/3. (Refr. overnight, at R.T. to 2:10)

. = unmot.

b. 3/3 5 PM transfer the B4 isolates (A11 – F15) to new coverglass, and incubate these overnight at R.T. Original in Frig. to 10A6; in 30° 10A6 —

P3.

By 6PM, these subclones had undergone 1-3 further divisions and were separated within the sqplaces.

(c) A4. Examine clones of A11-F15
But by this time, most were already immotile.

(E). Most have dried out. But of the 15 sets, only following were recorded as motile at this time:
C2, B2, B1. C2 probably inviable. C2 and B1 dried out, but B2 clones OK.

Trees (B4)

n = 4. B2 — C2, B1 + ? others [in ol at RT.]

F21 [5] [6] F22 D21 B21 E22 B33 E31 C32 D31

d. 2PM. B21. ca 10^3 total, 1 Fla$^+$ = A21-25 B22-25 E21, E22 C21-22
D21 ca 400 Fla$^+$ = D22-24 E24 C21?
F21 ca 500 1 Fla$^+$ = F22.
15 + 3

e. 4:20PM. Separate some subclones.
(Visit by Francis Bello from Fortune).

f. ~~9:05 AM~~ 2:30-5:30 PM 3/5. Ca 10^4 clones in each.
From 10 clones identify + separate Fla$^+$.

g. 8:30 PM — Research remaining clones for +. Record already transferred for new cycle over.

h. 10A6 Recurrence clones
only B33, C32 still semiclonal.

These carried to i = P6, j = A7.
Total n = 44, n = 42 resp.
involving 11, 7 actual transfers

Replication occurred beginning n = 3, 4? to
n = 10 (no greater than 19 poss.)

Detailed examination of early subclones seems now necessary. Also, repeat (n36) transfer of cells to motility agar to verify multiple trail origins. (Assume that most semiclones never get into ~~ag~~ soft agar)

Serum eff; phenol ~~tactism~~ taxis

DATE: March 6, 1954. REF:

1	2	3	4	5	6	7	8	9	10

A). Test efficacy of immobilization of SW680, 681 by homologous antisera. Nearly complete agglutination in slide droplets under oil. Individual immobilized bacteria are also seen. Some seem stuck at one end. Other single bacteria still swimming at first. Suggest agglut. in tubes, then transfer under oil.

[Also try more dilute susp. for ~~##~~ immobilization to determine whether single cells can be diagnosed by dilute serum!].

B) While setting up expt., separate droplets were set up as [cells SW681 / serum] & a polarization in the cell drop was noted.

As the serum droplet was entirely isolated, an oil-soluble component was suspected, namely the phenol preservative. This was promptly confirmed by the arrangement resulting: The polarity was transient, and cells randomized again after 1-2 hours.

3/7/54. Experiment c ½% phenol repeated c clearcut results. No serious effect on non-motile bacteria (SW967). Also noted moving bands of cell concentration becoming either apical or (later?) central. Clustering very marked. These might suggest either an optimum conc. zone or faster diffusion through oil vs. water.

SW681 diluted 1:50 in nonmotile SW967 showed same tactic effect but only for the ~~non~~motile cells (ca 5+ fold concentration distally.

Possible mechanisms? Cf phototaxis (Clayton)

3/8/54. In agglutination tubes, definite ring formed over phenol (+eosin + lactose) 10% ← ring in 15-20 minutes.
← clearing. Results are more clearcut under oil

March 8, 1954

Plate single motile cells in motility agar to learn possible multiple branching. Mixtures at R.T. 1200 – 3:00 PM

FA92-xSW666 } not seen!
FA92-xSW967 }

Refr. to 4:30 PM, Isolate 4:30 – 6 PM.

Most efficient isolation: let Fla⁻ settle, scan only top focal plane. Catches perhaps ½ all motile cells, but with a fraction of effort required for complete search.

By 6 PM, a dozen Fla⁺ picked out, some separated.

Refrigerate to 8:30 PM, then continue. Separate 13 cells total.

at 8:40 PM: 2P9.

			2P9.
A	1	①	.
	2	①	swarm
	3	①	.
	4	①	.
	5	①	.
B	1	②	3.
	2	① 1.	.
	3	① 1.	
	4	1.	swarm!
	5	①	.
C	1	①	
	2	①	
	3	①	

no tracks.

Expt. rather sloppy

Ca 9–9:20 PM, Fresh out drops, plate to motility plates. Add fresh agar to embed. Then immerse deeply in additional layers. Incubate 37°. (0.5% agar formula)

7/13/55 Analysis → $ diff

A1 ~~B~~ ∅

A2 ×

A3 ~~x~~ ∅

A4 see pedigree

A5.

36, D5 d., 47, ② 50. 100.

B1 — v.sh ① (8.14.)

B2

3. ①/6 ①/28

E3 ~~M~~ E5

B3 ① 1, ... 10⁴. 1. 10⁴.

rol v.i

B5 n.g., stayed motile

C1 diff ∅

C2 —

FA12 → x SW666
single cells added.

March 9, 1954.

Further survey of pattern of establishment of semiclones
Terminology: coverglasses now ruled in advance, (A-H)(1-5) and marked with serial numbers [101...]
12 × 666 kept overnight in frig to 9:45 AM.
Examined 10:20-10:55: no Fla+ seen. [Sample refrigerated and examined again, later also negative at 4:10 PM].
Pups. at room temperature 1:45 - 3 PM Isolations (ca. 3-4 hours).
Numerous Fla+ best isolated at uppermost focal plane.

[101] at ca 3:40 PM. collected:

	a	b. 4:50 PM	c 11 A10	d 4P10		e 10A11
A1	(+)	(1) (1)	✓	1. (1)	(1)/32 D3	A: (1)/10⁴
2	x ... Reservoir.			0. +	D.	BCD: 10⁴.
3	(1)	(+)	0	0	C. (1) D4	
4	(1)	(1) (+)	1.A (1).B 1.C 1.D		A. 15. → (D2),(D1); B. (2)/9.	
5	(1)	(1)	1 →	(1)/36. → (D5); (1)/47 → (E),(E2)		d. 10⁴
B 1	(1)	(1)	+ . 1. 1.	8. 14.	E5	10⁴.
2	(1)	(1)	0x → +. 3.	(2)/6.1 → E3 E4	(1)/28	10³. 10³. 10⁴.
3	(1)	1. 1.	1. → 1.	32.		10⁴. 10⁴.
4	(~)	1. 1. 1.	2. 1. 1.	0/8	1.	(14)/10³⁺ → H2
5	(1)	(1)	1.	1.		(1)
C 1	(1)	(1)	(1)	0		0
2	(1)	(1) (1)	1A (1)B	1C (1)D 16+ 22	8 1. more motile	100. 10⁴.
3				A2.		10⁴. 10⁴.

104? 9 E3-4-6 probably abandoned inadvertently. Record lost

b. Refrigerate 5 PM to 9:30 A10.
d-e R.T. overnight
e 10A11.

FA92 × 666 37° 9-12 N, Refr to 4 PM. No Fla+ seen.

FROM		10-12N11	N12	
D1	A2B ②/11.	②/10³ → G1G2	10⁴⁺	
D2		⑪/10³ ③→ H3	10⁴⁺	
D3	①/32 A2D	10⁴.	10⁴⁺	
D4	A2C ①	①	①	This all long with but n.g.
D5	A5 ①/36	d.	0	
E1	A5 ①/47	50.	10².	
E2		100.	10⁴.	
~~E3~~				
F1	B4A.	10³.	10³.	
G1	D1 ⑥/10³		10⁴.	
G2	D1		* 18/10³⁺	→ SP12 → [105] (G, H) (1-5) by SP13, each gave 20-10³.
H1				
H2	B4A ⑥/10³		25 (d).	
E3	①/6. B2		→ 10d.	
E4				no record on E4-5
E5	①/28 B2			
A3	D2 ③/10³		10⁵. No+ (incl trap)	

Confusion re G1-G2 H1-H2

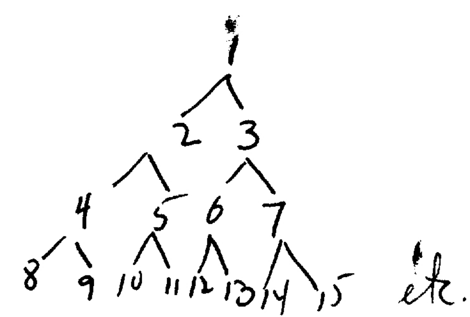
1 group is from B4, ⑥/10³

1 group is from D1-D2, ①/10³

Note: no H1 is listed.

∴ H2 is probably from B4. Whence F1? Maybe C3 - But unimportant.

Many of these pedigrees are thus shortened! (cf. 1142A-B!)

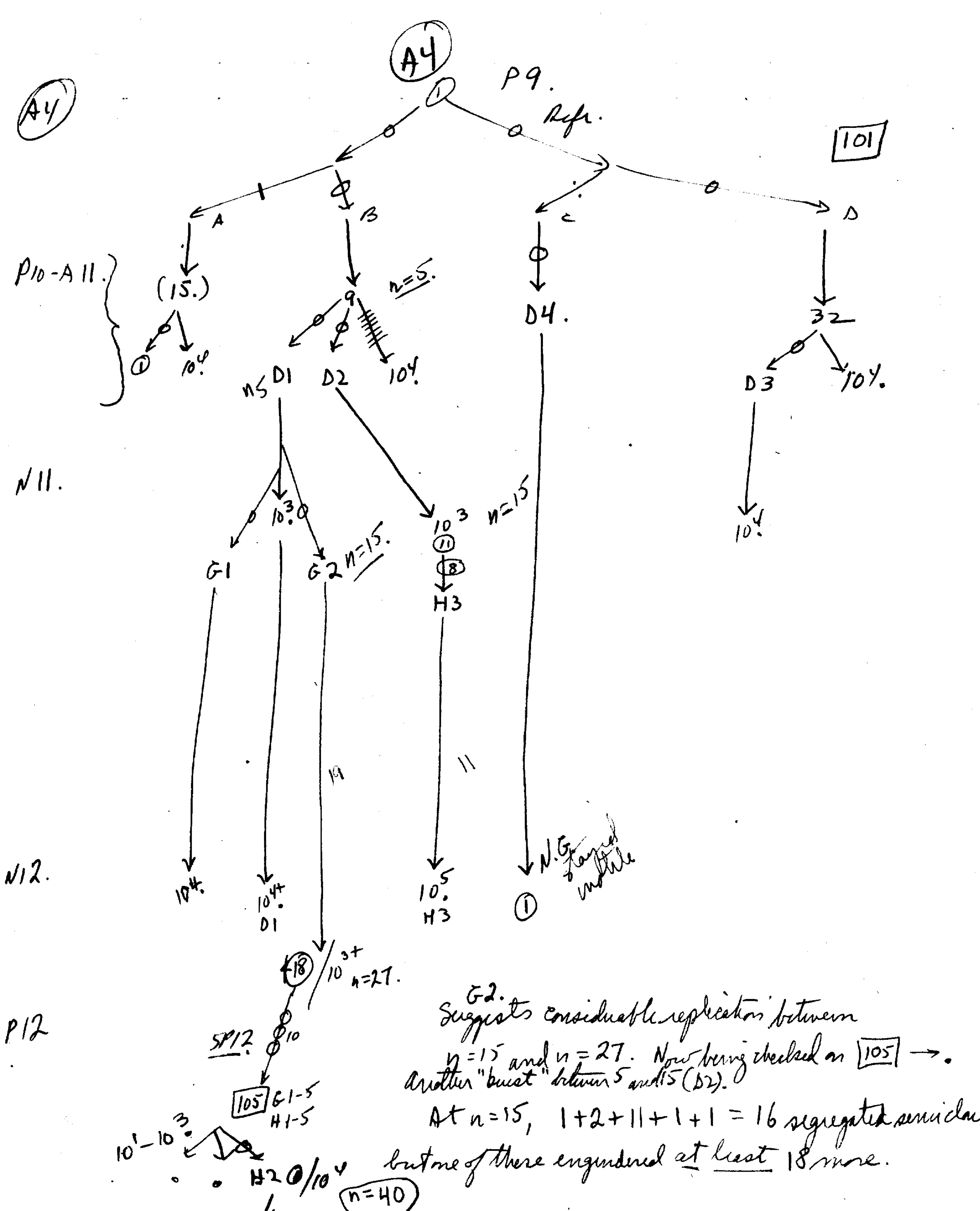


1141 A4 · line G2 showed replication ≮19 ≯27. Then only semiclonal for up to n = 40.

Total semiclonal yield:

Cells	4	5	6	7
#	1	20+11 31	1	1
Min fate: K6.		40,	15	7.
Time of division		19-27; 9-15..		

1141
pedigrees



(B4)

B4

(1)

n = 1
2
B4A
13
8
0 ·7 B4A
F1

not enough detail on these progeny!

Shows decision at between n = 3 and n ≯ 7.

10^3. (14) 10^3 13

(6)
H2 [7/55 (G2?) ⟶ (18) / 10^3. (i.e., replication to at least n_{16})
Notes are fairly clear that G2 was saved for other sequence.
4/24/56.]

25 d.

COMMENT: I do not yet have a really adequate pedigree on semiclone origins. Partly, it has been necessary to sketch out the background. Previous observations had suggested that division was restricted to $n < 10$.

Hyp: ① Irregular replication

② Segregation of particle — A: polytenic loci, aborted syphage ection

B: gene product, inherently heterocatalytic but particles.

Best basis of distinction would be total extent of replication and irregular later initiation of swarms to support A. Both might be possible!

From this plating, motile & nonmotile recovered.
F4 (mislabelled 1142E3).

PA 60 → x Fla^- gave +
9 → x did not.
22 → x Fla^- → H_1^b.
∴ $Fla_1^- H_1^b$; sib is $Fla^+ H_1^b$.

No + found from > 40 isolates from "E3"

60 → × SW666.
and plating

[104]
[105]
[106]

March 11, 1954.

PA 60B – × SW666 12·30 – 3'5 PM. Turn Refr. Ca 4:40 – 6:40 isolate + plate.
Use entrapment.
Plate EFGH on .4% motility agar. See over.
6:40 PM. 2 P12

(a)

Screen yield of swarms (1)

Ca 6P11.

		a			
A	1	1.			
	2	1.			
	3	(1)			
	4 }	(1)			
	5 }	(1)			
B	1				
	2	(1)			
	3	(1)			
	4	(1)			
C	2	(1)	(2)/500		
	3	(0)	(50)/10³!	→ 0 → [105] C1–5 5P12	
	4	(0)	(18)/10³		
	5	(0)	(1)/10⁴	~~+~~ 4/100	
D	1	(1)	10⁴: ~~[illegible]~~ motile!	→ 0 → [105] (D, E)(1–5). 5P12	
	2	(0)	(3)/10³		
	3	(0)	0/10³	plating also → no motile swarms.	
	4	(1)	0/10³		
	5	(1)	(1)/10³		

A–B: Essentially N.G!!

E–H plated. ←

Plate (hastily, no flush) (on surface 0.4% agar) Res. on [104] P12

		A12	A13	swarms → DCG from plating EH60
E	3	0		
	4	1.	:	0
	5	1.	:	0
F	1	1.	.	F/a⁻
	2	0	:	⊕/⁻
	3	1.		0
	4	swarm – to DCG to isol +/–.		0
	~~4~~ A4	0		0
G	1	0		0
	2	0		nonmotile.
	3	smeared..	.	0
	4	1.	3T?	0
	5	1.	2T?	0
H	1 [④]	1.	trails?	F/a⁻
	3 ⁴	0		⊕/⁻

But no appreciable trails were seen later. Thus v. few trails were manifested by direct count.

Good correlation !! 0/0 picked — trails not very striking.

Fluid trap method (had been tried before on possibly negative samples) now found to be most effective.
Use FA60–× as including another factor possibly linked to H_1. Swarm sibs wanted for crossover test.

5P12 — 3^{15}P13 16° 4P13 4P15

[105] not to [106] 4P14

42C3
C1 10^4
2 10^4 ① C1 50. no add'l +.
3 10^4
4 10^4
5 10^4 ① C2 ①/100 ①/2 50. 10^3

42D1
D 1 10^4 ② < C3 50.
2 10^4 ① C4 ①/200 ①/2 20. 10^3 ∴ ended semiclones.
3 10^4 C5 0
4 10^4
5 10^4 ① B5 4.

E 1 10^{3+} 3
2 10^4 → (F1) B1 28. 10
3 10^4 (1) B4 50.
4 10^4 (E3) B3 50.
5 10^4 (F2) B2 8.

∴ each was 1141H2① A1 100. 10^3

semiclonal not clone!

Pedigree results

By n = 10-15, many clones showed only a few +. Not followed further. However, C3 had ca. 50+. 5 sampled ~~...~~ terminated by n = 23

a) except for 2 which ~~...~~ terminated n ≥ 23 < 31 and 5 which was carried as a semiclone ~~...~~. ≯41.

n): Therefore replication ≮6 ≯10. Semiclones ~~...~~ ≯41.

max semiclones

b) D1. at n = 13, ca 2⁷ motile. 10 tested and showed repl. at 14.

∴ replication to ≯ 14. Semiclone then to ≯35. Others terminated earlier.

What was remarkable here was simply the very large crop of semiclones.

Need more data on distribution of motile in earlier divisions. It is now quite clear that replication does not extend beyond n = 15, usually ends sooner.

C3 → 10^3 → 5 (50) → 10⁴ C1 2 3 4 5; 10⁴ (1) / . . (1); c1 50. c2 100 (1) → (1) . ; 10^{13}. 50.

D1 → 10^4 (100) → residue plated: no sw. ; (10) → DE1-5; 10⁴: 3. ; 10⁴: 6 : (1) → A1, B2, B3 : 9^{24} ; 1-(2 sw)

110
107
112

DATE: 3/16/54.   REF:

FA60B → × SW666 (old susp) 11:25 – 1:45 at 37°. Refr. as 1143 stock
Isolate trapped motile cells to [107] A1–D5, E1 and plate.
[Should have labelled + followed residues but did not].
D1 = 4 app. NM cells E1 = 8 motile cells pooled. For most platings;
embed in layered .4% motility agar, ē chilling to immobilize. Layers seal perfectly.

E1: swarm D1 2 dup colonies.
not chilled 2/3 ē colonies. (contam?)
chilled: 14/16 had dup "colonies": no swarm, no distinct tracks
at 10A17. Reincubate. Medium is quite fluid. Φ 1 plate ē contam. swarm
A19: no tracks. See over for results on swarms.

Also, using old, refr. susp. (1141-42) Isolated A1–D3 in [110]
but decide later not to use. However D4, 5, E1–5, F1–5 isolated
ca 2:30 PM from ~~...~~ 1143 stock above.
~~At~~ Refrigerate at 5:55 PM. at 3:40 PM, all these were just
(1) except E2 (2) and F1 (2).

at 8P16, Check some of these, on [110] and transfer
[110] E1–5 to [107]. 110–F1 showed 0, (+); others not
checked. Incubate [107] at 29°; Refr [110] Residue 8PM – 9A17.
Then RT till index.

---

[107]: GH exa. n.g. (see protocol). 10:30A17

see b

E	2	*2+/300	to [112] B1, B2	To test further replication.
	3	50.		
	4	*7+/500	– 112: B3-4-5 – C5.	Inc 30° 12:30P17 to ..
	5	part of 2.		
F	1	(1) 10^3	→ A1.	
	2	250.		
	3	(1) / 10^3		
	4	10^3.		

107

E3

F1 2 3 4

10^3 300. ② 10^3.

① $\frac{}{10^3}$

3.

E4

20 . pd

E5

d.

suff for E1R

3/18 | 107 8P16 | 10^{30}A17 → 112

110 E2 { E2 + / 3 + / 4 + / 5 + } 4②/300 B1, B2; 50, 47/10^{2+} B3 4 5 C5. part d. 2

110 E3 { F1 + ①/10^3 A1 → immotile; 2 + 2-300.; 3 + ②/10^3; 4 ± 10^3.

110 E4 { G1 − p.d. 100; 2 − 200.; 3 − 60.; 4 + 20 p-d.

110 E5 { H1 + 50d; 2 + "; 3 + "

112	3P17 112	6A17 112	8^{15}P17 112	
B1	①/19	①/8	①/8	→ D1
B2	①/24	①/8	①/6 n=2	D2 v.sl.
B3	①/86	①/8	①/5 n=3	D3
B4	①/8	①/8	①/5 n=2	D4
B5	①/10	7.	40.	
C5	16.	50.	10^2.	
A1	3.		50.	

to 16° overnight.

110F1 ①

RT to 10^{30}A17

to 38 at 12^{30}P17 – 3PM
Then R.T. various times

~~1 30 P18~~ 112 | 11A18 to 3P18 | RT to 6P18. Refr. to 9^{30}P18 | 16° | A19 | P19

D1	①/100	0/2 ①/2 → E3.	①/29 → 114 G4	①/30 0/2	60.
D2	150.				
D3	①/10		ca 15.		
D4	150.	①/2 → E1. v.sl.	13. → 114 G3	10^2.	10^4.

Summary.

a) Platings: cells grew out in depagas; no traces

b) 8 clones: fun + at 10^{2-3} $_{\wedge}^{4}$ tested for later replicates (purify). 2 clones essentially n.g. (died) 2 clones followed:

110 E2 3P16.

107 — E2, E3, E4, E5 (n=2)

E2 → 300 K2+ (n_{10}); E3 → 50.; E4 → 500 K7+; E5 → 2. (d.)

n=<10

112 — B1, B2; B3, 4, 5, C5 (n_{11})

B1 → 1/19 → 1/8 → 1/8 → D1 → 1/100 → 1/2 → 1/2 → E3 → 1464 → 60. (29, 30, 2) n=40 P19

B2 → 1/4, 1/4, 1/6 (n=2) → 150.

B3 (n16) → 1/16, 1/8, 1/5 (n=3), 1/60 → 15.

4 → 1/8, 1/8, 1/5, 1/50 (n3), 1/2, 1/13 → 10^2.

5 → 1/10.

C5 → 16.

No replications after n≤10, and mostly prior. Total recap. 2, 1, 7, (1).

Total fate: 107E2 = 2 + 8 + 4,3,3,7,1,1,5,5,1

40 = 10+30 in one lineage and 10+6 in another

107E5. 10+16 = 26
10+20 = 30
10+3 = 13
10+0. = 10.

110E 3 not carried so much detail.

107 F1 → ①/10^3 ; F2 → 300. ; F3 → ②/10^3 not tested. ; F4 → 10^3.

∴ here no ~~later~~ decisions seen after n = 3 but not properly tested.. F1 shattered.

109

111
H4

60B → x SW666

3/17/54. Refrigerated 1143 susp. 940 AM. – 10^20 AM.

Then 30° – to 16°
8^30 P17

all residues unless indicated

109

A2 ✓ 52. ① → 111·A1 dried out.
3 ✓ n=6 ② → ..
5 ✓ 26 ① → .
B1 ✓ 1?
2 ✓ 25 ⑩ 4 → ....
SWARM 3 ✓ ㊿ swarm 1? → +also. Pure + Pure gns (A.B.) save
4 50 ⑪ isolated insertn A–K see below. R 10^5.
5 57. ③ → ... ... 3 not seen.

2^45 P18
Res. 10^4.
R 10^4; ①/300 ①/200 → D1, D2 see B1,2 no further +.
10^4.
+10/60 16/150

114

C 3 not exam.
5 " .

30° – 340 clones already large. Refrig. to 8^30 PM.

Note: manipulator broke down 9P17.
Ed Tatum visited A18.

3P18: ① from B4 to each of 11 droplets (A–K). No change
Ca. 250 each. Motile as indicated.

109B4 → 114

A	0
B	+15
C	0
D	0
E	0
F	3
G	2
H	1
I	1
J	4
K	1

D4 F3 E4 D3 ✓ C3 - F3

A1-2-3
A4-5 (B3 seconded at first division (but not necessarily this product)).
B5
C1
B1-4 B43
C2

		16 P18	A19	P19 (RT)
A	1	1.		1.
	2		①/8	13.
	3	1/25	1/2	10^3.
	4	0		0
	5	0		0
B	1	① 16.		200.
	2	①/26		60.
	3	82.		61.
	4	31.	①/10^3 1/2 → H2	
	5	①/26	1/150 1/8 → H3	
C	1	①/9		40.
	2	①/45 1/2	1/60 1/2 → H3	
	3	①/28	1/60 1/2	
	4	52.		10^3.
	5	①/20		44.
D	1	71.		10^3
	2	36.		10^3
	3	73.		10^3

D	4	21.	300
D	5	17.	10^3.
E	1	①/28	①/118:2^1 → F5
	2	28.	32.
	3	16.	10^2.
	4	22.	10^2.
	5	31.	400
F	2	33.	200
	3	①/34	70
G	1	7 150.	10^4
	2	60.	10^3
	3	10^2+	10^4
	4	①/30	60

H 114

A20

H 1.
2.
3
F 4-5.

N19: Sherman visited

8^{30} p21

A22

∴ One line reached $n = 59$
others $n = 33$, $n = 52$ before
primogeniture ended.
Isolate residual non-motile sibs to check
their propensity to give long descendances.

1144F2
G3

1144 Pedigrees

10 clones pedigree consideration.

A2 1/52 – 4.

A3 2/2⁶ no further replication. Fate: n = 13±.

A5 1/26 → 10⁴. | Limited semiclone (or others appeared and passed!)
n < 13

B1 ≮10/60 not pursued. Guess late early division apparent
ca 16/100

B2. ca 10/25 4 isolated/10³, A 0/10³ B ≮8 C ≮6 D ≮1⁷.

∴ substantial replication n > 5 ≮ 8 but not pursued.

B3. Pure swarm. Check H, status.

B4. N.i. B5. ③/57 lost.

Had planned to isolate clones at n = 3–5, but let things go too long ∴ picked the middle from B4.

Thus all clones not notable except B4.

B4

11⁺, ca. 39⁻

n = 6.

A B C D E F G H I J K.

at n = 14±. 0 15 0 0 0 3 2 (n=7) 1 1 4 1

C3 C4 C5 D1 D2 D3 D4 D5 A1 2 3
n14 n19 n17 n20

F4 n25

n

These same semiclones terminate, others divide to n ≮ 6+4 = 10, but [14] not expounded fully. No later division seems to n as high as 59!

J

E1 E2 E3 E4 E5 F2 F3. H I B1 2 3 4 K
n26 n19 n30 n17 n20 n27

F5; H1; H2; H3; 114

n29 n36 n32 n32 2P21

G5 7+5 +3+4 5+ 1+1+4 2+1 +5+1

115 H3 H2 H1 8P21

n48 n33 n41 115

G3 n=? G2 n48 → n52 F2

B2-2

3/19/54.

5-6PM. Isolates from pepsin-refrigerated from 3/18. Plant at R.T. to see whether gross semi clone pattern applies here. A few previous isolates were discouraging, but on agar this combination gives the longest tracks & should be the most suitable for transplant tests.

[116] 7 cells isolated 5P19, kept at RT overnight.

A20. 6/7 were ca 10^3. C1 had 2+ to [115] H4, H5.

N21: H4: lysed H5... 1. lng.

Time Pla+ x — in re growth

1148

[1/3]

3/17/54.

A; 2:05 PM Mix 60B, SW666 (old) in c.y. R.T.
(Isolate cells singly & small groups at 2^20-2^40.
Motile cells not seen in suspension at 2:40, (3:00), 3:35
Then R.T. By 5^40, ++ motile.
No substantial increase of isolated cells, but probably did in main drops after all. Will have to be done by plate count.

DATE: March 30, 1954. REF: [118].

For past few days have had poor luck c SW666x — to find motile cells. possibly suspensions too old, used too small drops?

3/28. Ca 3 hours then refrigerated to SW666x — FA60B.

① 3/29. 2 Fla+ → [118] E1, E2. P30: E1: 0 E2 ca ⑤/20. leave as is.

② 3/30. Suspension as above. 1144 H3 × — 60B. (= trail end sib of SW666x — 60B)

Isolations from 3:55 PM — ca 6 PM. Ca 1 division during this interval for some cells. Single motile cells to drops, a few sibs separated etc. 26° overnight. [118]

3/31 — log₁₀ cells — mot. — to fate [119].

A1 —
2
3 — 2 — 1? — .
4 — 2 — 0?
5 — 2 — 2

B1 — (4) — ① → B2
2 — 2 — •
3 — 2 — • — 1 → B1 ca 50, 20+?
4 — 2 C — A
5 — 0 A B — B, C 0

(margin: mito to 3 [119])

C1 — 4 — 1 → C1
2 — 2 — 1
3 — 2 — 1+ — C3
4 — (2.) — 1+, 1−
5 — (2 swishes)

D1 — 3.3 — 4 4 — ① D1
2 — 0
3 — (0)
4 — 4 — 1 — C 4
5 — 4 — • — •

E1 — 0
2 — 4+ — K1 — D2
3 — × — 3+ — K1 — D4
4 — 3 — •
5 — 3 — 1?

F1 — 4 — 1?
2 — 4, 4 — K1 — K2 E4 E5
3 — 1.3 — 1+
4 — 3, 2 — 1, 0 — E1.
5 — 0

G1 — 3 — ca 50%+
2 — 2, 3 — 1 — F3
3 — 2.3 — 4B — F4 (all 8)
4 — 3 — 2 — F5 (7)
5 — 2 — 1?

H1 — 1, 3 — •
2 — 4−, 4− — >100+ • — K2
3 — 3, 3 — all+. — to H1, 2 (5 ± each)
4 — 4 — 1 — G5
5 —

Did not search carefully for individ motile cells. • = 0 or 1 or 2...

This expt. designed for info on swarms.

Resumm. 4/5/54. 6PM

3/30/54. 35 motile cells to [118]. 8 clones had few or no +
3 failed
19 had a few
? 1. 48/200 (118G3) →8 119F4 → 121F3 42/104... (5).

4 definite success

1. pure S variable expr. 118B4 →tto 119G3 (20)/50 → 121G3. (pure motile. c contaminant came in later)
2. pure S G1 → 119G1 → 121G1-G4. any non-motile? prob not.
3. T+S! A2 119H3, [H5, F1, F2.] — from nonmotile branch
4. Pure S. H3 → 121 H1, H3. (2 initial sites. Both pure +.

Other lines were not followed up. [120] lost P3 interrupted analysis of possible segregating lines.

picked.

1. ~~not yet analyzed.~~ pure +. 119G3 Erratic expression of +. Same.
pure S 2: ~~[illegible]~~ 121G3, G4 same motile line.
3: 119 : H5, F1, F2 non motile branch
S.T. + 119H3 } motile branch ~~D1 single cell +.~~
− 121D3

4. 2 branches: 121H1, 121H3 both motile
pure S

DATE: 3/31/54. REF:

See 1147.

SW666, overnight culture. X-60B R.T., 10^{30} — 1. (2½ hours).

~~Tube~~ Set up 4:10 — 4:45 [1120] No + seen. R.T.

5;20. No + . heat c lamp (R.T. too low now? (ca 21-22° today)

10 5:45 Rare +

beginning > numerous ca 6+ PM.

A 1
2
3 5;52
4
5

B 20
2
3
4 6;03
5 6:10.

P1. 30 Trees of 10 cells, 2 failed, 2 not+, 4 few+, 2: B1, B4: — 10-20+/300 → d. few+/10^3.
— ≤30+/10^3.

no further multiplication on further transfer.

[120] lost P2. ∴ no swarms: 2 classes moderate exp. semi-slow

40

∴ 10 cells 8 viable no swarms.

50

Integrity of 124F5 and 124H2-3.

F5. (122E4)

Initially, ① 6.

① → 10⁴.

6 → $>40/10^4$

∴ $n = 13$

Ca $\frac{20+}{20-}$ —40→ 123C2

123C2 → 10^4 K7⁺ —7→ C5, 10⁴.

$\therefore \Delta n = \log \frac{10^4}{40} = 8$

$n = 21$

R2D5.

①①① ⑤

⑤ → 10^3. K35. Ca $\frac{40}{[20]}$ → C1

n = 11

C1 → 10^4 K8⁺ —10[7]→ D5 (123)

n = 19

D5 → 10^4 K4⁺ —0→ 124F5 ②/10⁴

0, lost

n = 29

① ① → 0

n = 42

125E1 | 125D1.
4. | 40.
↓
124H2 10^4. 10^4.
or incomplete. 124H3

P6:

became contam. i.e. coli C. accident recalled (stopped e.g. too fast, drops came off).

(might be contam coli)

To be checked ✓

probably contam: check DCL

see next page

/5/54
PM

April 2, 1954
60B × 666 10:00 – 12:20 at 30°. Refr.
~~? suspensions (same as 1148?)~~ Extra 20–30 mins at R.T.?
[122] ? Start to separate some at 2–4 cell stage, but too far along!
3:40 – 4:25 isolations [122]: A1–H4. F1, G5, H5 X.
37 single motile cells + 11 pooled at Z1.

all grew. SWARMS: 122 B5, F5, Z1. ∴ 3/48 swarms.

A few large uniclones also transferred:

[122] →	[123]	✓ = uninteresting
C2	A1–4	✓
D1	A5	✓
D3	B1–5	✓
D5	ABC(D) ×35 → C1	(8)/10⁴ → D5 ×4
E4	>40. C2	(7)/10⁴ → C5 ×? {(1) → lost; (1) → [124] F5}
F4	40+/10⁴ . C3. → (34)/10⁴	→ C4 ×40 {×42 → lost; (1) → 124 – H1,2,3.}

ca 10⁴/clone.
plated using no TTS! N4. (P4 ca 10³ each) P4 10⁴ each.

B5 ∴ pure +   D1. Almost pure +.   0/22 non motile.   ~~G B[illegible]~~

F5 ∴ + , [roughs! –] pure ↑   F1. Try to separate stationary cells.

3/24 stationary cells gave non-motile clones (E1, 2, 3).
E3 —(1)→ E4⁺ Rough?
[123]

F3 pure s.c+

see 1149E.

Also set up reconstruction mixture in E5, F5 (– from C1). Seem to give Fla > Fl₂⁺.

In this exp., I had intended to separate subclones, but these were too numerous and was finally abandoned. B5 and F5 are unseparated clones.

122FY

① ③

②/10Y

K40/10Y → 123C3 (4 40/10Y) —(3Y)→ C4

n = 13.

must have been considerable late replication

no further replication

4 49 / 10 3Y —(Y2)→ lost

—(7..)→ 124H2, 3. (A-F) → 40.3

F 10³ ①

→ 125H1

n = 21

P5, some of these ①/1000 (n = 31)

n = 31

P6. n = 33 125 H2. → 10Y.

Crossovers in "trackjogging".

1149B.
C

4/5/54. 35 non-motile clones from 1149 picked to broth P4. (Ignore rare semicolonal cells). Test controls × 60C P5. (DCG). cf. early expts. on macro-trailers.

c: also test 1147-3 branches: [119] - H5, F1, F2 [121] - D3.
(numbers 1, 2, 3, 4 written above H5, F1, F2, D3)

controls OK. 60C-× F1, H5, D3 all ++. 60B-× F2 ++.

Note FA60C (22/967) transmitted each culture but also the current stocks of SW666 and SW967. ∴ not valid test.

As a repetition 60B-× 967 : 0 (most expts.)
60C-× 967 ++. Not tried to identify this by
60C. 0 type of swarm.
967 0.

Tests continued by DCG — see her summary.

Not tried 90C -× 122 series. Each gave only b; each responded

JAN 24 1955

See note above on ambiguity of "60C", supposed to be 22/967

4/5/54. [E3] E1-2-3 are clones from a few stationary cells isolated in transduction clones [122] ~~B5~~ and F5. They appear rather rough. E4 is a motile clone from a cell in E3 at ca 200 cell stage. (Reversion?)

P5: test E1-3 for motility reversion and × FA60C. (cf 1149B.

However! E1-3 prove to be galactose positive! E4 is Gal⁻.

Unless a serious error has been made, at least E3 was segregating Fla⁺ Gal⁻ / Fla⁻ Gal⁺. Could latter be SW967? Whence? (crossing?) Test on EMB lactose.

There is a remote possibility that these are W2049 = Lac⁺ S^R. Droplets of 2049 + W2438 were on an adjacent coverglass that broke in course of expt. and conceivably might have contaminated some drops! Morphology of E1 is rough rods, ∴ different from 2438.

Great care must also be exercised in removing coverglasses for exchange.

✓ Lac⁺ S^R!

N6. FA60C = 22/967. But DCG finds control × 967 gave T+S. Possibly contaminated c FA22? She is rechecking.

60C × E1-3 gave no swarms

P6 Try SW967 × SW666. Mixed culture plated gave numerous swarms! New transductive phage? (Cf FA26!) — Ask Abele to check. — Yes. supernates × 967, not 666 to motility. Source of the phage? All 8 were Gal+ pm.

Transduction of lac_3 - W435. [1150]

W1409

April 11, 1954.

Two coincidences of Lp^s c Lp-linked mutations have been recorded: W-518 and W-1650.

A. A third coincidental mutation, W435 Lac_3^- has never been tested for transduction or linkage to Lp.

B. Recurrent Lp^s should be checked for other mutations, viz. auxotrophy. Cf. EML.

A: (1) Reisolate W435 from lyophil. First tube proved to be substantially Glu- but ca 1% Glu+. Test against λ (Hft Gal_2^-) and λ2 $^{MEMB}_{Glu}$ proved sensitive to both, no indication of transduction.

(2) Do. W1409 stock now repurified (lac_1^- lac_2^-) Lp_2^s λ 2^s; No transduction.

A (1) should be repeated on repurified culture; Lp-Gal-lac_3 linkage should be tested. [Cf. W1741-1744]. (W618 = $Gal_?^-$).

Conclude neither W435, W1409 not subject to transduction. EML will check Lac_3 for linkage to Lp-Gal, & DCG also will look for other mutations coupled c Lp^s.

B) DCG isolated 3 suppressors (Lac^+ Glu^-) from W1409. Tested by Boris - none of these are constitutive-lactase.

see EML program on isolated Lp^s-auxotrophs

April 12, 1954.

Written up 5/12/54.

No motile seen at 2 hours. First seen at 2½ hours! Begin isolations 2½ hours — 3:30. Some divided at least once by this interval! 40 isolated to clones. As far as possible, separate at 2-cell stage. Most clone pairs showed very few, occ. none of motiles. Record ~~numerous~~ clones with "numerous" motile at 10^3-10^4 cells.

	134 →		126 F4 (43) → Save! F3 → F5	
A1	(1) (1)			
A2 (f) →	Swarm studied stationary also		→ F3, F5	pure swarm
E3	(1) (1) →	(4) (8)		
E4	"	(7) (4)	(13)	
E5	"	(1) (4)	G1 (20)	
F3	"	(30) (6)	G4 (2+)	
H4	(1)	(50)	H3 (24)	
G1			H4 (26)	
F1			G3 (27)	
B2			F2 (12)	

See (margin)

No — formed from stationary cells of A2 (126 F3 - F5). Save as pure swarm.

Note (since Stolon's question) most numerous swarm clones separate quite unequally. E3, E4 most exceptionally. But we should get more explicit data on congeners of swarms.

Need better method to isolate early pedigree lines.

→ × paralytics

1,157.

spont. trailers?

April 14, 1954.

22 × 578. Pool A2 – F4. [127]

3h. 30° 9^30 – 12^30. Then isolate motile cells. Most invisible.

9 invisible + 5 — 14 dead

6 – nonmotile + 4 → 10 nonmotile at 10^3.

1 5+/20 – later 10^35.

3 (2–4). — 3 c semiclones. B1, C1, E2

1 ca 30 ought + (1?) → 10^3.

1 (how?) many +. F1.

On transfer of single cells, motility very sluggish & petered out so that direct study of phenotypic delay was not successful. Roughness?

Platings:

PA 22 × 578 trailers and swarms.

60 × 578 — 1S 0T; 1S 1T.

60 × 536 — 0

534 — 0

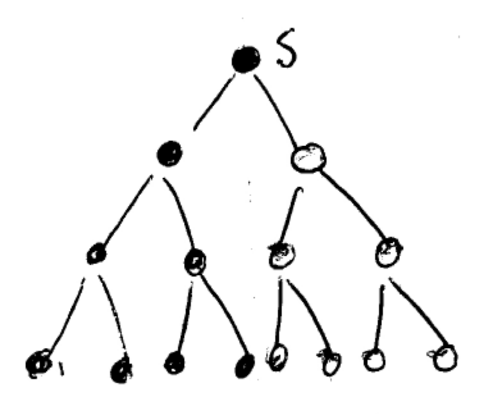
Controls:

578 0, 0, 1 sw no T.

580 0, 0

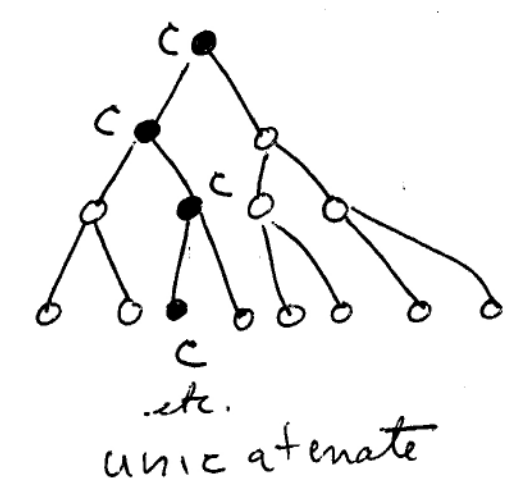
534 1 ? trailer of BHO5 note 5I colonies

536 1 Sw, 2T? relation sprinkling of sub-surface colonies.

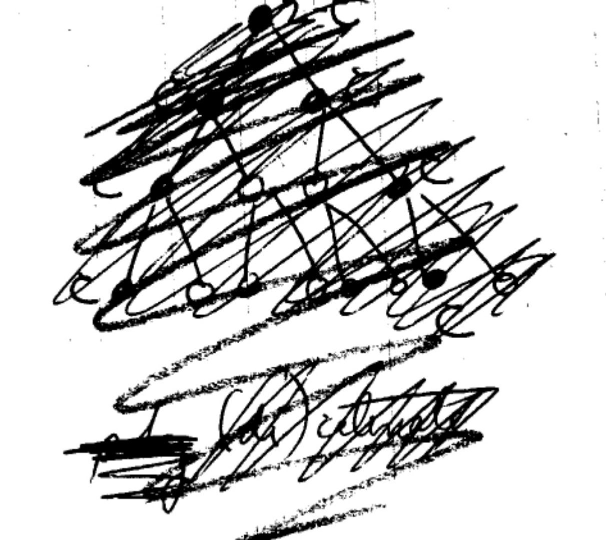


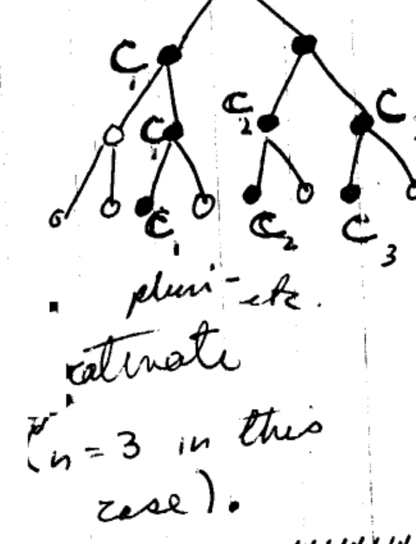
single clonal

segregational.



(single, unbranched chain)





pluri-catenate or ~~[illegible]~~

Strain C.

~~1144~~
" 1151

Note: away to ORNL week of 4/19/54.

4/4/54.

[123]. Isolate motile cells to G 2. → (grow+ P5.) [124] G1, G2.

(picks for selection on mot. agar.) Showed no motion.

W-2438 = C received from Murray for cytological comparison. Strain C W2049 as rec'd from Weigle is overtly different (SR, rough). I had set up W-2438 for possibility of selecting a more bacillary form, but after several hours noticed occasional motile bacteria. Picked these as stated. Ones appear erratically motile + stalky. Possibility of selecting more motile variants? In mot. agar, C is stationary.

N6 123 G2 in broth (37°): no motion at all. Inoc. from this to Penassay, incubate 1130AM 37°, 30°, 19°. for temperature effect.

8:30P6: at 37°: homogeneous suspension; very rare motile ±
30° rough mod. +
20° smooth fair. ++

Best chance for selection is at lower temperatures in agar ś gelatine. Use 20° culture as inoculum.
= 1151B.

N11: All cultures swarmed appreciably, 20° < others. Retransfer 30° and 37° swarms to the same, 3P11. P12: 30° showed faster spreading but still sluggish. P13 Both about ½ through tubes, 30 > 37.

4/30 4 transfers in gelatin-motility tubes, 37°.

DATE: 4/25/54.

REF:

D. Leave plating of W2441 c̄ Dot Goebring.

She has separated EMB lac 24h.

1. Lac⁻ — (v. slow + ?)

2. Stable lac⁺ pure + ?

3. " " prob +, −

4. Reversion " strong +

5. " " +, −.

Restreak these + prepare cultures for constitutive test. Hand over to Bon

0, 1, 2, 4.

DATE: April 13?/54. REF:

W2441 = "Lac₁⁻ Lac_c^c" as received from Monod.

P13 Streak out on EMB Lac., Gal. A+4: all Gal⁺. Lac:
colony types noted: separate #1 = Lac⁻ (v. faint pink at 24 hrs)
#2, 3 not pure. On re-streak

P1IV: both these lines showed sectored (slow Lac⁺), Lac⁻ colonies.

No pure + seen!

Crude NPGase test: intact cells			enzyme	
(Pine assay grown)	W1301 –	++	±
	#1	—	±
both r, –	#2,	—	±
	3	±	±	sec.

1151A. Restreak Lac⁻ from #1 | of W1427? Mal±.

B. Lac⁻, Lac± from #2.

C Lac⁻ " "

D Original W2441.

DCG purified Lac⁻ from plate D = W2455 and
Lac⁺ reversion from this (strong +) = 2456.

July 8, 1955. Resume

SRP tests on named cultures.

① Fredericq series = 776-96-108 (xW1177) 2 kept as wg 9,10

W 1377, 1395-97 x W1177

11/17/50 B/6 W1362 W1376 W1113

1/1/51 Ephrussi

? were Slopes's, other strains (W1028 etc.) were tested?

for first 1500, mostly only 1177 as parent.

→ × SW666

1212

chains

DATE: Jan 11, 1955.

REF: 214 / 217

Preliminary trials FA22 → × SW666 (TA42) No motiles seen. Recall that this shows low transduction titer (phage compatibility?)

FA37, and SW686 (induced lysate) → × SW666

Practice runs to help reorient what to look for.
(18) isolated. All grew; few gave any (1) at n = 10
See protocols.
1 gave 19 chains (vs 1 each in other subclones), did not continue to increase

1 swarm: F3 Probably pure but save for later review
Other splits: 7/4 6/3 7/2 7/1 Suggest non-randomness or mis-identification of "E" cells.

Concl. Note > 3 hours preincubation. Most economical procedure: many isolates, 2 or 4 subclones (no more!) each. Merely count chains in subclones at n = 10-15.

DATE: REF:

1 2 3 4 5 6 7 8 9 10

9-10. (Use 1ml cells diluted ~~1/10~~ 1/10 + .1ml phage 1/10.)

add .1ml phage + 1ml cells. Add 10ml broth. Spot ca 5 x 10^{-4} ml per sample ± add ~~fluid~~ broth about 3 x 10^{-3}. (9- 10+)

cells are 36h. SW666

No Tor S!

10

Recalibrate.

11-14 = .1ml FA37/1ml SW666 (old; dil. 1:1 c̄ broth) and 3 decade dilutions in SW666! Use flat drops, ~~pipet~~ loop D.

1/24		Mot agar		(.4%) agar	
	11	0^5 15^2 2^2 5^1 /8.		NM fall through agar	1 or 2 S/2
20	12	0^6 1T^1 2^2 7^1		swarms would be ill	0
	13	0^8		defined.	0
	14	0^8			0

JAN 24 1955 Plates reincubated. In 5 plates each of 9, 10, x ca. 10 spots each (100) only 2 swarms (late) no trails.

30 Note, however numerous poorly defined clusters under surface growth. Incidence should be compared c̄ SW666 s̄ phage and prolonged incubation!

A24 40 15 micro tubes etc of .4% agar c̄ ~~dilution 11.~~ (.1ml FA37 + 1ml SW666 (old)). Spot loop D.

50

FA37 → SW666
quantitative: ratios of Trails: Swarms

DATE: JAN 19 1955 REF:

1	2	3	4	5	6	7	8	9	10

Old susp. SW666 (Refr.).

A. 1 1ml SW666 + .1 ml FA37 . Test loopfuls (Dini's loops) on motility agar. Preliminary test to define range for counting T + S, and distribution of T.
2 .01 "
SP19 3 .001 "
4 .0001 "

JAN 20 1955 : Plates somewhat smeared.
1: Each spot swarmed.
2: 7 swarms / 7 spots
3 1 swarm / 7 spots } useful range?
4 No swarms: contam? }
wait for trails.

~~c fresh SW666 { SBAM pedo tube #4 and compare with 10-3 dilution of tube #1 (=5)~~
~~(difference in number of cells.~~

Use $1/2 \times 10^{-3}$ as standard level.

∴ ~~5 = 5×10^{-4} ml FA37 (in 0.1ml) + 1ml (fresh) SW666~~

6 = [(.1ml SW666 + .1ml FA) $\times 10^{-2}$] .1ml + 1ml broth.

5 = [(.1ml broth + .1ml FA37) $\times 10^{-2}$] .1ml + 1ml ~~broth~~ SW666

allow 6 to stand 20 minutes for adsorption.

1/21 7 ≐ 5.
8 ≐ 6

Mechanics

JAN 19 1955

1. Bruce affirms that isolated motiles do not form ~~chains~~ trails
Possibilities
(1) Only polycatenate cells form trails. Why no more branching than is seen?

(2) Any cell may form a trail *if it is active enough* and if it gets into agar.

Tests: (1) What is ratio of trails to swarms?

What is ratio of 1- catenates to swarms?
poly- "

(2) Get a ~~swarm~~ trail from a chain of intermediate n to be sure it is unicatenate.
May need to develop tactic procedures.

(3) Distribution of trails should not be random.
(Trouble: testing interfered by swarms. Use tubes? plates? spot plates? (not deep enough)

Try 37–XSW666. Y diluted c cells, broth, phenol.

Linkage. (Salmonella)

JAN 19 1955

JAN 1 1955

1. Discussion with Zinder: search for Fla⁻ - linked markers, using Demerec' lysates, and prospective search for new markers.

2. From Bruce's letters etc., H_1 linked Fla⁻ are:

SL13 = SW1048 para A.*

SW543-666 pB

SW553 - dublin

SL28 = SW1092 = heidelberg

* SW544 = Schwetze O

* SW966 (I have as b)

* rather poor; others are monophasic except SW1092.

3. Last spring (1130 ff.) looked for linkage & complementary crossovers as bases for pedigree analyses and thereby got into chain study.

Recollection of some trials with SW1092 but cannot promptly find the notes on it. Should repeat to evaluate suitability for linkage study (c̄ Zinder), & of allele Brustini.

DATE: 1/21/55 REF:

5: 2 6cm plates : 5 spots, 5?, (A, B / C, D)
2 10cm plates: 17 spots, 15 spots.

A. 0 swarms, smeared no obvious T.
B " "

D 3 swarms. , ~~0, T~~ 0^{12}; $(1S\,1T)^{13}$; $1S^{14}$; $1T^{15}$
Note: D13 showed numerous flares; motile cells obviously entered at various points.

maybe too fluid!

C 0^{15} 1S; 1T

6: A 6cm plate 0^4 (1S,T) (~~23~~2T,S) (1S)(1T) 8
B 0^3 $1S^6$ $1T1S^1$ 10
C 10cm 0^4 $1S^3$ $1S1T^1$ $1T^5$

one of these has multiple flares, maybe two swarms?

Totals		5	6
	O :	27	11
	S :	2	10
	T :	2	6
	ST :	1	3
	Σ	32	30.

T, S appear to occur independently; note much higher incidence of both classes in 6 of 5. 6 is at very low multiplicity. Note also the overall low incidence of tracks! Any earlier data?

DATE: 11/22/55 REF:

A22

7: A (pre "dried" plate) 0^10

B	0^9	47-0	37-0
C	0^10	2-T	2-T
D	89 (1T)		
E	89 1T		39

8A 0^9

B	0^10 1S multiple Haves	41-0
C	0^11 1S	5-S
D	09 2S 1T	1-T
E	0^11 1S	47

again note 8>7; reduced incidence of yesterday! Independence of Trails but low number re S is disturbing. Cf. earlier data.

Note; present expt entails large fluid volume + potential chains might proliferate without ever entering agar. Cf effect of inoculum volume

These experiments used loop B (Dino's) whose volume, full, is about .00351. loop D, full, is about .0023; flat = .00073; retention .00022, delivery from flat: ≐ .00051 Call this 5 × 10^-4 ml, and dose about 10x conc of phage over previous expts.

DATE: 1/20/55 REF:

1	2	3	4	5	6	7	8	9	10

(1) Plate ca 15 SW666 pup plate motility agar pour. c/s underlayer of NSA. In each case, discrete colonies with no doublets or spread arisen/formed.

1/21/55 add FA37 0.1ml to 1 plate: colonies still discrete d/4 A22.

A25 No swarms. colonies becoming more radiate; initially rosy to spheroids (in all planes).

1/20/55

Comments: Work to date seems to have emphasized the prolongation of chains rather than distribution of their sets! No trouble should be taken now with settled chains but search instead for continued lines of increase.

For ms, note we can detect division but not (directly) multiplication. Present data do not define where units are between gene and final effect.

TRAILS: Quant.

JAN 24 1955

901 FA24 → x 666 23T: 6S

975 FA22 → x SW666 12T/15.

∴ In sum SW666 x ← FA12 38T: 3 S

x ← FA22 42T: 3 S

x ← FA24

Review data on trails

JAN 24 1955

Impression that T>>S in many cases. Is SW666 exceptional?

—X967 gives great excess of trails.

See 1033 segregation.

Note "C" T not very numerous of S. "bb$^{or}_{\wedge}$ c" seems numerous swarms, no trails.

—XSW1048 120T: 10S.

See 999 T/S ratio of UV treated FA12 — UV$_0$:
29T : 0 swarms!
9T : 3 swarms!

"dinsin Ögar".
Has SW666 changed? Or is FA12 ≠ FA37?
of SW543?
c UV, remarked that 13T : 14S! difference?

Use SW666 trail!

997: FA21 —X SW666 41T : 10S 5/7 bp$^+$
FA22 —X SW534 30T : 2S

JAN 25 1955

Salmonella Comment.

Spent the last couple of days reviewing notes on Salmonella.

Many problems are left in mid air, e.g.

(1) Monophasics (
(2) H_1 duplications
(3) Fla⁻ mapping
(4) Specificity of transduction (by localization)
(5) phase variation; exhaustion;
(6) other phases → x
(7) Pullorum
(8) Kunzendorf c
(11) lysogeny-protection (coli proved better)
(12) heterogenotes (SW 686?)
(9) Misc H_1 → x monophasics (z_{28}; lw;)
(13) paralytics.
(10) backcross data
(14) especially chains.

Some of these are partly tackled

There is little in notes of immediate relevance to problem of trails except some inconsistencies in T:S ratios. See other comments for this and for comparison c pedigree data.

Trails: incidence

JAN 25 1955

, repeat comment that →x 967 gives many, long trails.

But has SW666 changed?

note: 999 FA12→x gave 38T : 3S
FA12UV →x 13T : 14S.

BADS claim → × SW541 [2×10^{-3} T/cell!]

.05 ml FA22 (ca 10^{10}) gave ca 500 papillae!

973

$\therefore \frac{500 \times 20}{10^{10}} = \frac{10^4}{10^{10}} = 10^{-6}$ papillae per phage.

BADS claim: 1 ml FA = 5×10^6 T ! and 1/5 as many swarms!

$5 \times 10^{10 (?)}$

SW541 is F.K. Copenhagen FK223
SW665 is stated to be Xyl - deriv.

JAN 26 1955

Note – BAOS remarked that

~~[illegible]~~

534 — × 553 gave T>>S (1T/17,000 particles!)

LT2 — × 541 many T, S. (claims 2×10^{-3} T/cell! 4×10^{-4} S/cell)

Try microscopically!

compare [illegible] yields. Any other markers for SW541? See notes. Ought to use 553 to demonstrate tracts from isolated motiles!

(consult Andy or Clive)

JAN 26 1955

M S comments.

Sw 54/1 x — TM2

3a. T > S. "several fold". counted 80-90 cols. in 15h. 37°.

∴ delay.

4b Trouble shooting (squirt oil at them.) too much interpretation.

4g "expts in wh few T/plate majority were single" — data?

4g he states adequate hyp. "prob. small".

5a earlier expts a & b; later 24 isolates: 1 n.g. 1 sw

15 < 10 7 > 10 (generally about 1/5 are "E" cells").

5c 8 cells isolated: sptsome 1:0 2:0 2:1 2:1 2:1 6:3.

30:3 44:3
E E

States random separation from non-E's how tell?

∴ E's catenate also.

6c how can n ≥ 10

7c ! ! critical

7d he has isolated E cells at 9th-22d generation.

→ x SW967 pul.

DATE: Jan 27, 1955 REF:

p26 FA22 ~~10⁻³ 1ml~~ .1ml + 1ml SW967. decimal dilutions in SW967.

loop D. ~~#1 ... tails/spot~~ #2: ~~...~~ spots: $0^4\ 1^2$
stokes $0^1\ 1^1\ 3^1$

#3 1T/10 spots and stokes #4: 0

A #1: spots $0^3\ 1^1\ 3^2$ sic
stokes $0^2\ 1^1\ 5^1$

~~FA~~50A

B ①: 11 spots S+3T ^{2} 1^2 ca5 ^{4} 2^3

② 8: $1^6\ 2^2$

③ 8 spots $0^7\ 1^1\ 2^1$

---

p27 Various media. Use FA50A, tube 2, flat loop D.

C

D = tube 3. #28, many trails appear to have ~~up~~ from ~~to~~ 30 to as much as 54 colonies per trail. (5^{30} - 9^{30} = 16 hours) which appears to be in excess of generation time.

DATE: REF:

1	2	3	4	5	6	7	8	9	10

A29.C
1 Motility agar standard
2 " " .30% agar 2 brown
3 " .35 blue
4 " .40 red
5 " .5 green
6 " .6 yellow
7 " .40 no gelatin.

C7: clusters (c side chains?)

5 spots like this; 2 ; 2 have no lateral clusters but numerous colonies under main spot

C6 or "clusters" under spots, short and small

C5 clusters, larger than 6

C4 not so large colonies nor as extended as C7. Interspersal of small and large colonies suggests minor branching. In general has appearance of concurrent clusters rather than single trail. A few possible initial branches but hard to interpret owing to numbers of swarms.

C3 swarms have more linear, or multitrain appearance. appear more restricted than 4.

DATE: REF:

1	2	3	4	5	6	7	8	9	10

C2 - numerous trails, somewhat fuzzy. # Clusters rather than linear origins. #

C1 Trails shorter, not readily diagnosed whether linear.

Conclusions 0.4% agar alone gives most extensive trails but somewhat fuzzy. Gelatin definitely retards motion. but 0.3% agar + gelatin though it has smaller microcolonies also shows up pretty well also. Considerable question on unilinearity of trails but will have to be settled by more precise methods. It is very difficult to say whether one trail or two.

D. Distribution of T's:

plate	0	1	2	3	n	SW.
1	8+2	5			1 polyc.	
2	5	4	3			
3	1	5				
4	8	5	1	1	4:1	
5	8	4	2			
6	5	6	1			1
7	5	4	0	1		
8	5	5	1			
9	8	4	1			
10	6	6	1			
	63	48	10	2	1	

ignore smears.

Total: 1 swarm.
68 T
126 spots.

some definitely nonlinear:

(over)

some zero's have minute colony (ies) just under surface.

Exp. 63 48 10 2 1

$\rho = \frac{68}{126}$

1216d

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

E same dil as D, spots on agar c/s gelatin:

$\dot{s}$ = 1,2
$\dot{c}$ = 3,4

1. 4T°/30 v. thin agar
2. 6T⁺/18. somewhat fuzzed but clustered, not charmed.
3. 3T' 1T²/18 more "linear" (more overgrowth, tighter gradient)
4 9T'/24. ; 1 "cloud" - reincubate

10

20

30

40

50

8a - presentance of chains. JAN 27 1955

10a "only E cells initiate trails"

Poisson formula applicable only when drops are of equal size + number of bacteria! Calculated 15 isolates and 3 trails $\doteq$

11/51 E cells.

10c I have seen groups but would interpret them differently

10d smeared.

Discussion - growing point.

Bruce found 3/15 motiles → trails

whatare the obligatorate initials?

—x SW-967
trails to agar

FEB 5 1955

1217

/216/

50B—x SW967 (fresh). 12^{30}-4^{40}.

FEB 5. motile cells fairly numerous. Let form single clones; H6 divided= a,b.

N6: examine for chains. Isolate as many motile cells as possible, and transfer these directly to ordinary motility agar, as individuals (B), as well as mass transfer from residual clone. (A)

.4= 10^4

Isolate		# of Fla+/-	A: Trails from clone (G / T)		B: pltd.	Growth, trails from singlechains.
A-6		0/.4	✓	0		
B-6		1/4			B4 1:	1g 0T
C-6		0/.3+	✓	0		
D-6		0/5.	✓	0		
E-6		0/4.	✓	diffuse		
F-6		22/.4	✓	diffuse	F1234E34 22: (10+12)	13g 0T
H-6	a	6/4	•		H124 7:	no T. How many seen? several same 42
	b	1/.4	•		H1245 1:	0
A-5		1/.4	✓	●	A4 1:	ng
B-5		4/.4	✓	0 long diffuse trail	B3 4:	3/4g 0T
C-5		4/.4	✓	1	c4 4:	1/4g 1?T seen
D-5		13/.4	✓	1 diffuse trail or clusters	D1234 13:	8/13g. no T 1? seen
E-5		n.g.				
F-5		0/.4				
G-5		0/.5				
G-6		7/.4	✓	3? scattered cols.	G34 7: (5+2)	4/7g. no T.

• not planted (picked residual residuum by mistake)

Incidence of trails in column A vs B probably reflects chemotactic stimulus from neighboring Fla⁻. Compare inoculations with and without added SW666 (for Fla⁻ medium)

Recovery of cells ≐ 30/53 > 50%!

(over)

Previous expts had been indecisive, thought it better now to transplant directly to determine ① how many trails per initial and ② role of taxis.

C1: 1 grew

5 colonies below again!

note C5 also had longer trail!

D1234 8 grew most 0, also

went downwards definitely

1218

FEB 8 1955

226

Plate subclonal cells ē SW666:
. 5 = group of 5 Fla+ 1-3 ē SW666; 4-6 s̄ O

4-6: 2 prolific T #3 1 T.

Add droplets to spots m motility agar ē SW666

No def. advantage of plating ē SW666!

(crossing?!)

	spots	T.	
G1	6	0	swarm
G3 } G4 }	4	0	1 has 8 scattered subsurface colonies

A2	2	all 0
6	2	
B1	2	
3	2	
4	2	
6	2	
C1	2	
4	2	
D4	2	
5	1	
E1	2	
3	2	
A1(x)	1	

	sp.	T:
F3	4	0
F4	4	0
5	4	0
G2	4	0
3	4	0
4	4	0
5	4	0

swarm m plate (contam?)

A3	4	0
4	4	1
5	4	
B2	4	0
5	4	
C2	4	
3	4	0
5	2	
6	3	
F1	2	
F6	1	
G6	2	0

(smeared)

H1
2
3 ea'l evolve 0
4
5
6

	spots	T
(swarm)	1	1
D1		
2	4	0
3		
E2		
4		
5		
6		
F2		

35 + 28 + 38 + 24 + 32

"157 s.c.i"

This proves a poor method indeed, only 1 clone each.

Misc: Serratia from D Stock A
Cl_a^R for Lichstein B.

1218

FEB 7 1955 & pre

A. W2745 = Edwards ~~55~~: 184 typed as "184 - Serratia marcescens" 1/27/55.

B. Prel. expt c̄ Cla 1mg/ml n.g. ditto Clam (chloroacetamide). Both sterilized in autoclave; former buffered c̄ $NaHCO_3$.

2/8/55: 10% sols Cla, Clam; 20% Clh ($Et<^{OH}_{Cl}$). —ml/20ml NA plate.

24h.

ClA:	.2 ml heavy growth	ClAm	.2 overall background numerous papillae + ~~outgrowths~~	ClH	.5 ml: n.g.
	.4 ml sparser, no pap.		.4 few colonies in heavy bush.		1 ml: n.g.
	1 ml NG		1 n.g.		

→ W2754

Remutate.

~~Isolate~~ 1218 B 1 from ClAm .4

Recodated and found to be:

	Glu A	G	Form A	G	Mtl A	G	Stl A	G
W2754	+	−		+	+	+	±	±
W1485	+	+		+	+	+	±	±

See letter to Lichstein FEB 12 1955

See also notes on papers by Grey, ES Penfold + Harden.

(over)

Av (see notes 180, 188) formate glucose EMB.

.4% – 1% formd n.v.g. ~~S. typhi – OK~~

S. wichita ± – ~~test~~

S. typhi – only weak ±) not sharp enough

difference. Suggest DCG worked out.

From platings on Cla, ClH test a few cols: all glu: AG+.

Chloroacetate soln – is probably decomposed on autoclaving

while ClH was not evenly mixed – gave pass purifying minoids.

Might try lower concentrations or purer material where F-G screening

method is worked out.

APR 22 1955 Send Ewing

APR 20 1955

W 2745

1218C1 = aerial cont

1218C2 = Johnson's strain 80K

3/28/55

DATE: REF:

1	2	3	4	5	6	7	8	9	10

~~wet~~ weight : 310 mg

suspend 310 mg in 10 ml dist H_2O = 31 mg/cc

dilute 1:10 = 3 mg/cc ; plate 300γ #1 plate 0.1cc onto NSA

dilute 1:10 = 300γ/ml ; plate 30γ #2 plate 0.1cc onto NSA

dilute 1:10 = 300γ/ml ; plate 12γ #3 plate 0.04cc onto NSA

dilute 1:10 = 30γ/ml ; plate 3γ #4 plate 0.1cc onto NSA

dilute 1:10 = 30γ/ml ; plate 1.2γ #5 plate 0.04 cc onto NSA

dilute 1:10 = 3γ/ml ; plate 0.3γ #6 plate 0.1cc onto NSA

dilute 1:10 = 3γ/ml ; plate 0.12γ #7 plate 0.04cc onto NSA

dilute 1:10 = 0.3γ/ml ; plate 0.03γ #8 plate 0.1cc onto NSA

10^6/ml original → 1 on # 8 $10^7 \leftarrow 10^6 \leftarrow 10^5 \leftarrow 10^4 \leftarrow 10^3 \leftarrow 10^2 \leftarrow 10 \leftarrow 1$

10^8/ml original → 100 on # 8

incubate 1 series at 37°C, second series at 30°C

after 2 days

No	30°C	37°C	average	#/mg
1	too crowded, i.e. nearly confluent			calc
2	– Not counted			calc
3				
4	~680	~784	~732	2.4×10^5
5	298	267	282	2.3×10^5
6	80	71	75	2.5×10^5
7	29	28	28	2.3×10^5
8	8	8	8	2.7×10^5

2.4×10^5 bacteria / 1 mg wet weight

no definite coloration yet (2 days)

replicate onto EMB lac for proportion of "coli"

Total	ferm	non	% ferm	
29	11	18	11/29	40%
68	19	49	19/68	28%
8	2	6	2/8	25%
28	4	24	4/28	14%

lac + (E. coli) 24% of population

133	36			27%
225	61	164	27%	
455	97	358	22%	24%
813	194			

no definite coloration yet (4 days)

50B —x SW-967
transfer motile initials to mot. agar

FEB 8 1955

227

The primary purpose of this experiment is to evaluate to addition of extra SW-967 cells to the explants, and to estimate the fraction of trail-forming clones per initial.

50B—x SW-967 9AM-1130 AM. Concentrate mixtures and trap. (This procedure works very (SW-546) well. Its main limitation is that 30-60 minutes are needed to entrap the motile cells.)

A1-F3 were collected to about 12:30, deposited no later than 12:50.

After lunch, collect to about 2 PM, and deposit F4-H6 (2/~~xxxx~~ square) by 2:10. At this time, earlier isolates <u>were mo</u>stly 2-celled.

Ca. 3 PM, transfer isolates, at random, to motility agar, either alone or with supplement of cells of SW-666 or SW-967.

FEB 9 1955

		No. of transfers	Grew	Trails
Series A-F3:	—	7	6	0
	SW666	7	—	0
	SW967	6	—	1
F-G-H	—	10	7	1
	SW666	10	—	1
	SW967	10	—	2

Totals:			
	—	13	1
	SW666	17	1
	SW967	16	3

} 5/50 transpl. (46).

cf 1218: 1/157 transpl.? why? Is SW666 inhib.?

Result is indecisive owing to small numbers. SW967 might be worth making a bal- mutant in.
Probably were at 2-4 cell stage when explanted.

This test had been suggested by the result in 1217A where 10 <u>clones</u> had given 4 trails, whereas isolated cells had given fewer or none. This should be repeated by direct comparison: let cells form large clones. Examine for prevalence of motiles but do not isolate. Explant in divided samples. Compare clones with initial transplants.

single cells: trails

1221

FEB 9 1955

230

50 → XS@ 967 9^{30} — 11^{45} — 12^{35}

Collect individual Fla+. Explant series A C E G

Let remainder form larger clones overnight.

Series	A		A'		C		C'		E		E'		G		G'	
	0	0	−	−	−	0	−	+	−	0	+	−	0	−	−	+
	0	−	−		0	0	−	−	0	0	−	−	0T	−	+	−
	0	−		+	T0	0	+	−	0	−	+	−	0	−	+	−
	−	−		+	−	−	⊕	⊕	−	0	+	−	0	0	−	−
	T0	−	+	+	−	0	+	−	0	−	+	*−	0	−	+	−
	−	0	±	−	−	0	−	⊕	0	0	+	+	0T	−	+	−
	0	0	−	−												
	−	−	+	−												
	−	0														
	0	0	−	∓												
	0	0														

0 grew

− n.g.

T trail A1-5

34 clones viable:

4 trails

In A' .... 24 viable. Total isolated = 58 = 34 + 24 (accidental concurrence!)

Note general reciprocity between A, A'.

Unfortunately a, b not precisely distinguished here; probably inverted

In second part of experiment, clones were examined and transferred in multiple drops to motility agar

+ = clone grew

⊕ many Fla+

[struck out]

In this series, 48 cells isolated, each was viable (sic!).

Motiles detected (probably some more): ① ① [struck out] ② ② ② ③ ① ① ⑳. Clones were about 10% each.

If at least 10 cells are needed for "E" type, then E = 1/48.

Detectable motiles after 13 generations = 9/48.

② Although none of these gave trails, the apparent incidence would be about 4/34 ≈ 6/48. >> number of clones with ca 10 motiles!

No swarms seen so far. (?) — This expt was partly spoiled by motile (contaminant?) in second part.

Results: (over)

FEB 15 1955 — Despite much labor, the expt. was inconclusive. Why no trails from the second group? Intent was to look for >1 T/cell clone. This seems patent from appearance of the trails in part (which appear to cluster or to flare out unlike earlier impressions (of other systems?)

→ × SW967. 1222

FEB 16 1955

Note 2/12, 2/15 failed to get any Fl+ from "SW967" (= ? single colony isolate). 10 → × 9 → × 50 → ×

Repeat, cf. "stock SW967" c this isolate.

FEB 16 1955 φ + cells 9 35 AM —

A [231] → Picked as single cells (probably many at 2-cell stage) to Motility Isolation Agar. (MIA) ca 2×48=96 picked to two plates.

B [ ] → clones (small droplets).

FEB 17 1955 A: (2 plates). Unfortunately MIA > 8 days old & probably too dry. Colonies totalled 44 on first plate (sic!) [How so high?] only 1 trail = B3a fairly strong and 17 on second plate, 1 trail H6 (several mots.)

(Results not very telling pres. owing to the agar.)

Totals (note discrepancy - medium diffusion? - or does the pos. that some of these were non-motile - seep. cells) bear. On second plate, viables were: E6ab, F 1a, 2ab, 3a, 5ab 6ab G 2b, 3a, 5ab, 6b. H6ab/.

C. P.L. also plated loop + ~~...~~ up p samples of FA 9, 50 → × SW967

Nothing trails seen (pres. agar!); no dissociating. pr tote: from 50 → × 1T, 1 swarm ~~spot~~ (sic; i cluster!) per 13 loop spots and nothing else. Save swarm as 1222C1.

D. Note "sci" suspension proved 'lysed-looking' and not further sens. to PLT22. SW967 and SW1139 are lp3. Store "sci" (see top of page) as 1222D1. Spend no more time on it now: it may merely be contaminated.

~~Summary of special stabs.~~ (1224)

Plate → × SW967
Spreading

FEB 17 1955

50 → × SW967 Usual routine.

Collect ca 50 Fla+ -100 in ~~100~~ ca .05 ml ~~H2O~~ broth, plate out on (old) ~~HC~~ MGA and MA no G (spread .01 ml samples)

	Colonies	C trails
MGA	1	0
	5	3
	6	2
	12	5
MA	7	0
	3	0
	4	0
	14	0

This was remarkably successful if each colony is of single cell origin! Does spreading influence the agar? (Can be directly tested). Should be repeated on a larger scale with fresh agar.

Save 1 trail-forming colony as 1224-A

5D-XSW967 1225

Effect of spreading etc

FEB 18 1955

.5 ml + .5 ml $10^{15}-12^{25}$ 37°.
Then R.T., Centrifuge, decant and add 0.1 ml broth. hold in Refrig. for subsq. use. (1057PM).

3^{30}PM Isolate Flat -2/15
500 (sic) isolated. transfer to 0.1 ml broth. Estimate final density at 2500/ml.

A). Effect of spreading. (Use loop D) etc.

see next page

FEB 19 1955 B) 96 micropipdrops left under oil → 14 clones. (+2?)
plate these on pres. spread agar. Microscopic survey,
2 clones were noted as ~~having~~ plurizational (ca 10?)

Fr. an est. of drops sampled once-2, 2/8 drops have cells.

14/96 in drops under oil.

A20,23: 14 were streaked out (5-10 drops) c̄ micropipette on 1 plate. ~~altogether~~, only 1 definite trail; some dubious isol. colonies.

2 clones (ca 10^{6+}) were spread out on ~~~~ MEA plates
1 clone gave about 6 isol small dry colonies and one cluster of 5-6

1 clone gave some indefinite isolated 1 colonies, and some definite but unimpressive: 1's: 8 2's: 5 3's: 1 (If these were collected together they would probably be more impressive.

1225

FEB 19 1955

Sat. The collected sample was used in various ways, partly directed by smearing of the plates. From yesterday's result it was wondered whether spreading the agar altered its surface to encourage tail formation.

1. Old plate 5 loops (D) then spread: 13 colonies, no T.
∴ est 2.6/loop

2. Fresh (poured Thursday) .01 ml, spread:

a. 6 tails 42 colonies (smeared).
2 " 48 " fairly discrete.

b. .01 ml not spread. (allowed to unmoor)
4 tails ? colonies (smeared)
35?
.02 ml little
5 tails badly smeared.

3. Spots (from micropipette: est ca 1 cell/4 spots ?)
100, not prespread - colonies? (smeared)
only 4 tails (per est. 25 cells).

pre-spread: 48 spots → 13 colonies
6 tails !

" 43 spots → only 3 colonies
0 tails

∴ nonrandom distr. of cells in pipsamples certain

sep. colonies per loop as noted:

8 loops. prespread 5, 1, 2, 1, 3, 2, 3, 1, 0, 2 = 20 cols.
1 T

4 of 1. < 8 " not prespread. 3, 0, 2, 0, 3, 0, 2, 3, 1, 2 = 16 cols
3 T.

[illegible] tail at 16 h.

How account for so many discrepancies:
extreme variation. gave T/C

1. Old plate, spread 0/13.
(Error by loop.).

2. Fresh plates spread
2x .01 ml . 8/90
Unspread
.03 ml 9/? (assume 135)

3 Fresh plates, pre-spread & incor.
with A) loop 1/20C
B) micropipette 0/3
6/13

4. Not prespread
A) loop 4/25?
B) mp. 3/16

No clear effect of respreading.

Estimates per loop agree:
13/5 20/8 16/8.

How about null fraction?
= 3/8 for 16/8 mean.

∴ ca 45 cells per .01 ml
(> estimated 2500/ml)
and makes this loop now
ca. 49/45 (21) × .01 ml
≐ .0005 ml [< former estimates]

Note extreme variability (+ sampling?)

~~Loop~~ mp. content est at .25/drop
∴ = .25/45 × .01 ml ≐ .00005
$= 5\times10^{-5}$ ml.

FEB 21 1955
(Mon.)

Collected 89 motile cells from same conc. suspension as 1225 (refr. over weekend). Transfer to 0.2 ml broth for plating exps. (Transfer directly from pipette, in two rinses, this time). Various platings.

1. Spread on MGA. (yellow = Fri poured; wh = Thurs.) 0.01 samples.

FEB 22 1955

	Colonies	T
Y	37	7
W	46	7
W	55	5
W	50	6
Y	–	4
Y	–	9 + 3 wh.
	138	38

∴ mediums are not different. Average T/C

$= \frac{38}{276} = .138 \; (\doteq 1/7)$

of 1225 = 8/90 ? 7/41.

Take 1/8 as rough average

2. Pour in MGA. 0.01 ml

a. thin layer, then cover	23	5
b. 1 thick layer.	38	5
	~~23~~ 61	10

all dup.

5 colonies had reached surface
2 c trails

TRAILS ARE V. INTERESTING.

3. Spread .01 ml c ca 10^8 SW967. → 9, 17 trails, many are very weak. Not unlikely the weak trails are provoked chemotactically.

4. Spots (loop).

4. Spots (loops) c/s prespreading plate surface.

		pres. C	T	s pres. C	T	
~~a. w~~						
a	w	3/4 (20)	1+1S		5	1 swarm on pres.
b.	w	~~10~~ 10/12	1	13/13	2	— 1 def. branched? Reincubate
C		(+ POH. .05%/drop)				
	20 spots laid 2 def. blank.	18	4	(20)	13	
d.		4/4	1	3/4	0	
	large p.p. drops	3/6	0	5/7	2	

1/c smeared from DOH side to other! may have carried rols. along.

no eff of DOH? altogether this plate shows 17/40; cf. 10/65 above. $\chi_1^2 = 7.4$ average cells ca 1+/loop $p < .01$

Try in shake tubes

Reincubate after R.T. 2 hours for qual. exam. fails.

In (2) 1 trail = 125 colonies at 17 hours!

FEB 27 1955 Some errors noted in the dupplatings too, though not long incubated.

FEB 23 1955
FEB 23 1955

Problem: ∄ 1 trail per clone? Would need to test clones of 10 - 100 cells.

A. T/C init = ca 1/8.

I

Approaches.

1. Most rigorous: Isolate single cells, let form clones and transfer individually. Too laborious!

2 A. Isolate single individuals. Transfer as singles to broth - tubes. Let grow to size n. Plate out

B. Let singles form clones before transfer. Then plate out. (Here uncertain what fraction of clones have developed although more clones are represented).

For this general approach 2A seems best. Can be contrasted with immediate platings of numerous inocula for concordance of ratios.

FEB 23 1955

II FEB 23 1955

Isolate motile cells but not singly. Plate out initials for T/C values. Dilute to samples of <u>how many</u> cells and let form clones. Plate these out at clone size <u>n</u>.

How many? if $\ll 1$ then most samples will be wasted

if $\doteq 1$ then expect only 1/8 to have an initial, though no independent check on density.

if > 1 then too high expectation of coincidences

III. Methods of plating?

1. Spread — restriction on volume; may get away c̄ respreading
2. <u>Pour plates</u> } Try these now.
3. <u>Shake tubes</u> }

plating SW967x— clones. 1227

(see 1237 summary)

FEB 23 1955

P22 Mix SW967 .5 ml x— .5 ml FA 50 1[45]. Then Refrig.
1224-A "

A23 Concentrate ca 10x for trapping.

11 AM drops fused.

try water? broth? buffer?

PM — A very many Flat were formed. Maybe too dilute some suspension or otherwise. Both tried. Altogether, ca 40 ⊕ were isolated singly.
Group A isolated ca. 12 N, → 2, 4 cells at 3 PM
B 2-3 PM → pres. 1 cell.
at 3-4 PM Isolate to 1 ml broth each
7:30 - 8 PM Plate in pour plates + shake tubes

A. tubes only	4 18mm (ca 20 ml) 4 13mm (ca 10 ml)	all had clones no trails. Minor trails seen in each but only near air (oven)

B.		plated	clones	blank	Comment
(B4)	~~tubes~~ plates 7	8	5	3	all show "minor trails"
(B1-2-3)	10 mm 3	9	7	2	all but 1 ? (B2) show numerous "minor trails"
					no ~~[illegible]~~ major trails (12 clones)

photographs at about 48 hours.

shake tubes probably OK for
major trails. For 16 hours, no growth
gradient. Later, colonies grow larger
near air and minor trails mostly seen
there.

---

13 tubes 7 large (20ml) - 2 blanks
4 10ml - 1 blank

---

1 head

clones ca 500 - 10^3 each!

So only 1T/20 clones! but note minor trails also.

FEB 23 1955

3[10] – 5[00] PM SW967 (old) .5 ml + .5 ml FA50. Refr.
ca 7 30 PM Enc. (ev home) in centrifuge. Refrig. when
not available.

8[55] traps set up.   By 10 PM all rel. few ⊕. Isol
c .15 + 2 + 2 and pour in shelsetube.
= (27C)
Yields → 14 clusters – all flowery tails!
+ ? swarms.

1228
i

FEB 24 1955

Stock of 1227 C. — 4.9. New stock

Incl. 34 tubes (10 ml) inadvertently left in cold water P24–A25. See 9A25 –

Clone procedure:

(1) Stock c̄ mottles ca 2 hours mix fresh cells & phage, conc. in centrifuge ca 10X. (Takes 2½ hours). Then ~~fuse~~ set up on c.g. for manipulator and set up trap drops. Freeze. Takes ca. 1 hour more to find many mottles. Thus expts. usually begin at ca 3½ hours! May keep "stock" after concentrating & store in refr. as indicated

(2) Collect up to 100 mottles. (A) Plant singly in drops (usually now in line on unmarked coverglass. Then promptly pick up from oil chamber with quartz pipette to .5 ml vols. of Pinassay. Incubate (3 hours at 37° OK)

(B) Deposit ca 100 cells directly in .5 ml broth from uppipette (removed from chamber) – (mount in syringe on stand & move the receptacle tube).

Add 10 ml MGA & pour plate at indicated time.

In last few weeks have been using hypodermic needles, syringes & coupling hardware etc. for convenience.

FEB 26 1955

Reexamine plates.

N26 Practically every plate has occasional colonies with 1-3 satellites (minor tails) [sketches] or [sketch] [sketch]. About 1/20 colonies show effect, but variably.

Three plates now show more definite tail possibilities

small plates

How many small plates were plated? ~~[struck]~~ As stands now, 1 blank, 9 c̄ clones. Yesterday I scanned through and did not notice any tails but might have overlooked. Clone size is 50-100. (49, 62, 132, 74, 28, 82)

Plate 1. Total count is <u>143.</u> Includes singles; minor ~~clone~~ tails:
O• } 13   O: to O:: } 4   also: [sketches]
and [sketch] (tight cluster regarded as major tail).

2. [sketches] 61 singles and [sketch]

Total count: 66

3. [sketch] and 7 minors.

Total count: 50

FEB 26 1955

Large plates — 36 blanks; 6 clones.

plate 4: Several multiple minors
(×8)

Other plates similar.

1 has few if any minors.

marked as
5T/81 colonies. 1 is beautifully linear — should be photoed. *

78 D. duct plate of pool.

now count 11T/[illegible] cols.

left overnight & photographed N27. Occasional minors but
FEB 27 1955
not fully developed.

also plates 1–3 ready for phot. (left on bench overnight).

FEB 27 1955 Examine the tubes of this expt. 34 tubes: 28 clones
None showed major tails, most had minor. (very low incidence — did low temp. interval have anything to do with it. Save a few tubes, $\bar{c}$ CH_2O, as 28E.

FEB 25 1955

Prep. Inc. 8^{50} – 11^{10} – 37° … 12^{15} fuse drops R.T.

fresh materials.

	Total.	No clone		Clones i T.
tubes ✗	5	4	[used uninoc tubes ?].	1. (227) A1 49 1 2's
plates 10 cm	6	5	"	1
plates 6 cm.	39	8		24

Plate out 5^{15} – see next page.

Later 4 more clones developed in tubes (call 29E) over

Singles isolated 1^{15} – 2PM + ~~pla~~ planted right away. (to ca 2:20PM)

3^{30}PM – Collect 114 cells – 3^{45} for B2. 4^{25} ditto 100 cells for B1. Plate immediately.

FEB 26 1955

10AM

B1 (100 cells gathered) 88 isol colonies (incl. 5 pairs) + 12 major trails.

B2 (114 gathered). 68 isolated, 7 prs; 1 3's; 12 major trails.

---

totals.	
151	1's
12	2's
1	3's
24	TRAILS
188	Total.

$T/C = \frac{24}{188} = 12.8\%$

(1/7.8)

Reincubate 10:15AM.

1 clone size

D: 3 groups of (100) planted on c.g. for further growth

1 #0 p 76 — transfer to 30° inc. as clones are only ca 2-5000.

FEB 27 1955 29E - 5 clones 1 c major trail = E 1

all show v. prominent minor trails Why delayed, unless unnoted.

(2's = ⚬⚬)

FEB 26 1955

C. SW967 plated as control on recurrence of minor trails, < 2's. 5P25.
A 26: (2 plates) < 1 too dense / 1 ca 400 cols. No MT, too dense re 2's.

photo neg.

A ~~B~~: 31 clones. 7 (sic) had major trails at 10^{45} AM. (A3-9)
Only 1 trail per each of these clones.

Remaining 24 clones: 15 had all singles. 6 had 2's (3_1; 2_2; 1_3 resp.) = A11-16

A-10. 1 had a ~~[illegible]~~ pattern near glass interface — definite cluster

call T.

A17 1 had a debris surface pattern probably splatter

A18 1 had what looks already like a minor trail

Remi. 11 Day.

Resume. 8 trails / 31 clones / 39 plates. All trails unique.

FEB 27 1955

~~Mates are left~~ in

1229C1 : This control also shows numerous "minor trails" — assume that SW967 produces spontaneous trails? Many are unmistakably distinct

O ⁘ These are therefore unrelated to transductions. Need to

do more test platings with other Fla⁻ stocks. (I had been suspicious of the very high incidence of clones with minor trails). Results on major trails are presumably still valid. Further comment on minor trails in the Transd. clones may therefore be superfluous.

1229 A: (small plates) Reincubated & examined 12⁵⁰ P 27.
A3-9 had major trails. Look at A 1-10, 17, 18. No comment unless something new.

A 3

4 — def. terminal branching appearance (spontaneous minors?)

5 — column of trails.

6 tighter cluster, tapering

7 edge of plate, loose cluster

8 loose cluster

9 edge of plate, branching vertical column

10 loose cluster & large in column.

Photograph 4, 5, 7, 8

see D

D MAR 6 1955 Plated, P4, each of 6
single colony isolations of SW967
from C. All (including stock SW967 earlier)
now show minor trails, though not
prominently (variations in fluidity of agar?)

Spontaneous trails from SW967

DATE: MAR 3 1955 REF:

1	2	3	4	5	6	7	8	9	10

3/1 A. Repeat 1229C: SW967 plated alone: same result: numerous spont. minor trails.

3/1 B. Plate out

	Colonies	A3 Minor Trs. (40 hrs)
SW967	✓	+
666	✓	–
1091	✓	–
1092	✓	–
1140	✓	–
541	✓	–
546	✓	swarmed (both plates).

under similar conditions (pour 50-100 per MGA, small plate).

P2 C. Concentrate SW967. look for motiles. Ca 1-2 found per conc. drop. Start clones from these. Reached ca 8-32 cells each by 8PM. Reisolate motiles where seen. Mostly unicatenate; 1 di-cat.
See protocols.

D. Reexamine same traps (left r.t. overnight) N3: no + seen) at this time.

see 1229D.

E C; did not last very long. Pick 3 of the ultimate clones = E1-3 (C5-D5-D4) and also pool others as E0 for comparison of incidence of spontaneous mt after selection.

DATE: MAR 3 1955 REF:

1	2	3	4	5	6	7	8	9	10

1228 Stock. Isolate ca 30 nums P2 — Pref. proteus.

A. ~~30~~ 47 clones (ca 2^5) plated in tubes + plates

22 were motile. Of 11 plates + 11 tubes viable,

P.4 4 + 4 = 8 major trails seen, all singly.

But minor trails interfere + should start new system (see 1232)

B. Restart same but abandon. Plate about 45 motile initials.

A5 (agar may have been fairly soft: professedly trails were violet

Inc. ca 430 to 10 AM (say 18 hours) Photographed to show extent of motiles. * **Not single clone**

→ x SW666 → x 541.
→ x 1040

MAR 5 1955

A. TM2 → x SW541
B. → x SW1140 (paralyzed)
C. FA37 → x SW666.

2 hours mix. 1 hour syn. Inc 10X. (to ca 2PM.)
1:1

Isolate ⊕ from C (2^{30} - 3^{30}). Pick to 1/ml penassay by 4^{03}
Incubate 3^{30} to c. 6^{30} - 6^{45} PM.

A showed few ⊕ in traps and B, none
Plate conc. susp. of these on mGA.

MAR 6 1955

Plates: A shows moderate T and S (ca 5 or 10 T : 1 S)
B " none.

C + (2 plates, "101" ⊕ cells plated in each.

3/6: 1. Too clouded by swarms for ~~accurate~~ precise count. Not poss. to estimate swarms. Definite ~~swarms~~ trails : 14
"Singles" and similar 67.
These include about 13 clusters of a few colonies (•• to ⁂). 81.

2. 1 (?) swarm occupies ca 1/5 of plate area.
Trails 13
"Singles" 65
(Includes 2's 1) 3
3's : 6
4 1
5 1)

Overall T/C = $\frac{27}{159}$ = 17% ≐ $\frac{1}{6}$

78.

(over)

A third group of 100 ⊕ was diluted in 1ml and samples plated in shake tubes.

1) .2 ml — swarm throughout. Discern 7 single + 2 (+1?) tails *

2) .2 ml — No sw. 11 "single" 3 tails.

3) .2 ml — Swarm top half. 3 tails 14 singles

4) .1 ml — Sw. ~~[illegible]~~ most of top. 6 s. 1 T. 1 large cluster

5) .1 ml — No sw. 6 s. 1 T. 1 med. cluster

6) .2 ml (residual). — 1 sw. (bottom ½) 15 singles 2 T (semi linear)

---

1) * [sketch]

not many linear

2). 1 more or less linear ¿ branching ?

of est. 100 cells thought ~~[illegible]~~ picked, then
20 inviable
5 swarms
13 tails
62± singles (incl. smaller clusters)

---

fr sample of 300 plated!

Save tubes under formalin (11R6)

Totals (Est.)

Sw.	TR.	Singles	
2	3	7	
0	3	11	
1	3	14	
1	1	6	+1?
0	1	6	1?
1	2	15	
5	13	59	2? / 79.

cf. (13 : ~~[illegible]~~) 78. before.

DATE: MAR 6 1955 REF:

Read individual clones (all in small plates).
16 plates negative; 25 c̄ clones.

		Count (2's = 2)
1.	1 trail plus several small clusters	65
2	1 trail only, terminal branch?	25
3°	0 trail several 2's	73
4	Several 2's, 1 5'. (would have been trail if aggreg.?)	8
5	1 trail (non-linear) 2 4's 1 3' ..	109
6	1 trail (non-linear) 1 3' several 2's..	44

and remaining 19 have only 1's & occasional 2's.

∴ (4 trails)/25. Expectation = 4. ✓ } All singly but often accompanied by clusters
No swarms " ≅ 1. } ? oligo-cultures?

Although not very productive this exptl. design is worth continuing.
Leave plates at RT for counting time.

Reincubate others.

Counts on these were (DCG). 81, 23, 21, 66, 87, 142, 44, 54, 69, 7, 143, 149, 140, 101, 32, 106, 10, 30, 17.

(Note variability - of lines / indirect selection) No addl trails seen.

In #7 however (count 66) one colony appeared like a solar system
hollow ball with studs of smaller peripheral colonies.

1232 X

opt set: Try to isolate to verify as Salmonella. photo not.

Having left at RT 24 hours, photograph some of above (2, 5, 6)
(2 shows trail; 5, 6 accessory clusters)

MAR 1~~4~~ 13 1955

Further tests on ~~*~~ SW1140.

X — FA 22
76
37
84
85

} no mottling overnight!

Would need FA 1140 to complete test; hold off now.

swd
old

→ x SW666 plate clones etc.

DATE: MAR 7 1955 REF:

Same stocks as 1232 (refr.) ⊕ now very prolific (assume negligible continued growth in such dense susp., at 4°C.)

A. Harvest 400 ⊕ to 2ml ca. 3PM. Refr. to 5:00 PM. Plate .1ml samples on MA, MGA.

B. Single ~~clones~~ cell transferred to ca 1:10 PM. Inc 37° to 4:30. Plate out. to 5PM.
of MA, MGA

MAR 8 1955 B:

group 1. MA (no gelatin) 2 plates negative 8 positive.

		Total Counts
1.	1. cluster c. 7 colonies all colonies tend to diffuse out.	20
	2 1 ? cluster at wall of plate	128
	3. ~~2~~ all singles	103
	4. " . 1 close cluster 4-5 cols.	125
	5 3 2's	53
	6 8 2's, 2 3's 17	125
	7. all singles. SW 18 also.	170
10 negative plates.	8 (now ~~MA~~ MGA) all singles.	(141)
	9 4 2's	118
	10 3 2's	335
	11 1 2' 7 total	~~4~~17
	12 2 swarms. Salm.? No	—
	13 2 2's	168.
	14 1 swarm, 1 small trail ⊗ 188 colonies total ~~swarm~~ Salm? NO	188
	15 Covered by swarm; also c. 100 colonies, no trails seen. Some lytic areas. Salmonid?	

~~these have no 2's, Trails~~, ...

A totals 100 mos,

4500 / 24 trails + clusters / 98 + 70%?

16 2 2's 200
17 2 2's 118
18 (MA): all singles
+ 16 addnl plates ≅ 2's or trails: Counts 208, 28, 95, 66, 75, 98, 207, 64, 198, 111, 214, 134, 32, 91, 89, 249.

34 plates c̄ clones. 13 s.

also ~~tubes~~ ~~5MA~~ 5MGA.
(1 swarm + ca 100 colonies) — (n₁).
1 cluster of 10, 4, 2, ca 100 colonies.
3: singles.

— cand. why so few trails? MA not v. satisfactory
(clones too large?) Compare A!

(ca 20/plate zale.)
A =.1ml.
MGA:
1 (.05ml) 6 trails, 2 (minor?) trails; 22 singles / 30 nosw.
2 (.1ml) at least 2 swarms (obscure most of plate)
! also 1 trail or swarm origins (place?)
3 3 swarms 1 surface trail 28 singles 2 3's 1 2!
MA 4 15 single or 2-3'. 7 trail or cluster

4 (.2ml) 1 swarm 7 clusters 12 trails 31 singles)

5 1 swarm 3 trails 14 singles.
manifestation doubtless better than on MGA but immobile colonies also too fuzzy.

DATE: MAR 8 1955 REF:

1	2	3	4	5	6	7	8	9	10

new prep. mixed 10^{40} – 12^{05} then centr. to 12^{35}. (strep.)

A. left on slide RT to 2^{05} pm. Traps fused.
harvest + transfer 50 ① by 3^{30}.
(ca 3^{30}?). Plate 5^{00} – 6^{10} 48 transfer plates

B. New traps. Harvest 200/2 ml. Plate .2 and .1 ml samples + plate in MGA, MGYA.

MAR 9 1955

A. Rather low recovery: 33 are negative (Remnant!)
Of 15 positive clones: 9 had only singles.
clone sizes v. small: 14, 11, 4, 7, 4, 24, 1, 18, 6

Remaining 6:

1. "Cluster trail at edge, ca 40 colonies grading in size, adj. to cluster of about 5 large, + 5 singles + cluster of 7

Total: 8

2. + + 8 singles +

(14)

3. + 4 singles

(8)

4. 6 singles

(8)

(over)

Try shorts – temp. effect on motility.

5 more diffuse trail reaching ground.

+ 6 singles +

6. and 6 clusters of 3 – c. 8 colonies each.

photograph! 6, 2, 3.

MAR 10 1955

On reincubation, 2 addl. positive clones appeared.
count: 16 singles
4 "
Remaining plates show no change except surface overgrowths. No minor trails or marked trail extension.

3/9/55.

		ml	T	clusters	singles cols		sel.
1	MGA	2		1	16	17	0
2	MGA	2	2	~~4~~	13	13	1 ?
3	contaminated	2	2		12	14	
4							
5	MG4A	2	3	1	18	2/8	
6	MG4A	2	1		7	8	
7		1	0		4	4	1?
8		1		1	6	7	
9		1	3		9	12	beaut. T.
10				3	6	9	
11		1	0	0	8	8	
12	MGA	1	18	1	2	13	
13	MGA	1	2	5	5	12	
14		1	7	1	1	9	1+

(1–6: overlay MGA)

(G4 = gel 4% rather than 8%)

149

Trails not greatly different MG4, MG8A.

13 sec! (18) trails 2 colonies Save for photog.
How such an odd one? Abnormal dist. of cells, or cooler agar?

14 ditto. Could they have been exch between MGA, MG4A?
May have to repeat expt. cf. when chilled

<u>Conclusion</u>: (1) Effect of gelatin concentration is indecisive
(2) 6 clones/15/48 had trails. But distinction between <u>major</u> and <u>minor</u> trails may not be so clearcut as most of these clones did have several aggregations. Note: clones <u>small</u>.
(3). Why low survival yield, but apparently selection

MAR 9 1955

New prepn.

A. Harvest 200 ⊕/ml + plate MGA. Inc. from c. 11AM to 5PM, then to RT. overnight.

B. 57 single cells planted. c 11AM to be followed microscopically. Examine at 3PM — variable size; mostly quiescent. Cells have grown mostly by enlargement. At 6PM, clone size again v. variable (some only 1 or 2, others & 1000). 3 clones picked as most numerous motiles. These were plated c. 6:30 PM. Unfortunately the MGA was floccy + plates could not be accurately interpreted. 1 may have had + well marked trail. Expt. needs to be repeated.

Limited incubation allows swarms to be limited and counted.

A. Inoc est.:

Inoc est.	T	SW	Clusters:	1-Cells	Total
100	2	1	8,4,3,2[6]	65	~~91 89~~ 77
10				4	4
20		2	2[1]	15	18
10		2		1	3
20	2		3[1]	15	~~20~~ 18
20	1	2	2 3, 3[1]	33	40
* 10			2,4,5	9	12
190	5	7	18	142	~~18~~ 172

but agar rather poor lumpy and hard to score. Why so few trails here?

Conclusions: plating of selected clones may be promising method but needs to be repeated.

MAR 11 1955

MC8A (pipe ~~ɑe~~ ~~Ɑel.de~~) B

~~± Tween. 0.2%~~ ~~[illegible]~~

Plated from inoculum calc. at 100/ml. Inc. ca 11-12 hours then R.T.

− Tween.

Est input	SW	T	C(2,3...)	1's	Total
40	2	3	$2^5\ 3^1$	35	46
20	2*	2	$2^5\ 3^2$	10	~~[illegible]~~ 21
25	3 **	0	$2^2\ 3^1$	14	20
85	7	5	$2^{12}\ 3^4$	59	8 ~~6~~ 7 ~~[illegible]~~

* sw had 1's in center

+ Tween

Est input	SW	T	C(2,3...)	1's	Total
10	~~2~~ 3	0	2^4	2	9
10	0	1	2^1	3	5
20	1*	3	2^1	17	22
40	1*	2	$2^2 3^1$	26	32
20	3*†	0	2^3	12	18
100	8	6	11 1	60	86

† ~~Trail~~ sw had 2 in center

‡ sw, at center had 0°° and ♀ nearby center.

Tween had no certain effect. how about survival?
but could be systematic error of sampling?
Swarms were all about 1-2cm diam, somewhat variable even on 1 plate.
Note very low incidence of trails. Too early selection of ⊕?

86	~~[illegible]~~ 100
87	85

Should recover Fla subs from A swarms: plate out residues of the drops for full test.

PA37 → SW666 selected clones Effect of tween 80. | Misc. observations 1237

MAR 10 1955
MAR 10 1955

[232]

Prepn. t-b-b 3/9/55. (Quite fresh < 2 hours before incub.)

A. Freeze taps 11[0]. Isolate to [232] 50 by 11[47] AM.

(A–D) 2/row (a, b); 1–6. = 48 isolates. Incubate at 12[40].

B. Isolate 400 (+) to 12[40]. To 2 ml pen assay. Plate samples (≐ 40, 20, 10 cells) in MGA c/s Tween 80 .01%. Incubate from 1[05] PM — 11 PM.

Examine ca 4[30] PM to select clones for plating. Isolate: (all have c. 10^{2+} cells and:)

1	A3a	SW	Inc to 11 PM then R.T.	SW. + 1's B hot. 10[30] AM.
2	B6a	SW		SW. + 1's Shows 3 clusters (c. 2, 3, 4)
11	B3a	(20)		
4	B2b	(10)		SW. + 1's
5	C1a	(15)	Inc overnight	1's
6	C2a	(15)		1's
7	C4b	(10)		1's, 2
8	C3b	(20)		1's
9	D2a	(10)		T
10	D3b	(10)		T
3	A5b			

as well as 10 more quiescent clones as controls.
(107/366; 11/200; 7/19; SW/COL)

MAR 11 1955

A. Controls all had singles only. A3a, B2b, B6a all had swarms and colonies. A3a shows flares best; Isolate is selection for later plating. The other two do so less strikingly. Arrangement should isolate the Fla⁻ sibs.

Among remaining plates, 5, 6, 8 show singles only. 7 has 1 2'

9: [sketch]

TRAILS IN 9, 10

10 [sketch]

In selected groups 2T/7
unselected 0/10
hardly a dramatic result though warranting more extensive study.

Reexamine 232.

1) A3a - act. swarm   ̄c residual F1a probable

4) B2b ✓

2) B6a act. sw. —

3) A5b also has numerous s motiles (c. 20% or more) ✓ → swarms.

other clones are not unmotile

(most have no evident ⊕ on casual examination

some a few etc.)

Try, B5a, has ca.	$10^4/10^4$	No trails.
B6b	$10^4/10^3$	
~~B~~		
C2a	$/10^5$	

MAR 12 1955

MAR 12 1955 single colonies from residual drops of clones A3a ~~B2b~~ B6a and A5b are being tested for motility and saved ̄c selection ~~also~~ also stab originals as 1237-A.

4/1: 1237 - 1A +
1B +
2 + } all b not ̄c

MAR 12 1955

~~Isolates~~ Fla+ and − from 1-4.

Streaked on NSA, test single colonies on MGA ca 4-5 hours.
Compare original plating counts

1	{1+A 16− 1+B: ~~17~~−	later a second +
3	3+: 29−	
4 ~~2~~	0+: 25−	
2 ~~4~~	7+: 27−	

can be used for flag production in unselected motile lines

diff may depend on selective residue.

pool +, − to stab.

~~[illegible] 24.~~

Isolate 4+ by selection. (original mixed clones are also preserved for possible later need).

MAR 13 1955

see 1417 1+A is still mixed.

MAR 1 2 1955

Bearings:

Since Jan 1, I have been primarily occupied with Salmonella trails. An important question has been the uniqueness of the"E particle". This would be hard to establish by quantitative data on the clones directly, and I have been principally concerned with looking at platings of small clones in motility agar.
The results with SW-967 are not fully reliable owing to spontaneous "minor trails". This work has been done only since Febr. 23. Before that, from Jan. 11,I was mostly fiddling around. I must have been preoccupied with otherkinds of experiments too, or writing or what not, since relatively few experiments are recorded. There are also some experiments on crosses of heterogenotes, but DCG did most of the routine on these. From Jan-Febr., there were a number of misc, experiments on conditions of plating etc., which amounted to very little. There were some indications of major and minor trails. Also developed technique of trapping from conc. cell susp.

Summary of clone platings. (trails per clones/ per platings) and comments mass pla.

1227: 1/20/25 T: unique

1228 3/9/10 T: majors unique, noticed addl minors 13/84/ 100?

1229 8/31/39 All T unique 24/198/200

1229C: spont minors

Total
—x 967 12/60/74 All major trails unique. some dist. non-linear however. Confusion with spontaneous minors.

—x SW966 Almost as prolific source of motiles

1232 4/16/25 Single majors, but other clusters. Clone size c. 2[6] 27/159/202 + 5sw.
5Sw/13/79/100

1233 1/34/47 Single small trails; a few other clusters. c. 26

1234 6/15/48 Definite concurrence of smaller trails or larger clusters; hard to define. Av.Cl.S. c. 23-4. 30/149/200 3 sw. (not random)

1236 57 clones, follow microsc. plate only 3 NVG. 5/172 /200 18 cl., 7 sw.

1237 (eff. Tween) This prepn. seems ng. although very fresh. Should compare directly with 1234 prepn. 11/ 173 /185 15 sw.
48 clones. followed micros. 4 gave swarms (segr. non mot.)
10 quiescent clones gave only singles; 6 with fairly numerous motiles gave 2 T's, + 1 with clusters.

MAR 12 1955

The principal point is perhaps best met by experiments like 1234, plating fairly early. A corrélation of trails with pluricatenates like 1237 might be worthwhile, but more laboraious.

In view of sluggish motility of early log phase cells, this should perhaps be done with earlier clones in aged medium.

In some prelim. expts. yesterday, I noticed that TM2 transferred to aged medium supernate was more actively motile, particularly showing a more jerky motion with shorter free path. Examination of TM2 in motility agar suggests that many cells are directly immobilized, others move in apparent interstices, but still more slowly than normal. There must be a considerable accidental factor, and cell with numerous motile progeny naturally has best chance to propagate a trail. Since genetically competent TM2 are immobilized, there can hardly be immediate correlation of genotype (or pluricatenation) and ability to move. Should watch trails in situ if possible. Why not?

Plans: continue with experiments like 1234. Compare this prepn. with 1237 in yield of trails. Continue with medium effects. Set up trails in situ, selecting cells which remain motile in gel. to see if these form the most chains.

→ x 1140 n.s. why?

Do not forget many other carryovers:

EM
Gal, Nelson
heterogenote crosses
Hfr x F- !!!

G&C
DAr
Gal Salmonella.

DATE: REF:

a) 3/14/55 Collected ca (100) from 1234, 1236 preps for comparison + plated in MGA. (app. left out at RT so result is unsigned.) Later brief incubation showed 1234: 6 swarms/87 (100)
1236: 35/68/97.
T not detected.

3/15/55

b) New prep. 1238 (22 hours)
Freeze 1:40 Dool. (late) 3:20 – 3:50 A) 200/2 ml ~~B) 50 (1)'s~~ but lost by error.

Running out of gelatin!

Plate out A samples. (Noted remarkable incidence of trails, might be related to quality of medium. agar was granular loose from reheatings!)

	Est. mac.	C	T	Sw.	Cols.	Σ
S.C. (B1)	100	2-3	82	5	4	95
	20	1	4	1	6	12
	20	11	4	1	3	18
	10	2	4		3	9
	10	3	3	1	4	11
α.	10					
	15	1	3	1	5	10
	185	21	100	9	25	155

See photographs.
Note variability.

many swarms were centered $\bar{c}$ C or T

Suggests variation of 1238C

Trails in (B1) particularly florescent.

1238
C-D
E

DATE: March 16 1955 (Wed.) REF:

New suspn. (ca 9-11) Fuse drops 12^{30}

to 3^{30} coll. (500) in 2.5 ml Plate, .25 ml samples

a). in MGA variously diluted. (Plating yesterday had shown remarkable incidence of large trails) Incubate to 4PM. Then R.T. refrig. (Plates 0 c .25 ml). Figures indicate amount of NSB in this ~~½-1 ml of samples.)~~ (-.25 ml).

Est input 50 cells.

NSB	Clusters	Cols.	T.	Sw.	Σ
0	2	53	2 (v. sh.)	2	59
1	2	55	1 "	0	58
2		59	2	3	64
5	5	46	6	0	57
7.5	18	39	11	3	71
10	11	19	19	3	52
Input (50) 0	1	93	1	8	107
(100) 0		66	2	3	

Sw less compact (NSB 2); sw diffuse (NSB 7.5), " (NSB 10); TRAILS flourish!

Basic agar must have been unusually stiff. Swarmers in NSB0 v. compact also. Thus incidence and quality of trails increases with decreasing agar concentration!

---

also B) Fuse 12^{30} collect to 2^{30} (1). P. drops to 3 PM Incubate to ? 5^{30} PM. (Some refrig. to 1215 PM Thurs for plating:

plated 12:15 Thurs.

C: 13 blanks 4 contam.) novis cols. 1 swarm + 18 clones } all except C1 singles only

C1 has 1 major (v. prof.) trail 6 moderate 4 or 5 clusters + 20 clusters
No shot - owing to contaminant also.

D 8 blanks, 5 clones.

1238C1 - see photo.

T_1 c. 400 colonies

$T_{...}$ << 50 ~~[illegible]~~

DATE: Mar 17, 1955. Thurs REF:

1 2 3 4 5 6 7 8 9 10

Met Kornberg visit later in PM. ca (1000)/ml cool. but no used since 1240 pepns. was fresher next day.

1238 pepns. Freeze traps 12N. Spotted out 1:40 Clones picked and incubated c. 2^{30} – 6PM ($3\frac{1}{2}$ hours). Plate in MGA ± diluent USB. (c. 10ml: 15 MGA). Also noted that these dessimilated cells showed internal structure (nuclear?)

MGA standard still .4% Agar 8% Gelatin

A). MGA str96t. | 1 contam, 12 blank, 16 clones add. | 2 swarmed < No def. colonies; 2 large plaques / " " (vague sev. initials; 1 plaque

all singles. no frails

20

30

B) MGA 60% | 1 swarm – 7 plaques 4 single cols. | 8 blank, 8 clones add.

40 1. all singles
2 " "
3
4 " "
5 " "
→ (6) at least 11 frails, 1 major. * sa photog. > 110 cols!/ T_2 c. 30
7 all singles

Total 48.

3/22/55

Counts on motility plates:

1239A:

11	103
61	140
144	67
119	41
17	181
37	115
54	92
196	51

1239B:

97
225
60
134
100
102

1238D:

22	39
36	25
40	6
15	44
52	6
6	61
13	13
9	18
44	39

Plates marked "C"

9
9
2
31

DATE: March 19, 1937 Fri REF:

1 2 3 4 5 6 7 8 9 10

~~Isol~~ (1000)/ml. and plate .1ml, .2ml samples. Inc c. 2–11 PM then RT

1. MGA (100). 4 swarms, 56 singles (remains of yesterday's MGA [Ref.] equally stiff)
12 2's
8 3's
5 short trails c 6 clusters

2 MGA (200) (somewhat loose; – cf. swarms) 6 swarms 23 Trails ext.
33 singles 4 2's 7 3's 6 clusters

3. MGA (200) [firm!) 4 compact swarms 2 or 3 short trails, rest singles
dilute 40%

4 MGA Wilson trails nebulous, swarms limited 47 trails 3 sw (1 c T)
26 singles 6 clusters
5 3's

5 MA (4.5%) 3 sw: 1 T, 1 center ;

ℓ c G mils
incubate 3^15 6 6^oc

Out of gelatin! Use MA (.45% agar) and Wilson gelatin (n.s)
(a) (b)

two groups: (a). 9 plates (2 large) are blank ; 19 plates (6 large) have clones

a) (5) 19 plates photo
① covered by a swarm ; 3 zones of lysis. In addition ca. 18 all trails of some extent, some considerable. May be hard to photograph.

② 7 + c^10, c^9, c^4 + pyramidal cluster, & 50.

3. 7 + c^2, c^6, c^4

4° 12 + c^6 c^6, c^4 ; c^9 ; c^12, c^7, (a with T ca 102), c^9, c^6

⑤ 32 + (T^+) ; + c^7 c^8 c^6 c^3 c^11 c^2 c^4 c^6 c^7

also (14)
51 (1 c?), 22 (1 c), 9 + 2 c [✿ ✿], 63, 94, 18 + ccc ;
15 ✿ ; 23 ; 16 + ccc ; 33 ; 13 (incl 2'), 60, 11, 14 ;

50

(Gen)

D) in 10,1 sm gelatin agar. (V. sloppy - gelatin must bind agar!). 9 blanks 10 doses.

1. 5 short tails + 9 cols
* 2. 13 prol. tails + 4 cols.
3. 4 short " 7 cols.
(4) 12 prol. 7 cols.
5 2 mod. " 23 cols
6 14 tails 11 cols
7 1 good t. + 8 cols.
8 12 " t 18 cols
9 1 linear t 15 cols
(10.)
[illegible]. 7 tails . 0 cols!

these tails oft. linear

Conclusions: 1938-40.

MAR 21 1955

1. Incidence of trails among motile initials plated varies with the fluidity of agar. Addition of 40% diluent gives very high incidence. In any event, agar that is hardening tends to be quite inhomogeneous, if maintained at critical temperature.

2. Single clones can give at least one trail + large clusters in harder agar, and numerous trails in softer. This is clairly an unreliable criterion for singularity of catenation of higher order.

Further plans:

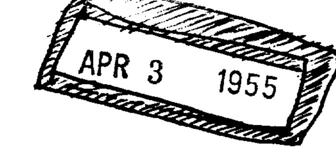
1. A few more tests of fluidity and related variables for photographic documentation.

2. Shift studies to direct pedigrees; need some further data on irritants; inh. of cell size growth and chemotactics.

3. EM transfers.

4. Write it up!

But will doubtless spend this week cleaning up away from lab.



What happened last week? N.G.

1. Out of gelatin
2. No good idea what to do next on chimeras!

3. Change medium < D(0) – flattens out too far (wets glass – how counteract?)
Penassay 10% – poor growth.

Out-survival in H_2O seemed limited. Metal poisoning? – Try purer water.

Problem: don't want to follow ind. pedigrees more than 3–4 generations but minimum size drops allow too many cells ultimately. Should have 1.val clones of about 300 cells. Try partly exhausted medium.

4. Serum effect

i /TML of course miff owing to H_2 1,2

b < 1237-2 (H_1^b). at first almost completely inhibited, but same probably inhibition by anti-i at 1:100. with overnight growth, see active motility and agglutinated clumps

– May still be worth trying at 1:100. (serum titer ca 10^4).

APR 1 1955

Serum effects

(1) 1237-2 = b (2) TM2 = i

1. Try against serums 1/100 in broth.

	o	b	i
(1)			
(2)			

b cells ~~almost~~ completely inh by b serum, partly by i

i cells partly inh by i or b (1, 2 comp?)

Plumb
Trains
Pos. Eff

37-X866

1241 (E)
MAR 31 1955

New preps. 1/2 : 1/2 — Inc. 10:44 — Centr. 11:45 — Decant. 12:10 Refr.

Collect in 10% broth (E); D/0 (F).

OOOO
A B C D

[illegible] 2:45 (31) by 2:55 spot at A↓, B.

E) 22 deposited to 3:05

F (35) to 3:15 Spot at D, C, C'

to 3:25

Start R.T.

5:30 ; 7:30 – 8:30 / E: only 1-4 cells saw. mostly (1)

F 1-4 (often (2)) far more active!

spreads [illegible]

Viscosity?

9:30 A1 Poisoning to [illegible] very little growth

→ x Sw b66

Misc.

3/28 NG

3/29. 3) 10% broth dispos. 9. A) Pen assay 40 ①'s, same split, 1-4. No striking many chemical, not closely examined (too many cells, > 10⁴/.) but 3 swarm-clones A < ⊕ 1, ⊕ 2; B < ⊕ 1, ⊕ 2; C / ⊕.

save for later check of identity + homogeneity.

3/30 cf. 10%, 100% Pen assay: needle troubles

Φ 12/58

DATE: APR 5 1955 REF:

New prepns. a): from aerated SW-666, b) from unaerated culture.
Both + 1:1 FA-37 10:20- 11:35-12:10 Refr. (Incub. in rot.)

Note: Rotator now standard for aeration.

A): Prepn. a). Fuse traps 12:30, collect ca. 100 motiles, but use to spot (B)
2-2:15
DCG pick to 3:30. Incub. in .5 ml Penassay to c. 6:15 PM. Plate in MGA ±
40% NSA as diluent.

B): Same collection, plant in spent broth (SW666 Aer.) to cf. total clone size. St. RT.

C): Prepn. b) Fuse 4 PM. Collect 5:30-6:00 (some needle tr.) This prepn at least as good as a, probably better.

1000 (±) / 1 ml. Plate 0.1 ml samples in large Plates; .05 in small. Compare MGA, + 40% NSA dil., + 60% NSA dil. Incub. 37° c. 6:45 – 11PM/ Then RT to P6; then refr. for analysis. (dil. MGA very soupy!)

APR 6 1955
Hold A,C for study. (Spent broth = overnight SW666A, 60° 30 mins., the sediment and decant. Numerous fine granules still present).

B { 11:15 -12:15 AM Examine B). Note that clones are limited to 100-1000, while Penassay gives at least 10x as many. 18 clones (in spent broth) examined: (sequence not retained).

3 – 0's 2: about 100 Fla⁻. 5 had 1+/c. 100, of which 4 isolated to broth for plating clones. (4?) 3 had 2+ (+ Cl, 2,3), each isol.

C14 had 29+/1000 C13 23/1000 C6 12/-00. (These ~~mixtx~~ collected and plated without further growth.

C5 had 10+/-00. Plant individually, pick to broth for clones. (C5-1-2 maybe 2/0 instead of 1/1).

(Inc. 12:30 – 3:00 PM. Plate in MGA-40). }

1242A

APR 6 1955

DATE: REF:

Note: OCG picked in sequence but this was undesignated for plating: Assume cold

#'s, are empty:

(18) 1, 2, 5, 6, 8, 11, 12, 14, 23, 24, 25, 28, 31, 35, 41, 44, 45, 46.

note: 6n + 1, 6n + 3 (n = 0-7) were MGA all others MGA + 40% NSB.

(1, 7, 13, 19, 25, 31, 37, 43
3, 9, 15, 21, 27, 33, 39, 45)

swarms (or cont): 9 (plaque c. 50 1's) ; 13 (c. 4 1's opp. ca 4 ⊕'s)

26: + c. 10 colonies, incl. 5-6 trails

29 + c 20 trails, few 1's.

(7) 33: patchy lysis, swarm + 3 3's, 11.

36: Prob cont. ; No colonies - cont.

39 swarm + c. 100 singles + short clusters. .

MGA: 3 ca 60 no T 2 2's.

7 6 1's

15 c. 60 1's

19 c. 45 1's,

21 6 1's 8 C's (3-6) 1 T[35] 1 T[10] (closeby).

27 1 major trail + 9 C's (3-7) + 6 1-2's.

37 1 T ; 8 1's 3 2-3's.

43 11 1's 2 2's

(note contaminated ... to gar?)

MGA 60% 40% NSB

4. ca 20-30 profuse trails ~~[illegible]~~ 50 1's. somewhat dessicated

10 >100 all 1's { 24, 10, 24, 23, 14, 16, 15, 10, 19, 24, 30, 47, 13, 11, 100, 150, 6, ~~[illegible]~~, 23, ~~[illegible]~~, 14, 15, 16 and others. (colonies per trail.)

(11) save for phot.

37 - 1's 5 C (<10) 21 T's.

18 9 T's 38 1's

18 seems somewhat tighter agar, 12 T ; 3 clusters (3-7); 38 1's

12/2 A

DATE: REF:

1	2	3	4	5	6	7	8	9	10

20 1T ([drawing of colonies]) ; 60± 1's

22 (partly slopped) Profuse trails about 1:3 1's

(30) Profuse trails, 16T, 5C (3-5), 551's {T's include c.80, 80}

32 " " 7T, 1C, 561's.

34 45 1's

38 17T's 3C's 45 1's

40 >500/plate ; some prob. trails but too crowded to count.

42 14T's 33 1's (rough counts).

save for photos 11, 18, 30.

Test "swarmers" in B tac in order
9, 13, 24, 29, 33, (3), 39 others ✓
X

APR 9 1955

{ Note BADS letter — #11 illustrates clone c profuse trails. But this was incubated 15 hours. (Usually no progression over c. 8 hours but must be controlled! Need check on trail progression at R.T.) of "mcp trails" }

37 -X 666

1242
+B

APR 5 1955

(1) New prep A non aerated SW666 x — FA37 } Rotate 10^{20} to 11^{35}.
B non aerated . x — 1:1

(Rotator now in op'n and generally used for aeration rather than bubbling)

10^{20} - 11^{35} - 12^{10} Refr.

(2). Freeze drops A - 12^{30}

see 1242

APR 7 1955 9^{30}AM Score series C.

Note: many plates have small swarms, doubtless contaminants. Save for characterization & cf. 1243 A.

C1 2 plates A 10^2 1's; no clear T. 1 fuzzy stuff of 8 near glass. B. c. 60 1's

C2 A 60 1's 1?T (fuzzy:) } terminal chains?
B ditto

C3' A. c 40 v.s. 1's & B the same — many cont? scr.

C3 a " "
b " "
c " " Heavy contam. } N.G.

(QU)

C – 2. 30 1's no T

2 – 0

2 – 2. 40 1's. no T.

Terminal (+) have poor chance of starting a longish trail.
Medium OK.

---

25 – 1 } 20 small 1's + cont swarms
2 } ditto
3 " "
4. " "
5 40 1's " "
6 " " " "
7 " " " "
8 0 " "
9 30 1's " "
10 30' 1's " "

(small angles prob. also cont.)

5/10 plants → clones. All of these had presumably petered out & gave no trails at this point.
cf. 6, 13, 14.

1242B

DATE: APR 7 1955 REF:

	1	2	3	4	5	6	7	8	9	10

Pk6: 7 1's 6 clusters 3 trails

Σ 16

Input:

def. linear

prob linear

? linear

Pk13 16 1's 1 globular cluster

3 linear clusters (4 ?), and

Σ 25

prob uni chain

total 16 1's, 8 "trails", 1 cluster

Pk14 See photos. Most trails linear in aspect. ~~to~~

21 1's 12 T's 5 C.

But not C13 nebula!

10

20

30

40

50

DATE: APR 6 1955 REF:

3:40—6 PM Trap 1000 ⊕ / 1 ml. Plate 0.1 ml sampler
25 ml total volume.

			T	C	1's	SW	Σ
—1	MGA		88	12	9	5	114
2	MGA	(0.03 ml)					
—(3)	10 ml Penassay		129	0	8	12	149
4	" H_2O						
—5	" Boiling						
—6	" NSB						
7	5 " NSB	mismeasured?					
8	7.5 " "						
9	12.5 " "						
10	15 " "						

3 gave best development of trails. Use routinely from now on. Layers evidently too shallow for extreme gas.

Note: this batch of MGA already showed deposit of gelled agar and was probably inordinately soft to start with.

4-5-6 agreed in showing almost 100% trails! all worth photos.
St. room temp to bring out swarms more sharply.

This expt. n.v.g. for comparison of agar density owing to looseness of original MGA.

DATE: APR 8 1955 REF:

	1	2 i's	3 i's	4 T's	5 sed	6 Σ	7
1				3+		68	
				11		64	
				1		54	
			2^2	0		20	
2				1		43	
			1^{2-3}	0		78	
				0		62	
				2 linear		43	
3				0		59	
			3	0		67	
4				2 ?.s		12	
				4 s.		20	
5						0	.
						0	.
6	0					0	.
				8 short		46	
7				0		49	
				0		47	
8				1 sh.		82	
				2 sh.		65	
9						0	:
						0	.
10				11 short		65	
						0	.
11			✓	0		62	
			✓	0		0	..
12				0		0	.
			1^3	0		44	
13				0		0	.
				0		6	
14				1		20	
				1 ectus.		17	
15						0	:
						0	.
16				6 m.s. - linear		53	
				5 " , 1 glob.		41	
17						0	.
			1^2			24	

Inc. 5:30 - 11 PM, then RT. Scored 9 A 8. This dil. MGA may be stiff

Owing to probable stiffness of agar, expt is inconclusive.

1-2 prob. worth photographing.

Photography 1244'

APR 8

DATE: 1243-3 REF: (lt. value 3-25)

1	2	3	4	5	6	7	8	9	10

1243 for calib. f6.3 - f9 at 1/20 +X film found best (as indicated by light meter!) for large plates, on light box. Leica set-up no lens ~~[illegible]~~ in front. Bellows 019 - 6 mms. for large.

Use 1242 ~~#3~~ #1-6
#3 = sample.

f8 1/20 +X.

10

1243 1-6. f8.
1 = #1 c 1½ secs.
2 #1 1/20 "
3
4
5
6 etc.

Same slopes

EMB large plate (margin etc. fitting?) 1/20 f4.5

20

1242C -0
-0
-40
-40
-60
-60
-60

30

1238 B1 marked.

small plates ~~[illegible]~~ with lens #3, no ext tube.
1238C1 ...
1244A1 (4), A2 (4), (marked).
1242 C13

4, 9. wavy solutions!

50

DATE: 4-9. APR 9 1955 REF:

1	2	3	4	5	6	7	8	9	10

Photography 35mm Leica setup again per yesterday
Large plates ~~at~~ no front lens.

(3)(4)(5)(6)(7)(8)(9)

1243 1-6 1243 : 3, 4, 5, 6, 7, 2 f/8 1/20 sec.

7 λ-6A6

7

HF 7-6

ags came out beautifully
D19 dev. 8 minutes

EMB f/3.5 2744-x
last + 2769
1426A + 6.
~~1243-6 again~~

EMB overexposed

1238B1

1242C -0
-0
-40
-40
-60
-60

small plates +1 3 lens.

1241-1 (4)
-2 (4)

1242A 11, 30, 18

1242 C6, C13, C14

1231B

Resume 1242 - 44.

APR 9 1955

1242

A) clones from single initials.

18 empty

7 had swarms (diffused) + trails [some contain.)

8 clones as MGA standard: some T's, usually poorly developed.

13 " " MGA 60%.

* 20T; 50 1's	100 1's
9T 38 1	75 1's
* 12T 3C 38 1	>500 1's, T's.
1T 60 1	
>T	
* 16T 5C 55 1's	
# (32) 7T 1C 58 1's	
17T 3C 45 1	
14T 33 1.	
*# 11 21T 5C 37 1's (count complexity of trails).	

*photographed.

Terminals

B) Plant in spent broth 18 planted. (17?) Looks for F/a+ / c 10³ F/a-

3 0's. 3 0⁺ 5 1⁺ 3 2⁺ and

groups of 29, 23, 12 from 3 others. (C6) (C13) (C14) Plant these as groups or clone in MGA60. From singles, No clear trails from clones. Groups:

C6, 13, 14 See photos. { 3T 6C 71
1T⁺⁺ 8T 161
not counted }

Nothing at all!

APR 9 1955

ctd.

1242C Platings in MGA, 60%, 40% in small and large plates. Swarms [large plates:] rather messy but photographed. MGA-0 showed compact sw, no T. 40, 60 about equivalent development of trails

Small plates ~~not yet studied~~ equally messy, suggest that MGA40 is sufficiently dilute to bring out most trails; more profuse at MGA60.

1243. Is simply group initials, various media. See pictures Pers assay o days as diluent. (1-6).

1244.(B) Like 1243 but excess non motiles interfered. MGA standard rather stiff; MGA 40-50 optimal

A) Sib clones. Agar probably too stiff but photogr. sequence 1-4, 24.

New notes on Bruce - { abstract together?
or MGB } c. 4/10/55

1. my cells don't get stuck
2. they stay motile - usually both n_1's are ⊕
3. don't like "replicas" of genes.

"We have never obs. E cells in > 1 subline ~~[limited observation]~~". How many E clones have been derived

B claims one case of E at n_{22}
only 1?

Need my own data on E+E or E+S in 1 clone

style; numerical calculations; final conclusion first.

Where are pedigrees?

No time now to clean up pedigrees.

37-X 666 / serum and clones.

1245

DATE: APR 12 1955

REF:

1	2	3	4	5	6	7	8	9	10

A. Misc. tests on serum, diluents:
Water whether tap, this lab distilled, or enzyme institute double distilled appears to be suitable diluent (contra "spent broth") to limit growth. Inocula c. 10 - 20 cells retained motility, did not flatten unduly and growth was limited. (Unavoidable contamination with broth of isolation.)

B. 9, 27 ⊕ 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 N.G. In B2, noted clone at center as above but at periphery, net and cords of long cells and filaments (somatic serum?) This was also noted later in i-serum.

C 12 X 2B pyrim. Freeze dogs 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) - held to 5/31/55 for photo and counting.
9 blanks. (probably picked late)

serum inhibition of trails

1240.
1246

digests found 12 30
(4:45 figures)

DATE: APR 13 1955 REF:

A. Serum effect microscopic. Buttals isolated from
B } sel 1245 (c. 2 30)
FA11 → x SW666; FA37 → x SW666 and put in 1:100 i, b.
(A) (B)
serums in broth. b serums inactivated both very quickly;
i after 30-90 seconds (usually).

Then tried plating. (by c. 3 45). Harvest c. 300/ml A; 500/ml B

(but motile broke + 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 1:1000 5 swarms (sl. 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 .1 ml samples (do.) (1-6).
1 swarms too messy; about 50% T;
2 (MGA) [5 swarms, 56 1's; 7 short trails]; [4S; 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!
i serum inhibits b trails!
abandon i → b system for this study

DATE: APR 13 1955 REF:

37 X 666

ca 50 plates — D. SW967 plate in MGA, MGA60 for minor trails in <24 hours — 5PM. but too crowded.

E Inc. continuously ditto check SW666 MGA60 only — at 2 days, no minor trails, some diffusion: colonies are

ca 10 plate — F 1237A+ for flares — incubate 5PM – 10AM. MGA only
note singles, not pure?

G control SW673 " .

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

APR 14 1955 Platings of c. 50 isolates each in MGA60 + .01 ml serum.
all show swarms.

	trails
a	+
b	−
c	+
d	+
i	−
k	+
r	+
j 2	+
B-0	±

a, c, or d would be quite good for further test.

(c. 5^{30} PM)

D Replate P 14 — A15: excellent development of minor trails in 60% MGA; intact only in MGA but will probably show later.

1249 D1A for photog. — Refrigerated 10 AM. 5/15. 60% MGA. Reincubate sister plates

Needs to be done in my own expts.

APR 13 1955

Today

Serum effects ✓

Swarm sibs *

Flares ✓

Terminals start *

E.M.

SW917 / MtA60 ✓ ✓

Paired sublaces for multiplicity of "E" *
(Use c TZ ?)

Isolations in water ?

Viability pH 4

Viscosity fx.

Trails in ϕ^+_x (viability of sublines' multi, stiff somatam)

APR 15 1955

Notes: Phase Var - TH2 ph2 monoph?

Mention to Bruce 6/i — of. kumyndorf.

Inc. Tax

Fla^{+} H_{1}^{a}

↓

Fla_{1}^{-} H_{1}^{b}

a, b

$\frac{+a}{-b}$

a, b mcp

Serum inhibitors a → x

DATE: APR 14 1955 REF:

	1	2	3	4	5	6	7	8	9	10

APR 15 1955

14: n.g.

4/15: Freeze drops, FA76a (S. miami a) → x SW666 11AM.
FA37 → x ...

Preliminaries
see 1250, 1252

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

Rerecu's # 4'
431

3 cells isolated

1131 ? ~~cells pitted~~. mit ~~this expt~~.

~~) pedigree to n_3~~

1) pedigree to n_3. 1/8 gave motile on transfer, found to be mixed +/−. (8+ : 12 −) Flat: H_1^i
− ~~is~~ H_1^b. ∴ segregation at $n_{4>}$!

2) n_{13} : all −

3) n_{13} : all −

1132 2 cells. followed divity to about n_3:
1) showed 1 chain to n_3 ; n_{13}^-
2) 2 sibs both motile, catenated to ~~n_3~~ n_3, n_8 both. n_{13}^-.

11~3

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

1131) 1) 1 pedigree to n_3. 1/8 ~~[illegible]~~ gave swarm, mixed $Fla^+ H_1^i$ / $Fla^- H_1^b$. Segr & n_4.

2) 2 cells → n_{13}^-.

n_0 = (initial chain)

1132) 1) 1 chain ~~$n_1 \to n_3 < n_{13}$~~ $n_0 - n_3 < n_{13}$

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 coil.

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

1) $n_0 - n_1 > n_1$.

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

initials only ↑

1134 C3) $n_4 > n_5$ < 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

C5)₁ $n_0 - n_{23} > n_{33}$.

C4)₅ $n_4 > n_5$ ..... $n_5 > n_{10}$ [5 isolates $n_{10} > n_{18}$]. 16, 50 intermed.
n_5 \ $n_{16} > n_{26}$

A3)₁ $n_1 - n_3 - n_8$? not certd.

B5)₁₀ $n_{13} > n_{15}$ < $n_{15} > n_{20}$ / (4) $n_{19} > n_{26}$

$n_{15} > n_{18}$ (6) < n_{28}. / n_{18}. / 8 ~~[illegible]~~

Branch ~~1~~ (1). Branch (2) (3) ~~Branch (4)~~

Foci's 4/15/55

1134 A5 / 7 { $n_2 > n_{12} - n_{12} > n_{28}$ (2, 3 rd.)
+1? $n_2 > n_{12}$ — $n_{12} < n_{19}$ / $n_{12} > n_{19}$ / n_{40}.

(100θ?)

(14 resolutions, 6 days [refr. ovens])

A4 { ~~$n_1' > n_{14} - n_{16}$~~
~~$n_1' > n_{14} - n_{19}$~~

$A4_2$ $n_1' > n_5$ < n_{17}. / n_{20}.

1138 most rool dried. 1 clone saved (B4) also had trouble drying.

B4 .F12
$n_4 > n_{14} - n_{42}$.

$n_6 > n_{14}$ < $n_{17} > n_{27}$ / $n_{18} > n_{23}$ / 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

$n_{27}+n_{15}$ n_{45}

1138 addl chain ~~tota~~ after n16

"

n6 B21

4/400

n14

D22 D23 D24 E24

11. B31 14. 7. 104 C31

23.

10^3.

104 A34

1.

300.

B21

A21	3 C23 lost	4 C33. < 5
22	2 C24. ~~B3~~	
23	1.	> 13
24	3 C25	13 A32. > 1
25	2 D25 ~~2 A33~~	13 A33. > 2.
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 chainlengths n16

A21	19.	
A22	22. ~~> 27~~	> 27
A23	17 > ~~13~~.	> 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.	

$H_1^a \rightarrow x$ 1250

DATE: APR 20 1955 REF:

FA93 (SW940 4,5,12 a:–) →x SW666 . 11AM - 12^15 - 12^40 ?

FA37 " "

A) 93 →x 2^35 fuse drops. 5PM Harvest = 5×10³/10ml.

B) Same dilute 500/ml. = 500/1ml .2 ml samples.
B5 = 1ml / 6.

C) 37-x (SW1257) 400/.4ml
1. 0.1ml MO-A60
2 ± "
3 .001 a
4 .001 b

plate c 3^35 PM

5/31 The results here are not tabulated but are i below.

APR 23 1955

Repeated.

		T				T.	
B1	–	++		C 1,2,3	–	++ >90%	
3	a	+ (reduced in % + extent)		3	a	++	large trails no units.
4	b	–		4	b.	–*	– dried out before any growth
5	b	– (Recheck carefully – seen)					

(margin: * some small late trails?)

[initials] 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. [initials] It is possible that early chains are b and later are a or vice versa.

Save C1 23 B1 B3 for belated photos 5/31.

5/31

from 5 plates of B5 and 2B4 looks for any

trails or suspicious.

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

B4/a ꝏ

b ꝏ

c ꝏ

These may have had early ~~b's~~ a phenotype with little enough b to survive in presence of anti b.

Plates to DCG to attempt reisolation of these trails.

6/4. DCG found (on replating isolates)

a: gave few 1's and many clusters (ca 100 cols.) (after 3 hours incubation 37° then overnight at 23°. MGA) photographed.

b: pure 1's

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

DATE: APR 20 1955 REF:

	1	2	3	4	5	6	7	8	9	10

See 1257 pages.

3:05 fuse drops

APR 21 1955

1252 pages.

fuse drops 1:45 2:10 - 2:35 spot 56 (1)

D C B A

[diagram: spots in columns D and A, (4) ↑]

A ~~A~~ 1 - 15
D 1 - 11.

Examine 5:30 Most active ~~—~~ without being haevilly are

{ A 2, 3, 4, 5, 7 B1 B7 B9 C 1 2 3 4 8 14
{ D 2, 5. Check for correlation c̄ swarm, trail.

5:15 P22 - Pick swarm clones + plate samples
est 10^4/~~ml~~ 10ml = 10^3/ml plate .02 ml, .1 ml samples in MCA

1257.

activity is jostling

DATE: REF:

	1	2	3	APR 2 2 1955	log 10	6	7	8	9	10
A 1	(+) + uf			–	0					
2	(1) (1) 1.0			3	4					
3	(1)+ (1) F	v. active		10	4					
4	(1)			10	4					
5	(11) F			4	4					
	T 1.			1	4					
	(T) 1			0	4	Research for (+) by trap:			0	
	11.			11	4					
	uf			–	0					
10	1.	vs		0	4		..		0	
	F			–	0					
	+. 1. 1.			6	4					
	11.	duty		14	4					
	11.			1	4					
15	11.			0	3 pd					
B 1	(11) +	v.a.		4	4					
	uf			–	0					
	1.			18	4					
	++ ...			1	4					
20	TT +.			0	3+					
	1T			12	4					
	(1) 1.			(1)	4					
	11.			3+	4					
	(+) +.			2	4					
	1.	duly		1	40					
	uf T			28	4					
	1 (1)	rot		1	4					
	T (1)	slow		3	6					
35 C 1	uf (1)			c. 30	4					
C2	(\\) (1)			swarm	20% - 3+.					
	(11)			4	0					
	1.			–	4					
	uf TT			–	0					
	(11)			1	0					
	T 1.			0	~~100~~ (200) 3					
40	+.	duly		20	4					
C11	+ ...			1 50% swr	4					
12	(1)			2	3+					
13	(1) uf			–	0					
15 D 18	(1) 1...	"D1" →		40+	3+ (500)	Proved to be swarm now (10%)				4/10
2	1.	2		1	3+		4/23.			
3	1.	3		sgnalse	<1	Inv. uf				
4	1.	4		3	4					
50	1. (.)	5		0	3 lyp.					
	1.	6		8	4					
	1 T	7		Swarm? 100	3		✓	10% mot		
	+.	8		SW 250%	4		✓			(over)
	+.	9		8	4					
	1.	10		10	4					
	1.	11		–	0					
11	u1.	12		–	0					

Save swarms
D1
D7
D8
C2
C11

① from samples directly to bioassay
(plated precise, over)
in stabs for whole clone
in MGA tubes for pooled motile
pool Pla⁻ cols. from MGA over for nonmot.

Replate
D1 - see infus
D8 - too crowded to count swarms
ca. 50%
to recover components strains 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	10 1's ~~only~~; 2 vs T. Ooo
B12	28	22	4	10)	11 + 1 vs T 1 cluster
C9	20	28	6	5	1 tail, def. multi's but compact 28 1's
C1	30	37			4 v.s. tails Ooo. 31 1's
* D1	40	45	10	10)	34 swarms + 13 nm. No tails.

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

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 51 sw — 250 1's
D~~6~~ 7	100?	—	10	[No sw 1st pl D6...]
D8	50+	10%	4	.02 3 1's / .2 3 sw; ~~35~~

swarms are plated but not counted.

Repeats

The data may be grouped as follows:

mots.	clones	
inv	A1 9 11 B2 11 15 C3 5 6 14 D 10 11 ; D3 D5	12+ snl
0	A10 15 B5 C8 D4 A7 ~~xx~~	6
1	A6 14 B4 7 10 12 C7 10 D2	9
2	B9 C12	2
3	A2 B8 14 C15 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 ~~xx~~ see above	~~4~~ 3
sw	D1 D7 D8 C2 C11	

C15-D1 conf. in tally but not pltg.

Inits. 3 active:

D1 swarm: Since 45/55? F/a+ were removed from D1 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 ≳ 10%. Late segregation?

April 21, 1955. True fine trails.

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

(a) Since a does not inhibit b→xb, probably specific.
Is b effect specific? Would need a $Fla_1^- H_1^{i}$, e.g.
(Repeat c S. heidelberg initials?).

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

(2). Late branching? Pedigrees + platings of {N_{10}, N_{13}} isolates

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

(4). Are all segregates H_1^b (a) trailends
(b) swarm sibs — esp. of $H_1^b Fl^+$.
(c) look for b-resistant trails.

Today: (A) Replate a/. and of a/ i→xb.
(B) Start (2.)

Tonight Review notes — summarize for (4).

Does b (minnesota) serum also inhibit $H_1^a Fla^+ \to \times H_1^b$ trails.

any $Fla^- H_1^{nonb}$?

DATE: APR 22 1955 REF: 1250

"b" New Preps FA10 → × sw666 10^{10} - 12^{15} - 12^{40} Pref.
Frese trypsin 240. Harvest 4^{45} ε. 800/ml.
mc. c. 5^{30} - 10^{00}

A. FA10 → × sw666 (.1ml) β a → ×. (.2ml) ε. 102 cells/plate

			A b→×b	9A23: trails B. a→×b.
1. —		—	++	++. 10 swarms, nonecentered
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 c dilution.

B preps. may be late, segregated.

photog! ∴ b, minnesota also inhibits completely. Reaction may be specific for → × H_1^b but this cannot be verified unless a $Fla^- H_1^{nonb}$ can be isolated. (rather of 1250B5 platings?).

C Search for 1b trailets. 1252A2 (1) (2) 1252B2 (3) each show 1 wave edge (prob. serum not diff.)
2 1. Oo
1 4. Oooo } no marked swarms.
3 Oo.

1250B5 4 plates

See 1256.

~~5/31 from 1250B4, B5 search carefully.~~

photos of 1252A, B 5/31

Tetrazolium- formazan chains preliminary

APR 22 1955
APR 23 1955

P22. Prepare stained cultures. Add 1 ml overnight culture to 7 ml broth + TZ .005%
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. Maán 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 termihally marked. (Z = formazan granule)
Hunt for rare medial marked= 7,8

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.s. owing to dirty agar — hard to find individual cells. Try methods instead. (cf. test 1-zygote).
Disadvantage of oil chamber is solubility of released Z in oil.

But prel. observations above bear out earlier concept as regular mode of fission

① (diagram) →

② most Z ~~to~~ unipolar

③ Z dies out.

DATE: APR 2 5 1955 REF:

Standard system now: SW-940 (FA 93; H_1^a) ~~aa~~ —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 ~~s~~ stained (1PM). Sediment to harvest pellet. (31/2 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. ~~Zxxxxxxx~~ Z chains were followed for 4 to 6 fissions. 1 clone gave a swarm (c. 50% motile) = 31B/ E = preponderance of motiles (>10)

Of 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

1, 2, 3, 4, 5, z

or, if cell is

4 | 3 | 1 | 0 z

The result agrees with random expectation, but possibility of a negative correlation of z (after n3 or n4) should be retested. Original notion if E were polar was expectation of:

4 | 0 | 0 | 4 E

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

) Also see data on splitting of intermediate ⊕ and variations in number of ⊕ among clones; partitions among subclones.

APR 25 1955

1254

does this necessitate 6/56

DATE: 8PM.

REF:

	#	app.	3	total	0 5	6	7	8	9	10
1.	5	0 glass unlam.			4					
	4				4/1					
	4				4					
	3	00 no trans.			3					
	8				4	→ 1/2.				
	4				4					
10	2				5					
	4 5(2³)				5	bires 4/16 mot.		c. 0/8	d 1/4	
	4				5					
	4				4					
15	4	(all 4 mot)			4					
	8.	only mot in a.	bires 3/8		4					
18	2 u.	2 mot in b.								
19	New ... cool formold temp 9PM									
20										
21	7				4					
22	5 2²		b. res 8/32		4	8/2	→ . 6. (1⁺, 1⁻)			
23	5 2²		3/8		5					
24	8									
25	16 2 u.				4					
26	4				4					
27	8 2 u.									
28	8 2 u.									
29										
30	4				6					
31	11 2³	u.			3					
32	4				6 (1 var. already)					

Reexamine at 8PM and isolate residual Z from mid. # of var Z. to rightmost drop.

St. RT.

E.G. 31 was

23

conclude 6/56

B A

Z

x

44+
77−

Reconstruction:
in general, rightmost is Z chain cell.
the others are successively later sibs of it.

e.g.

D C B A Z

DATE: APR 26 1955 10^30 AM. REF: 2

	A		B		C		D		E		F	7	8	⊕	9	n	10
1	0	4	4	4	(25)	4	0	–		29	22 → 3^05 PM.			2		4	Z died
2	0	3	1	4	1	4	0	4		2						4	
3	0	–	~~3~~	~~4~~						–						0	
4	2	4	(15)	4	1	4	0	4		18	too close to pick.			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		28	3	0					23	23 → tube for plating			1		2	*
11	1		(18)	4	0		2		1.	22	18 →			1		5	*
12	0		1		0		1		0	3	2					6	
13	0		3	3	0	3	0	3	0	3	3					5	
14	1		(38)	2	1	4	2	4	.	37	28 →			1		4	
15	(44)	4	3	4	2	4	1	4.		50	43 →			3-4		2	
16	0	–								–						(0)	
17	2	4	3	4	0	4	1	4		6						5	
18	(17)	4	12	4	6	4				26	17 →	6 →				2	
19	1. /100, (3).															7	
21	1		0		1		0			2						4	
22	0		4		0		0			0						4	
23	1		0	3'	(22)	4	0		0	23		21 →		2		6	*
24	2		2		0	2+	0			4						5	
25	3		1							4						1	
26	1		0	3	0	3	0	4		1						4	
27	(42)		1		2		2			47		41 →	Z n/ Z out			0Z	×
28	1		5		0					6						0	×
29	–									–						5	
30	0		2		0	3	0		0	2	0					5	
31	0	4	sw	3	0	•				sw no trails	Picked sw later - pure ?1					4	
32																	
	×	↑	×	↑	×	↑	×	↑	total chains in whole clone.								* Medial granule

Left margin: ⊕ / logn; 4 unless 37.; 12^30 / 1^15

⊕ is chain in which persistent motiles appeared.

n = number of fissions for which Z was followed. (not necessarily isolated!)

Save + Recover Fla⁻ sibs in 31B swarm. Plating of sample from the clone gave (1 ml) (.01 ml.) 44 swarms : 77 colonies, no trails.

1/56 ↑ must be $\log_{10}$ Fla⁻ in clone ⊗ must be Fla⁺

TZ, methocel; divided clones 1255

DATE: APR 28 1955 REF:

1 2 3 4 5 6 7 8 9 10

Preliminary expts 4/28 showed that 2% Methocel 4000 immobilized cells so they would stay together after fission and form rubclones. Use to test distribution of Z in a chain, and to isolate after n3 or n4. Make up Methocel in Penassay. The methocel completely immobilizes the bacteria. ?What concentration allows Fla+ to swim?

10

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

Abandoned.

20

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 anomalies.

30 Conc. Most cells stain unipolar. Rare (5% bipolar, subpolar). Most chains show terminal granule. Occ. 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 controls.

40

50

Z chains

APR 28 1955

DATE: 2-3PM 5PM — REF:

(+)	1	2	3	4
A1 A	to A2A			
B		II_4	to L.	~~A4B~~ B5A
C		(III)	1 to XU	A4A
D		II	R	
B1 A		i		
B			0	
C				
D				
C1 A		II		
B				
C				
D				
A2 A	moves.			
B				
C				
D				
A3 A				
B2 A				
B				
C				
D				
B3 A				
B				
C				
D				
C2 A				
B				
C	moves.			

Exp. n.g. too much slippage perhaps fluid added to method drop or motility present. Let go — at here.
c. 5P27

DATE: REF:

⊝ c. 2 30M 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									

DATE: APR 28 1955 REF:

1	2	3	4	5	6	7	8	9	10

1 1252A2 } a-x b
2 "
3 1252B2 } trails? in b serum.

Test by two regimes. Isolate Fla$^-$ H_1^x

B) x — FA10 (H_1^b) + b serum ⟶ ?⟶ Fla$^+$ H_1^b

A) x — FA93 H_1^a + a serum. If x = a, no swarms. If x = b, H_1^x = b.

Results:

1 - Not pure motile. Gave to DCO
2 - Pure motile . } { B no swarm
3 - " " . } { A swarm ⟶

20

30

40

50

DATE: MAY 3 1955 REF:

1 2 3 4 5 6 7 8 9 10

A. Staining in situ W1177.
a. in mixture c .005% TZ under oil — No stg. overnight Some inhibition ?

b. In methocel 4000 20% (in Penassay) + c .1% TZ, isolated colonies only (resistant ?) — stained only in center.

? are these conditions too aerobic ? Otherwise potentiate toxicity of TZ.

Should reisolate colonies; compare growth c, s TZ under oil.

B. Chains from isolated cells. (See c. 4-6 any Then after)
1. In scattered region many chains showed interstitial lethals. (effect of cold ?)

2. Few Z granules now seen.
3. Isolates ruined by spread of growth under them (film of moisture from drops !) A - B - C

D 2 is tangle
D 4 c 20 cells, Z term.
5 n.g.
6 ng
7, ng
10

E 1. tangle, Z term.
2 - 5 n.g.
6 no Z
7 no Z
8 no Z
9 n.g.

A4

Z chains. etc.

Motility in Methocel soln.

DATE: MAY 4 1955 REF:

attempts at leaving cells in situ, according in relation to
A. 28μ MMM beads, in Methocel 4000 cps 2% in Anassay.
General conclusions as stated ~~attached~~ — see 1208 p[illegible].
Some Z⁺ chains c. 100 cells terminal Z cell still intact. ~~Infectibl~~ [illegible] noted — probably more frequent after refrigeration but this is not settled by direct reobservation.

B. 5/6/55. Set up to repeat 1254. Began OK but slow to divide at RT (though warm) & later lost c-g.

C. " Methocel 400 cps seems to slow up motile cells (1257 A I+). Put trials as selector for E cells. But most used 4000 in trials.

D. See 5/8/55. E - a serum: did not completely inhibit initial [illegible] uptake.

1) Possible use of Scotchlite MMM beads as reference markers — there is a slow drift; Maybe better to use 3% Methocel 4000 rather than 2%

2) Wasted expt. in correlating E, Z chains

3) Prelim expt on screening E, $ cells by viscous medium strikingly successful. See ff.

4) But most time last week or 10 days was wasted exc. to improve general impressions & technique.

5/6/00 1258

Lab plans: what to doS? Things are a mess.

1. Currently enmeshed in the fate of Z granules. Can these really givr any important information? By following a granule during the growth of a single cell, one might get a clue as to whether groth is interstitial or bipolar (jn a few cases). To distinguih, one might have to show increasing separation between two granules, before fission in a single cell and thismay be difficult.
It is already clear that 1) terminal granules usually remain terminal, and that this is the most common type, already suggesting a polarity in the cell. Occasionally, bi-antipolar cells are seen (more commonly that bi-synpolar), suggesting that the two poles share something distinct from the fissile center. However, the basic interest in the Z granule for the current problem is the possible correlation with E, and this, if anything is what should be pursued for now. Later it may be conveneint to try to repeat experiments with a polar-flagellated organism.
Another sideline is to use the chains in stiff medium to study other problems, chiefly lethalitym both spontaneous and UV. Also look for data on growth of branched cells. (Twort)
2. More pertinent: 1),look for divided E further. 2) diagnose E,S cells by viscous media. 3) transfer intermediate chains for electron microscopy 4) clean up serotypes of co-segregants-- collect more? 5) For 4 and others need to complete review of data and write up.

3.TODAY: Clean up what is accumulated to look at and photograph.

Start new preps. of 93--x w/wo TZ. Use for divided clones and for Z correlation.

(Sat 5/7/55- Sun 5/8/55----)

Use TZ stained prepn. 5/6. 12n7 Checked first with 1237A1+ for swarm motility. In this series, used 2% methocel 400, diluted c. 1/10 with penassay.

a) use methocel for trap; b) isolate initials in broth trap, then trANSFER to mcl.

The latter was found ineffective (probably still too stiff); By 4 PM, had isolated 13 cells still sluggishly motile in mcl trap, and 7 addl. which were at a distance from reservoir but not now motile. swarm cells were sluggishly motile in this methocel conc., about 50-70% were directly inhibited. This oln. probably wets glass more effectively, at any rate it tends to spread, and a few of the motiles below may be contaminants from 1237A1+/

The motile residuals above were planted in individual drops of broth for class. as Sw. or E cells.

N4: found, in first group: 6 swarms, 3 E, 2 ng, 2 Ɇ.
second 4 E 1 ng 2 Ɇ

Total 6 S 7E 3ng 4 Ɇ

which demonstrates strong selection against Ɇ cells

Detailed counts:

	growth	motiles
1.	4+	9
2	3 + sw	swarm, 50%?
3	4+	2
4	ng	
5	4+	12 (from Z cell, but Z nf)
6	map 3+	0,1
7	sn, 1 mot cell	
8	20 sw	16, sev. shakes, prob. sw
9	sw sw	
10	sw sw	
11	like 8 sw	
12	500	12
13	200 sw sw	
21	4+	4
22	ng	
23	4+	18
24	4+	24
25	4+	2
26	4+	15
27	4+	16

(1o4)

The occasion was also used to plant about 25 single motiles (removed before test below--perhaps should have been left in it) for concordance on immediate and later motility of dividing chain cell. About 12 usable cases no discrepancies, some to one or two later divisions. As none gave two motiles, pres, none of these were E . Of remainder, most gave two app. nm at this division--it may be possible to reexamine these drops tomorrow. What is significance of this crisis in termination? Is is growth in fresh medium? (May still need a good exhausted medium to keep cell size small.)

P8 These were then used in tests for residual motility in mcl. Unf., 1,5 were wasted in 5½% mcl 15 (calc. visc 200) which proved also to inh. swarms. Further tests were then made with mcl 400, 1.8% and 1% (1:1 penassay), the latter being adopted as it permits almost full motility of motile swarms(from above). (This may be too fluid for accurate discrimination against Ɇ, as will be seen). From E: 12,23,24 26, 27, cells reisolated which remained mot; were planted for further classification. → none proved definite E cells. See further below.

Until this is worked out no great in further pedigrees!

—x SW 666 initials in methocel
and Z chains

b a serum.

DATE: May 9, 1955 REF:

1 2 3 4 5 6 7 8 9 10

Prestained prepn. used. (at Z 9:45-12N; phage to 1:20, centr. and refr. 1:40)
(also another preph. unstained = A.

Note immoderate spreading out of methocel droplets. isolate initials in 1% methocel 400 (1:1 2%: penassay).

A. isol. from unstained, plant out in droplets individually.

B. isol. Z-stained initials. to c. 3PM, some fresh isol. C to 4:30
me t in single drops on initial cg. first, transfer latter as families to isolation cg . Ditto for A-- plant out descendants.

D. 5PM B above, in broth traps: pick c. 4000 initials (somewhat late now for tests) in serums.

(Klein visited 5/10-11.)

D: Almost all initials are inhibited in a or b serum, though cells may continue to spin for a few minutes. 7 cells did persist in b, planted out. 3 proved viable swarms. isolate as 1259 D1-3. See DCG for results of platings(after picking to broth) in MGA. 2 persisters in a, but neither viable.

E. same as B-D but not sublined

A: Held to 5/11 for examin, and may have partly diminished therefore.

13 clones 2? E clones. 1% Methocel probably too thin..

B: Most isolates grew out; had been separated once or twice at n_2-n_4. However, of
C: 38 isolations, 3 ng; 5 swarms; only 4E, none interesting except:
E-clones were reexamined for content. In B2, sib to swarms had 22 motiles, transferred to 238 E2,3; 4; 5(sw). The motiles in E2 tested, all gave rise to inviable or E clones & therefore certainly not sw. cells

not methocel

6$ originally looked as if only c. 100+/10^4 but these later proved to be swarms. The clone was not recovered (owing to drying out) to verify original low assay.

B2 0119 111 (22)/10^4
a swarm b 10^4. c (22)/.
Z E
swarm

Otherwise, detailed numbers of intermediates were not recorded.
F: 5/10 interm motiles tested in a serum: at least (28) from 5 clones were immbo. but 2/4 from B15 were not! However, two tested swarms were inhibited; specificity of serum should be rechecked.

5/12/58

Also saved 1259B1 (= b8). Swarm- test purity by plating
B2a,B (= c5 z cell removed at n_5 = nonmot,b) (not not certain record)

5/17

DCG found D1-3 all motile but with confusing clusters. B1: no definite swarms B2b "all clusters"; a pure non-motile. Will have to be rechecked on return

(over)

E: 34 isolates planted w/o lineage afterward.

4E (9,11,15,16)
15E (1, 4,3,1,1,6,7,3,3,5,5,2,1,3,1,4,....)
5 Sw
5 ng.

Only conclusion: medium not adequately selective. Try 1½% methocel 400
(v.i.: 1260)

1259 summary to 5/16

5/12 Plated in MGA

5/13 Picked possible singles. Plates were incubated too short a time at 37°; D1 & D2 had singles, swarms, & clusters; D3, B1, & B2b singles & clusters only. Counts:

	Clusters + swarms	Singles	Singles picked
D1	51	3	2
D2	91	1	1
D3	59	5	4
B1	32	21	8
B2b	90	17	8

(Singles picked: these spotted on MGA)

5/14 All "singles" picked 5/13 & spotted on MGA were motile (Spots had appearance of "clusters" rather than swarms).

Plated again: All original broths, + some of singles picked 5/13 (D1, 2; D2, 1; D3, 2; B1, 2; B2b, 2.)
Incubated 3 hrs at 37°, overnight at 22°, then refrigerated until examined 5/16.

5/16 Results of 5/14 platings:
Original broths:
- D1 Swarms, centered swarms, & col. c̄ "satellites"
- D2 ~ D1, higher proportion of swarms.
- D3 ~ D1.
- B1 trails, clusters, apparent singles; no swarms
- B2a pure non-motile
- B2b All clusters

Presumed Fla⁻:
- D1 (1) All clusters } no singles
- D1 (2) Clusters, swarms } no singles
- D2 Clusters, swarms, no singles
- D3 (1) Clusters, swarms } no singles
- D3 (2) " " } no singles
- B1 (1) } Clusters, trails or satellites; possibly some singles; no swarms.
- B1 (2) } Clusters, trails or satellites; possibly some singles; no swarms.

N. - 62 MAY 10 1955 Best resume page

- 1259B. ABCDE

Sm abc 3 swarms, 5E / 34 isolates

disappointing. No E conv. Z. But write out detail anyhow

B1 | (III) → (37+) → 13/104. → E

B2 | (1) III → | II → (5)/104, later (22) 1/37, sw E?

B10 | | → but only 1 drop seen. 6/56. no

B15 Rec. confused. sw E

C1 II | → only 3 drops? (prob Lng.);

C2 III | → 1/9 → letters

C4 | 9 → But only 1 drop, + (100)/104!

C5 | inf → /22 → whence 3 drops? (3d has 1) prob. 22 sw.

C8 | | IIII. sw sw

(over)

C4 (+) many to [238] H2 }
Residue to F2, F3 } Hold for full analyses if needed.

C and B were evidently confused yesterday!!

MAY 10 1955

Do not save these swarms owing to possible confusions.

But study closely B2 and C4.

B2 is swarm / 10⁴. ⁴⁄(5)/10⁴

(1) ... / i → " 10%.

↘ i (5)/10⁴

n_2 n_3 prob.

C4 is (1) → $\frac{>7(100)}{10^4}$.

---

[238] divid before piling

--x SW666 in methocel

Partitions

May 13. New prepn., unstained. (probably usual, about 90-120mins.)

Fuse drops 2:30 Collect to 3:30. Cf 1259D motile.

No initial was nearly as active as 59D. Pick those that have moved the furthest, not necessarily v. active now. Estimated yield, 10% of broth yield.

Note : to compensate for spreading of methocel solution, use cg. that has been greased (human), then flamed. This works well, especially with larger drops, but smaller drops are too convex for best visualization. Intention was partly to look for early chains (E) in the methocel, but time did not allow and most isolates were made to broth directly/(A, B resp.) Lineages were separated at n_2-$_3$.

A: 1,2,3,6 ok. Partitions at n_1:

14+ :1 6:5 ng snakes. Later transferred entire clones to get fullest estimate of motiles.

A1 came out +(14):6 Sepn at n_1 =

B1-14,21-36. 4 ng. Mostly non E. Records show at first scanning:

2:4;1 14 2:1 3 7:20 0;4;1 1;3;d 3 24+ 2:1 1:0 5

sw;sw;sw;sw (1260B33 later DCG verified purity of each). 6:5 7:+

Underscores were rechecked (on ungreased slide!) and following definitobe values for splits on these:

1:20 8:20 2:2 4:12 3:2 7: 26 Therefore no equal splits.

General totals:

E 5
ng 4
sw 1
~~E~~

33

Little if any selection for E in 1½% methocel.400. Need 2% which probably totally stops many motile cells.

No new experiments after 5/14 Trip to NY 5/18 - 5/29. Resume lab work 6/3

→ x SW666
Methocel

JUN 1 1955
MAY 31 1955

Pupa 93 x SW666 (1:1) 10^{05}–11^{45} (12^{30} sic) Rf. c c. 4PM.
c 4^{30}–5^{45} isol. residual motiles (in antigen). Est. discrimination factor

A) Note: to prevent spread of methocel, e.g. are lightly greased with fingers (on one side); flamed; oil added. However, motile selection seemed most effective where there was appreciable wetting and spreading of the drop on the coverglass.
Isolates transferred to fresh pen-assay drops c 6PM. Inc 30°
Counts of (+) / 10^{3}–10^{4}: 4, 20, 20, 3, 46, 4, 6, 2, 50; 3, 20, 18, 3, 20, 7, 11, 13, 10, swarm!

JUN 2 1955

Σ: (6 E : 3 ∉ : 1 swarm + 2 inviable)

∴ with 2% methocel 400 there is effective discrimination. At this conc, Fla+ (1237A1+) was greatly slowed down (10x ?) but most cells did continue to move.

[Note — to this point considerable interruption in continuity of work was occasioned by ① trip to NY for ascites meeting ② breakdown of manipulator — hole in diaphragm, temporarily repaired].

∴ continue pedigree studies on preselected initials.

Swarm: indirect plating of clone, in 1 ml, .01 ml gave 44 swarms / 265 singles
again note low ratio. Preclude competition!
(see photo — plate had been held at RT overnight, inc 2½ hours 37°, then RT 4 hours.

McL 400 2% solution.
Pedigrees.

JUN 2 1955

A). grow in pen assay B. grow in McL. 1261 pups.
Plant to fine drops c. 12^{10} PM.

No cells continuously motile like Fla^+ clone were seen. Picked the most active. If continued, probably need to regulate the degree of wetting. Did chain pedigrees to 43 - 45 on 34 minute transferred to broth.
P3, scan for E, ϕ, swarm. Found only 3 E clones.

A 3 B 8 C 2

A3: A → (1); → B (2), C (40)
B8: A → 0 1 0 2; → 40 (30), (4)
C2: → (→ 0 A, 36 ?), (→ (1), → (25) (2)); → Lost, 3 → A6 0

∴ splits are 1:42 3:34 and 28?:0 (known 1:27) 2:25

One swarm clone C4, already pure. DCG checked purity of each clone by plating.

C4 → (++), (++), (++) (++), (++), (+), (++)

Save! as 1262 C1-5 for H, check.

Swim tests were only 3E, 1S : 22 ϕ and 10 ϕ (= lethal)
3E ↓ 43, 37+, 28
22 ↓ [11-0; 5-1; 2-2, 3, 4, 6, 7].,

the experiment was quite unsuccessful.

Again review Salmonella data to get paper out of the way.

July 13 1955

A) → Should first get general picture of experiments + what they were!

Write out 1138 B4

? → x SW666 Lp$^+$

Note diminished motility of large cells. Occ. early isol 1. → ①/10^3...
(remarks on growth cycle) e.g. 1141 B4

7/55

	Σ	a	b	c	d
1141 A4 v.p.					
A5	3 {	1	1	5	—
		2	6	6	11
B1	1	0	1	1	1
B3		0	1	1	1
B4 vp					
A1, A3, B5, C1 n.g. (stayed motile)					
C2	2	1	2	2	av 3.

(A4) (v.p.) Σ = 33.

*① first suture!

	Σ	a	b	c	d
A_2	1	2	2	19*	—
B_2 – $D1_{15}$	18	6	16	16	29
– G2 15	1	20	27	27-29 / 40	37 / 45
C_2	1 n.g.	2	2	2	2
B D2 5	11	9	15	15	29
D_2	1	2	2	7	20
G2		16	23 / 23	33 / 24-26	47. / 34

note partition: 19 : 11
or 1 : (19 : 11) : 1 : 1

But cannot use as G2 may be listed as from B4!
B4 If the latter, then

This datum is unreliable.

subscript = point of this branch in the pedigree.

7/13/55

		Σ					
1142. (9)	C3	>50 5 tested		6	10	31	36
	D1	>100 10 tested		7	24	41	45
	C4	10 2,1,3,1,0,0		3	10	—	— no record
1143	E3	5		2	2	12	14
				3	12	—	—
			s_1	2	2	2	7
	E2	11	s_1	2	2	3	3d
			s_2	3	10	16	23
				3	10	42	48
			s_7	5	11	27	31
				5	11	31	38
				5	11	14	—
				5	11	—	15

1144

Leifson cultures.

1272

SEP 9 1955

all 6 cultures grow as well or better at 30° as at 37 except 205.

For preliminary comparisons, reinoculate H1, H300, H32, H37

1:5 in broth + reincubate 9AM –

1272

SEP 8 1955

Leifson's slides Seeliststtes [illegible] figures [illegible]

H1 A. 1200x aposl. P. aeruginosa typ.

no clear antipolar pairs. sprouting?

polar monotr. unip.

H 300

ditto cells larger than above.

H32 vibrio? multitrich, antipolar "alcaligenes" (Lophobacter)

both

H37 large cells.

H205. more usual

rare

prob. intermediate polar multitrich some bipolar!

H430 usually unipolar

(over)

See [242] protocols.

Conclusions

a) 1 pedigree possibly

```
              +
            /   \
           +     −
          / \   / \
         +   − +   +
        /\  /\ /\  /\
       + − + + + − + −
```

b) No great regularity; some + < − +

some + < + +

Could be studied further

H 1 is best culture