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/~~mmk~~ square) by 2:10. At this time, earlier isolates were mostly 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.	~~[illegible]~~	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

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

5/50 transpl. (46)

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

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

single cells: trails 1221

FEB 9 1955 230

50 → XSW 967 9^{50} — 11^{45} — 12^{35}

Collect individual Fla+. Explant series ACEG

Let remainder form layer clones overnight.

Series	A		A'		C		C'		E		E'		G		G'	
	0	0	−	−	−	0	−	+	−	0	+	−	0	−	−	+
0 gaw	0	−	−		0	0	−	−	0	0	−	−	0T	−	+	−
	0	−		+	T0	0	+	−	0	−	+	−	0	−	+	−
− n.g.	−	−		+	−	−	⊕	⊕	−	0	+	−	0	0	−	−
T trail A1-5	T0	−	+	+	−	0	+	−	0	−	+	+ −	0	−	+	−
	−	0	+	−	−	0	−	⊕	0	0	+	+	0T	−	+	−
	0	0	−	−												
	−	−	+	−												
34 clones viable:	−	0														
	0	0	−	∓												
4 trails	0	0														

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 root agar

+ = clone gaw

⊕ many Fla+

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

Motiles detected (probably some more): ① ① ~~[illegible]~~ ② ② ② ③ ① ① ⑳. Clones were about 10^4 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 i.e. 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 misinclusive. 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 1 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 Fla+ from "SW967" (= ? single colony isolate). 10 → x, 9 → x, 50 → x

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

FEB 16 1955 φ + cells 9^35 AM —

A [231] → Pick as single cells (probably many at 2-cell stage) to Motility Gelatin Agar. (MGA) Ca 2×48=96 picked to two plates.

B [ ] → clones (small droplets).

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

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

Totals (note discrepancy – medium difference? – or does the pres. that some of these were non-motile – see protocols) bear. On second plate, viables were: E6ab, F 1a, 2ab, 3a, 5ab 6ab G 2b, 3a, 5ab, 6b. H6ab).

C. P16 also plated loop + ~~[illegible]~~ wip sampler of FA 9, 50 → x SW967

No strong trails seen (pres. agar!); needs repeating. In toto: from 50 → x 1T, 1 swarm ~~[illegible]~~ (sic; i cluster!) per 13 loop spots and nothing else. Save swarm as 1222 C1.

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

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

Plate → x SW967

Spreading

FEB 17 1955

50 → x SW967 Usual routine.

Collect ca 50 Fla+ in ~~100~~ ca .05 ml ~~H2O~~ broth, plate
-100

out on (old) ~~##~~ MGA and MA no G (spread .01 ml samples)

	Colonies	Cc 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

50 – XSW 967 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 subseq. use. (105 PM).

3³⁰ PM Isolate Flat – 4¹⁵
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 upip drops left under oil → 14 clones. (+ 2 ?)
plate these on pres spread agar. In rough screening 2 clones were noted as ~~having~~ phenicatinate (Eq 10 ?)

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

From est. of drops replaced on c-g., 2/8 drops have cells.

14/96 in drops made anew.

2 clones (eq 10^{6+}) were spread out on ~~the~~ MG A plates
1 clone gave about 6 isol small deep 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 trail 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 trails 42 colonies (smeared).
2 " 48 " fairly discrete.

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

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

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

∴ non random distr. of cells in pipsums certain 4 of 1.

" 43 spots → only 3 colonies
0 trails

sep. colonies per loop as noted:

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

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

>50 colonies/trail at 16 h.

How account for so many descriparcies:

extreme variation. gave T/C

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

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

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

2. Fresh plates, spread
2x .01 ml 8/90

Unspread
.03 ml 9/? (assume 135)

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

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

Note extreme variability (& sampling?)

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

~~loop~~ up. content est at .25/drop
∴ = $\frac{.25}{45} \times .01$ ml ≐ .00005
= 5×10^{-5} ml.

No clear effect of respreading.

FEB 21 1955
(Mm.)

Collected 896 motile cells from same conc. suspension as 1225 (refr. over weekend). Transfer to 0.2 ml broth for plating cups. (Transfer directly from pipette, in two runs, this time). Various platings.

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

	Colonies	T.
Y	37	7
W	46	7
W	55	5
W	50	6
Y	–	4
Y	–	9 + 3 wh.
	138_3	38_6

∴ media are not different. Average T/C $= \frac{38}{276} = .138$ ($\cong 1/7$)

f 1225 = 8/90 ? 7/41.

Take 1/8 as rough average

FEB 22 1955

2. Pour in MGA. 0.01 ml

a. thin layer, then cover	23	5
b. 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 (loop D) c/s prespreading plate surface.

~~c. w~~

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

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

no eff of DOH?

altogether this plate shows 17/40; cf. 10/65 above.

$\chi^2_1 = 7.4$

average cells ca 1+/loop

$p < .01$

Try in chalcetubes

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

In (2) 1 trail ≐ 125 μcolonies at 17 hours!

FEB 27 1955 Some unions noted in the drop platings too, though not long incubated.

FEB 23 1955
FEB 23 1955

Problem: $\not>$ 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 Isolate single individuals. A. 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 initials for concordance of ratios.

FEB 23 1955

FEB 23 1955

II 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 $\ll 1$ than 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 SW967 x— clones. 1227

see 1237 summary

FEB 22 1955
FEB 23 1955

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

A23 Concentrate ca 10x for trapping.

try water?
broth?
buffer?

11 AM drops fused.

PM - N'v'ly many Flat wire found. Maybe too dilute some suspension or otherwise. Both tried. Altogether, ca 40 ⊕ wire isolated singly.
Group A isolated ca. 12N, → 2, 4 cells at 3 PM
B 2-3 PM → pers. 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 18 mm (ca 20 ml) } all had clones
4 12 mm (ca 10 ml) } no trails. Minor trails seen in each but only near air (over)

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

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

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. (extreme) in centrifuge. Keep refr. when not available.

8^{55} traps set up. By 10 PM still rel. few ⊕. Isol c. 15 + 2 + 2 and pour in shelse tube.

(27C)

Yields → 14 clusters – all flowery trails!
+ ? swarm.

1228
i

FEB 24 1955

Stock of 1227 C. — n.g. Newstock

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

Clone procedure:

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

(2) Collect up to 100 motiles. (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 Penassay. Incubate (3 hours at 37° OK)

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

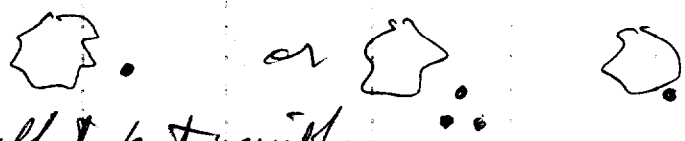
Add 10 ml MGA & pour plate at indicated time.

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)



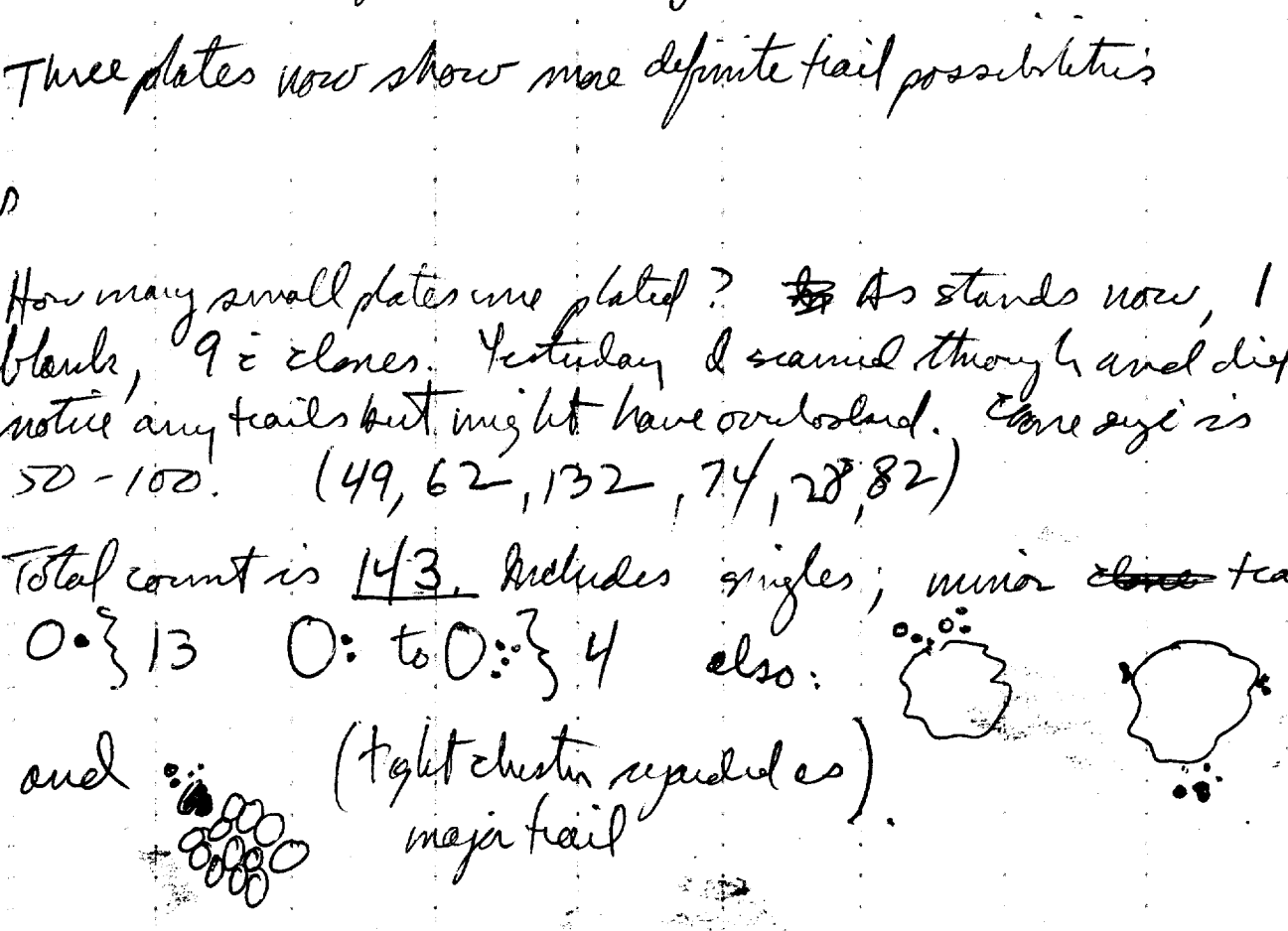
about 1/20 colonies show effect, but variably.

Three plates now show more definite tail possibilities

small plates

How many small plates are plated? ~~to~~ As stands now, 1 blank, 9 ± 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 143. Includes singles; minor ~~clone~~ tails:



O• } 13 O: to O:: } 4 also:

and (tight cluster regarded as major tail).

2. 61 singles and

Total count: 66

3. and 7 minors.

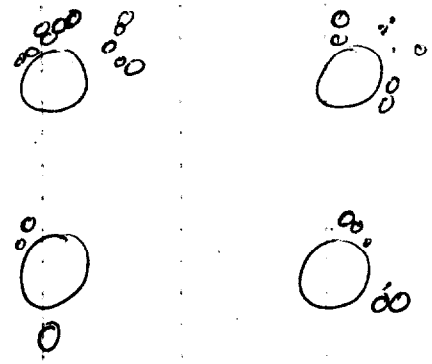
Total count: 50

12 28
3

FEB 26 1955

Large plates — 3 blanks; 6 clones.

plate 4: Several multiple minors (&8)



Other plates similar.

1 has few if any minors.

28 D. direct plate of pool.

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

now count 11T/~~[illegible]~~ cols.

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

FEB 27 1955

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, i.e. CH_2O, as 28E.

FEB 25 1955

Prep Conc. 8^{50} (37°) – 11^{10} – ... 12^{15} fuse drops R.T.

fresh materials.

	Total.	No clone	[used uninoc tubes ?]	Clones is T.
tubes	5	~~4~~		1: (227) A1
plates 10 cm	6	5	"	1 49 1 2's
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 80; 12 major trails.

---

totals.

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

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

$(1/7.8)$

Renumbate 10:15AM.

/ clone size

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

1 40 p 26 – 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 misnoted.

(2's = ∞)

FEB 26 1955

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

photo ng.

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

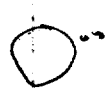
Remaining 24 clones: 15 had all singles. 6 had 2's (3_1; 2_2; 1_3 resp.) = A11-16

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

call T.

A17 1 had a debrois surface pattern probably splatter

A18 1 had what looks already like a minor trail



Remi. 11 AM.

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

1229

FEB 27 1955

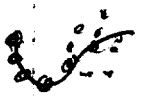
~~Plates are left in~~

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

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.

1229A: (small plates) Reincubated & examined 12^50 P27.
A3-9 had major trails. Look at H 1-10, 17, 18. No comment unless something new.

A3

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 8 large n columns.

Photograph 4, 5, 8

see D

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

Spontaneous trails from SW967 1230

DATE: MAR 3 1955 REF:

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	✓	swamped (both plates).

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

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

D. Reexamine same traps (left. r.t. overnight) N3: no + sem) 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:

1228 Stock. Isolate ca 30 mins P2 — Refr. pro tem.

A. ~~30~~ 47 clones (ca 2⁵) plated in tubes + plates

P4 22 were inviable. Of 11 plates + 14 tubes viable,

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: profuse clusters were noted

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

→ x SW666 → x 541.

→ x 1040

1232

MAR 5 1955

A. TM2 → x SW541

B. → x SW1140 (paralyzed)

C. FA37 → x SW666.

2 hours inc. 1 hour ref. Conc 10X. (to ca 2PM.)
1:1

Isolate ⊕ from C (2^{30} - 3^{30}). Pick to 1/ml penassay by 4^{03}
Incubate 37° 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.

2/6: 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 1 ml and samples plated in shake tubes.

(1) .2 ml	swarm throughout. Discern 7 single + 2 (+1?) trails*
2) .2 ml	No sw. 11 "single" 3 trails.
3) .2 ml	Swarm top half. 3 trails 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) 12 ml (residual).	1 sw. (bottom 1/2) 15 singles 2 T (semi linear)

(1) *

not nearly linear

2). 1 more or less linear & branching?

of est. 100 cells thought ~~pic~~ picked, then

20 inviable
5 swarms
13 trails
62± singles (incl. smaller clusters)

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.

Save tubes under formalin (11R6)

for sample of 300 plated!

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

DATE: MAR 6 1955 REF:

Read individual clones (all in small plates).
16 plates negative; 25 c 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, 15'. (would have been trail if aggreg.?)	8
5	1 trail (non-linear) 2 4's 13' ...	109
6	1 trail (non-linear) 13' 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. } ? ≐ oligocatenates ?

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

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 - if linear / 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 Salmonelle.

prob not.

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

13
MAR 1~~4~~ 1955

Further tests on → SW1140.

X— FA 22
766
37
84
85
—

no mutty overnight!

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

would
would

→ SW666 plate clones etc. 1233

DATE: MAR 7 1955 REF:

Same stock 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 0.1ml samples in MA, MGA.

B. Single ~~clones~~ cells transferred to ca 1.10 PM. Inc 37° to 4.30 Plate out. to 5PM.

cf 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. all singles	103
	4. " . 1 close cluster 4-5 col.	125
	5 3 2's	53
	6 8 2's, 2 3's 1 7	125
	7. all singles. see 18 also.	170
11 negative plates.	8 (mix MA MGA) all singles.	(141)
	9 4 2's	118
	10 3 2's	335
	11 1 2' 7 total	7
	12 2 swarms. Salm.? No	
	13 2 2's	168.
	14 1 swarm, 1 small frail 188 colonies total Salm? NO swarm	188
	15 Covered by swarm also c. 100 colonies, no frails seen. some lytic areas. ✓ Salmonella	

~~others have no 2's, Frails~~ ...

A ~~totals~~ 100 mor,

4 sw / 24 trails + clusters / 98 + 20%?

DATE: REF: 1233. 2

	1	2	3	4	5	6	7	8	9	10
16	2 2's							200		
17	2 2's							118		

18 (MA) : all singles

+ 16 addnl. plates s 2's or trails : Counts 208, 28, 95, 66, 75, 98, 207, 64, 198, 111, 214, 134, 32, 91, 89, 249.

34 plates c clones. 13 s.

also ~~10~~ tubes ~~5~~ 5 MGA.

1 swarm + ca 100 Colonies (n1).

1 cluster of 10, 4, 2, ca 100 colonies.

3 : singles.

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

A (ca 20 / plate calc.) = .1 ml.

MGA:
1 (.05 ml) 6 trails, 2 (minor?) trails; 22 singles / 30 no sw.
2 (.1 ml) at least 2 swarms (obscure most of plate)
: also 1 trail or swarm origin (Pure?)
3 3 swarms 1 surface trail 28 singles 23's 12!

MA 15 single or 2-3'. 7 trail or cluster

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

5 1 swarm 5 trails 14 singles.
manifestation doubtless better than in MGA but minor colonies also too fuzzy.

DATE: MAR 8 1955 REF:

1	2	3	4	5	6	7	8	9	10

new pyps. mix ed 10^{40} – 12^{05} Then centr to 12^{35}. (stock rfs.)

A Left on slide RT to 2^{05} PM. Traps fused.
harvest + transfer 50 ① by 3^{30}.
(ca 3 PM). Plate 5^{00} – 6^{10} 48 transfers plated

B. New traps. Harvest 200/2 ml. Plate .2 and .1 ml samples + plate in MG-A, MG4A.

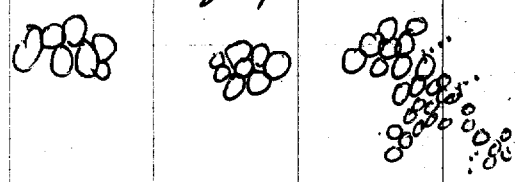
A. Gather low recovery: 33 are negative (Remmit!)
Of 15 positive clones: 9 had only singles.
done sizes v. small: 14, 14, 4, 7, 4, 24, 1, 18, 6

MAR 9 1955

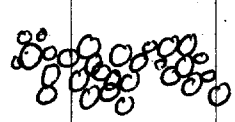
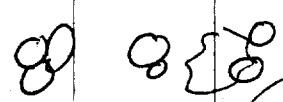
Remaining 6:

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

shock Try temp effect on motility

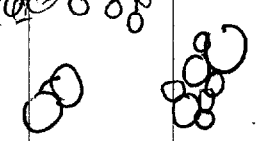


Total : 8

2. +  + 8 singles + 

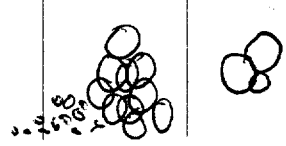
14

3. + 4 singles



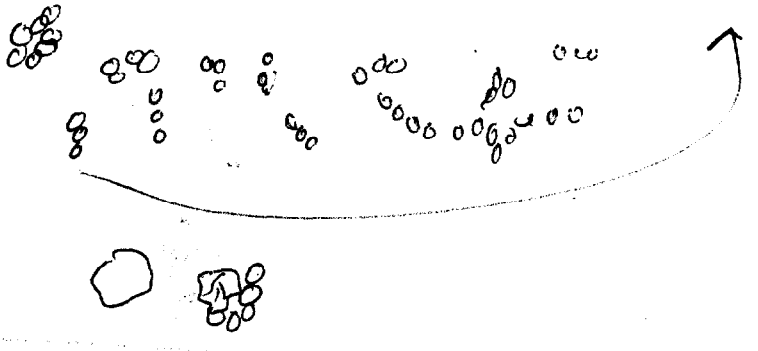
8

8

4.  6 singles

(over)

5 more diffuse tail reaching ground.



+ 6 singles +

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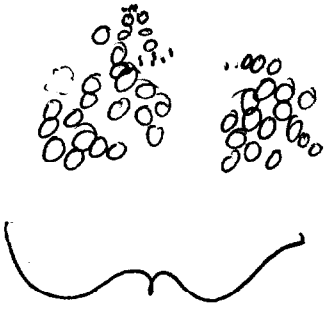
6.  and 6 clusters of 3 – c. 8 colonies each.

photograph! 6, 2, 3.

MAR 10 1955

On remutation, 2 addl. positive clones appeared.
Count: 16 singles
4 "

Remaining plates show no change except surface overgrowths. No minor tails or marked tail extension.

3/9/55.

G4 = gel 4% rather than 8%

		ml	T	Clusters	Cols (singles)		Sec.	
1	overlay MGA	.2		1	16	17	0	
2	MGA	.2	2	~~2~~	13	15	1 ?	
3		.2	2		12	14		
4		contaminated						
5	MG4A	.2	3	1	18	21		
6		.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	1	1	2	13		
13		.1	2	5	5	12		
14		.1	7	1	1	9	1+	
						149		

Trails not greatly different MG4, MG8A.

13 sec! 18 trails 2 colonies Save for photogr.

How such an odd one? Abnormal distr. of cells, or cooler agar?

14 ditto. Could they have been exch between MGA, MG4A?

May have to repeat expt. cf. when chilled

Conclusion: ① Effect of gelatin concentration is indecisive

② 6 clones/15/48 had trails. But distinction between major and minor trails may not be so clear cut as most of these clones did have several aggregations. Note: clones small.

③ Why low survival yield, but apparently selective