1219

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/~~square~~ 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.

	No. of transfers.	~~Exam~~	Grew	Trails
FEB 9 1955 Series A-F3:	—	7	6	0
	SW666	4	—	0
	SW967	6	—	1
F-6-H	—	10	7	1
	SW666	10	—	1
	SW967	10	—	2

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

Totals:	—		13	1
	SW666		17	1
	SW967		16	3

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 from large clones. Examine for prevalence of motiles but do not isolate. Explant in deirided sampler. Compare clones with initial transplants.

single cells: trails 1221

FEB 9 1955 230

50 → Sc 967 9:22 — 11:45 — 12:35

Collect individual Fla+. Explant series ACEG

Let remainder form layer, clones overnight.

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

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

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 not again

+ = clone

⊕ may Fla+

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

Motiles detected (probably some more): ① ① ~~5 6 20~~ ② ② ② ③ ① ① ⑳. Clones were about 10/each.

If at least 20 cells are coded for "E" type", then E = 1/48.

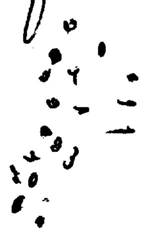
Detectable motile after 13 generations = 9/48.

② although none of these gave trails, the apparent incidence would be about 4/34 ≐ 6/48. ⇒ number of clones ≥ 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?))

FEB 16 1955

Note 2/12, 2/15 failed to get any Fla+ from
"SW967" (= ? single colony isolate). 10 → ×, 9 → ×, 50 → ×
Repeat, cf. "stock SW967" c this isolate.

FEB 16 1955 φ + cells 9³⁵ AM —

A [231] → Picked as single cells (probably many at 2-cell stage) to Motility Isolation Agar. (MIA) ca 2×48=96 put and 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 – ser protocols) bear. On second plate, viables were: E6ab, F1a, 2ab, 3a, 5ab 6ab G2b, 3a, 5ab, 6b. H6ab/.

C. P.16 also plated logs + ~~...~~ ... samples of FA 9, 50 → × SW967
Nothing trails seen (pres. agar!); undo ... ... pr tote: from 50–× 1T, 1 swarm ~~...~~ (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.

~~Streptococcus~~ (1224)

Plate → SW967

Spreading

FEB 17 1955

50 → SW967 Usual routine.

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

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

This was reasonably 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 tail-forming colony as 1224-A

SD - XSW967

1225

Effect of spreading etc

FEB 1 8 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. (10 5? PM).

3^{30} PM Isolate flat - 2/15
500 (sic) isolated. transfer to 0.1 ml broth. Estimate final density at 2000/ml.

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

see next page

FEB 1 9 1955 B) 96 micropip drops left under oil → 14 clones. (+ 2?)

plate these on pres. spread agar. Microscopic screening: 2 clones were noted as ~~having~~ pluricolonate (ca 10?)

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

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

From est. of drops up taken ca. e-2, 2/8 drops have cells.

14/96 in drops made anew.

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.

FEB 19 1955

Sat The collected sample was used in various ways, partly dictated by remains of the plates. From yesterday's result it was wondered if whether spreading the agar altered its surface to discourage trail formation.

1. Old plate 5 loops (0) 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 remain)
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? (smear)
only 4 trails (pr est. 25 cells).

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

" 43 spots → only 3 colonies
0 trails

sep. colonies pr loop as noted:

prespread 5, 1, 2, 1, 3, 2, 3, 1, 0, 2 = 20 cols.
1 T
>10 colonies/trail at 16 h.

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

∴ non-random distr. of cells in pipettes contain 4 ± 1.

How account for so many descrepancies:

extreme variation: gave T/C

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

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

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

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

No clear effect of spreading.

Estimates per loop agree:
13/5 20/8 16/18.
How about mul fractions?
= 3/8 for 16/18 mean.

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

Note extreme variability (+ sampling?)

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

1226

FEB 21 1955
(Mm.)

Collected 895 motile cells from same conc. suspension as 1225 (refr. over weekend). Transfer to 0.2 ml broth for plating expts. (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 + 3 wh.
Y	–	
	138_3	38_6

∴ mediums are not diffuent. Average T/C $= \frac{38}{276} = .138 \left(\cong 1/7\right)$

% 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. 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 all over week. Not unlikely the motile trails are provoked chemotactically.

4. Spots (loops).

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

		pres. C	pres. T	s pres. C	s pres. T	
a	w	3/4 (20)	1+1S		5	1 swarm or pres.
b.	w	10/12	1	13/13	2	— 1 def. branched? Reincubate
C	(+ POH .05% drop) 20 spots laid 2 def. blanks	18	4	(20)	13	no eff of DOH?
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 rods along.

altogether this plate shows 17/40; cf. 10/65 above. $\chi_1^2 = 7.4$ average cells ca 1+/loop $p < .01$

Try in shaker tubes

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

In ② 1 fail = 125 colonies at 17 hours!

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

FEB 23 1955
FEB 23 1955

Problem: ≯ 1 trait per clone? Would need to test clones of 10 – 100 cells.

A. T/C$_{limit}$ = 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 both tubes. Let grow to size n. Plate out

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

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

FEB 23 1955

FEB 23 1955

II

Isolate motile cells but not singly. Plate out mixtals for T/C values. Dilute to samples of how many cells and let form clones. Plate these out at clone size $\underline{u}$.

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 nuclear density.

if > 1 then too high expectation of coincidences

III. Methods of plating?

1. Spread – restriction in volume; may get away in respiratory
2. Pour plates } Try these now.
3. Shake tubes }

plating SW967 x— clones 1227

FEB 22 1955
FEB 23 1955

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

A23 Concentrate ca 10x for topping. try water?
broth?
buffer?

11 AM drops fused.

PM — Nicely many Flat were found. 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 → pur. 1 cell.
at 3-4 PM Isolate to 1 ml broth each
4:30-8 PM Plate in pour plates + shake tubes

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

B. tubes

		plated	clones	blank	Treatment
(B4)	body plates 10 cm	8	5	3	all show "minor trails"
(B1-2-3)	10 cm plates	9	7	2	all but 1 ?(B2) show numerous "minor trails"

photographs at about 48 hours.

major
no ~~all~~ trails (12 clones)

see 1237 summary

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

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

Shed

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 FD50. Refr.
ca 730 PM Enc. (ex home) in centrifuge. Refrig. 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. — 4 g. Newstock

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

Clone procedure:

(1) Store i motiles ca 2 hours mix fresh cells & phage, conc. in centrifuge ca 10×. (Takes 2½ hours). Then ~~free~~ set up on c.g. for manipulator and set up hop drops. Free. Takes ca. 1 hour more to find many motiles. Thus cysts, usually begins at ca 3½ hours! May keep "Stock" after concentrating & store in refr. as indicated

(2) Collect up to 100 motiles. (A) Plant single hop drops (usually row 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° 0.1°)

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

Add 10 ml MBA & pour plate at indicated time.

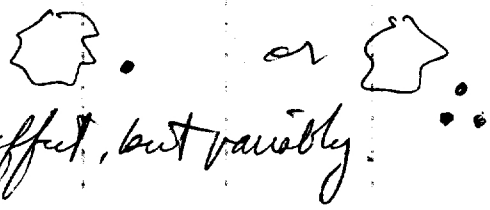
At last few works have been using hypodermic needles, syringes & coupling hardware etc. for convenience.

1228
2

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

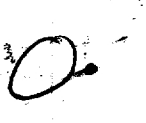
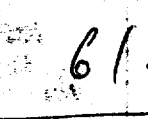
How many small plates are plated? ~~15~~ As stands now, 1 blank, 9 ± clones. Yesterday I scanned through and did not notice any tails but might have overlooked. Clone size is 50-100. (49, 62, 132, 74, 28, 82)

Plate 1. Total count is 143. Includes singles; minor ~~clone~~ tails:

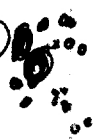
 13  to 4 also:

and  (tight cluster regarded as major tail).

2.   61 singles and 

Total Count: 66

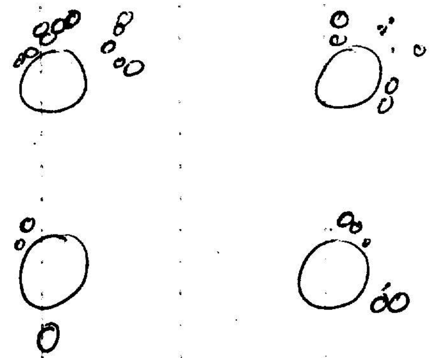
3.  and 7 minors.

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.

28 D. direct plate of pool.

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

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, c̄ CH_2O, as 28E.

FEB 25 1955

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

fresh materials.

		Total.	No clone		Clones ī T.
tubes	✗	5	~~4~~	[uninoc. tubes ?] used	1. (227 A1)
plates 10 cm	↘	6	5	"	1 (44 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)

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

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

140 p M – transfer to 30° Inc. as clones are only ca 2-5000.

FEB 27 1955 29E – 5 clones 1 c map trail = E 1

all show v. prominent runner trails Why delayed, unless

annotated.

(2's = ●●)

FEB 26 1955

Sw967 plated as control on recurrence of minor tails. 5P25.

C. A 26: (2 plates) < 1 too dense (< 2's)
1 ca 400 cols. No MT, too dense to 2's

plating.

A 25: 31 clones. 7 (six) had major tails at 10⁴⁵ AM. (A3-9)
Only 1 tail 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 ~~blotchy~~ 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 tail 

Remi. 11 B4.

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

1229

FEB 27 1955

~~Makes case left in~~

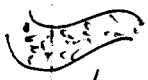
1229C1 : This control also shows numerous "minor trails" — assume that SV967 produces spontaneous trails? Many are unmistakably distinct

These are therefore unrelated to transductions. Need to do more test platings with other Hfr strains. (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) Remounted & examined 12 SD P 27.
A3-9 had major trails. Looked at H 1-10, 17, 18. No comment unless something new.

A3

4  def. terminal branching appearance (spontaneous minors?)

5  column of trails.

6 tight cluster, tapering

7 edge of plate, loose cluster

8 loose cluster

9 edge of plate, branching vertical column

10 loose cluster & large vertical column.

Photograph 4,578

SEE D

D MAR C 1955 Plated, P4, each of 6
single colony isolations of SW967
from C. albicans (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 plate 1 alone: same result: numerous spont. minor trails.

3/1 B. Plate out SW967 Colonies A3 Minor Trs. (40hrs)
666 + —
1091 — —
1092 — —
1140 — —
541 — —
546 — Swarmed (both plates).

under similar conditions (pour 50-100 per MBA, 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. Resistate motiles where seen. mostly unicellulate; 1 di-cell. See protocols.

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

see 1229D.

E C; did not test 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 nemis P2 — Pref. protein.

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

P.Y. 22 were movable. Of 11 plates + 11 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

→ SW666 → S41.
→ 1040

MAR 5 1955

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

2 hours sun. 1 hour refr. Enc 10X. (to ca 2PM.)
1:1

Isolate ⊕ from C (2³⁰ - 3³⁰). Pick to Vrml penetrasyn by 4⁰³
Incubate 3⁷⁰ to c. 6³⁰ - 6⁴⁵ PM.

A showed few ⊕ in traps and B, none
Plate enc. susp. of these on m6 A.

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 precise count. Not poss. to estimate swarms.

Definite trails		14
"Singles" and similar		67.
		81.

There include about 13 clusters of a few colonies (to).

2. 1 (?) swarm occupies ca 1/5 of plate area.

Trails		
"Singles"		13
(Includes 2's	1)	65
3's	6	
4	1	
5	1	
		78.

Overall T/C = 27/159 = 17% ≐ 1/6

(over)

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

① .2 ml	swarm throughout. Descem 7 single + 2 (+1?) tails*
② .2 ml	No sw. 11 "single" 3 trails.
③ .2 ml	Swarm top half. 3 trails 14 singles
④ .1 ml	Sw. ~~tail~~ most of top. 6 s. 1 T. 1 large cluster
⑤ .1 ml	No sw. 6 s. 1 T. 1 med. cluster
⑥ 1.2 ml (residual).	1 sw. (bottom 1/2) 15 singles 2 T (semi linear)

① 

not many linear

② 1 more or less linear "branching"?

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.

Some tubes w/ dark formation (IIR6)

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

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

for sample of 300 plated!

cf. (13 : ~~72~~) 78. better.

DATE: MAR 6 1955 REF:

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

		count (2's = 2)
1.	1 tail plus several small clusters	~~65~~
2	1 tail only, terminal branch?	25
3	0 tail several 2's	73
4	several 2's, 15'. (would have been tail if aggreg.?)	8
5	1 tail (non-linear) 24's 13' ..	109
6	1 tail (non-linear) 13' several 2's ..	44

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

∴ (4 tails)/25. Expectation = 4. } all singly but often accompanied by clusters
No swarms " ≅ 1. } ? oligoclonates ?

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

Remember altus.

Counts on these ones (DCG). 81, 23, 21, 66, 87, 142, 44, 51, 69, 7, 143, 149, 140, 101, 32, 106, 10, 30, 17.

(Note variability - z/two / inherent selection) No addl tails seen.
Yes #7 however (count 66) one colony appeared like a solar system
hollow ball with studs of smaller pigmented colonies.

1232 X

opt out:  Try to isolate to verify as Salmonella. not

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

MAR 1~~4~~ 13 1955

Further tests on ~~→~~ SW1140.

~~X~~— FA 22
766
37
84
85
—

no mottling overnight!

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

said
sold

→ SW666 plate clones etc. 1233

DATE: MAR 7 1955 REF:

1 2 3 4 5 6 7 8 9 10

Same strain as 1232 (ref.) ④ now very prolific (assume negligible continued growth in incubator resp., at 4°C.)

A. Harvest 400 ④ to 2ml ca. 3PM. Ref. to 500 PM. Plate 0.1ml samples. MA, MGA.

10 B. Single ~~all~~ transferred to ca 1:00 PM. Inc 37° to 4:30 PM. Plate out. to 500 PM.
of MA, MGA.

MAR 8 1955 B.

20 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 cols. 125
30 5 3 2's 53
6 8 2's, 2 3's 17 125
7. all singles. SW 18 also. 170

MGA
10 negative plates. 8 (min MA MGA) all singles. (141)
9 4 2's 118
10 3 2's 335
11 1 2' 7 total 497
12 2 swarms. Salm.? No ———
13 2 2's 168.
14 1 swarm, small trail 188 colonies total Salm? NO swarm 188
15 Covered by swarm also c. 100 colonies, no trails seen. some lytic areas. Salmonella?

~~other than no 2's, trails~~, ...

A states

100 mor.

4sec / 24 trails + clusters | 98+70%?

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 c 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 ~~[illegible]~~ tubes. ~~5MGA~~ 5MGA.

(1 swarm + ca 100 colonies) — (n_1).

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

3 : singles.

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

---

A (ca 20/plate scale) = .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 origin (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 nonmotile colonies also too fuzzy.

DATE: MAR 8 1955 REF:

new pyrex. mixed 10^{40} – 12^{05} then centri to 12^{35}. (start up.)

A left in slide RT to 2^{05} pm. T traps fused. harvest + transfer 50 ① by 3^{30}. (ca 3^h 4). Plate 5^{00} – 6^{10} 48 transfer plates

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

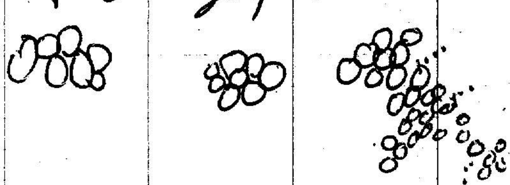
MAR 9 1955

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

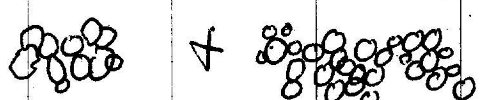
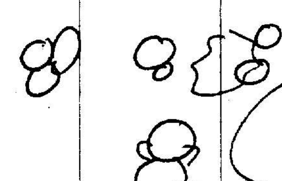
Remaining 6:

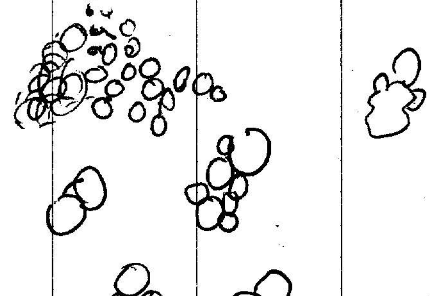
Try shorter develop. effect on motility

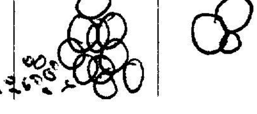
1. "Cluster" trail at edge, ca 40 colonies grading in size, adj. to cluster of about 5 large, + 5 singles + cluster of 7



Total : 8

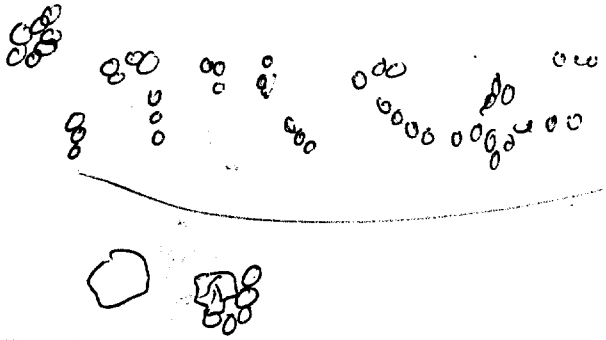
2.  + 8 singles +  (14)

3.  + 4 singles (8)

4.  6 singles (8)

(over)

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

Remaining plates show no change except surface overgrowths. No more trails or marked tail extensions.

3/9/55.

			ml	T	clusters	singles cols		scd.
1	} overlay MGA	} MGA	2		1	16	17	0
2			2	2	~~4~~	13	15	1 ?
3		contaminated	2	2		12	14	
4								
5		} MG4A	2	3	1	18	2/8	
6			2	1		7		
7			1	0		4	4	1 ?
8			1		1	6	7	
9			1	3		9	12	Beaut.-T.
10					3	6	9	
11		} MGA.	1	0	0	8	8	
12			1	18	1	2	13	
13			1	2	5	5	12	
14			1	7	1	1	9	1+
							149	

G4 = gel 4% rather than 8%

Trails not greatly different MG4, MG8A.

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

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

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