9
MAR 1955

New preps.

A. Harvest 200 ⊕/sml + plate MOA. Inc. from c. 11AM to 5PM, then to R.T. 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 sizes again v. variable (some only 1 or 2, others ≥1000). 3 clones picked as most numerous motiles. These were plated c. 6:30PM. Unfortunately the MOA was floccy + plates could not be accurately interpreted. 1 may have had + will need trial. Expt. needs to be repeated.

Limited incubation allows swarmers to be limited and counted.

A.

Harvest:	T	SW	Clusters:	1-Cells.	Total
100	2	1	8, 4, 3, 2⁶	65	~~88~~ 77
10				4	
20		2	2'	15	18
10		2		1	3
20	2		3'	15	~~20~~ 18
20	1	2	2, 3, 3'	33	40
~~*~~ 10			2, 4, 5	9	12
190	5	7	18	142	~~180~~ 172

but again rather poor lumpy and hard to prove. Why so few trails here?

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

MAR 11 1955

MC8A (pipe ~~…~~ Ocl. dc) B ~~+ Tween .01%~~ ~~[illegible]~~

Plated from inoculum cols. 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's and 8 nearby center.

Tween had no certain effect. How about survival? But could be system. error of sampling?

86	~~[illegible]~~ 100
87	85

Swarms were all about 1-2 cm diam, somewhat variable even on 1 plate.

Note very low incidence of trails. Too early selection of ⊕?

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

Pupn stk 3/9/55. (Suite fresh < 2 hours type incub.)

A. Fuse tops 11¹⁰. Isolate to 232 50 by 11⁴⁷ AM.

(A–D) 2/row (a,b); 1–6. = 48 isolates. Incubate at 12⁴⁰.

B. Isolate 700 ⊕ to 12⁴⁰. To 2ml penicillin broth. Plate samples (≐ 40, 20, 10 cells) in MOA c/s Tween 80 .01% Incubate from 1⁰⁵ PM — HPM.

Examine ca 4³⁰ PM to select clones for plating. Isolate: (all have c. 10^{2+} cells and:

1	A3a	SW	
2	B6a	SW	} Inc to 11 PM then R.T.
11	B3a	20	
4	B2b	10	
5	C1a	15	
6	C2a	15	
7	C4b	10	} Inc overnight
8	C3b	20	
9	D2a	10	
10	D3b	10	
3	A5b		

SW. + 1's D half. 10^{30} A 1-1.
SW. + 1's shows 3 clusters (c. 2, 3, 4).
SW. 1's, 1's, 2's, 1's

T
T

as well as 10 more quiescent clones as controls.

MAR 11 1955

(107/366; 11/200; 7/19 SW COL)

A. Controls all had singles only. A3a, B2b, B6a all had swarms and colonies. A3a shows flares best: Isolate is selected for later plating. The other two do so less strikingly. Inoculum should isolate the F1a⁻ sibs.

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

9:

TRAILS IN 9, 10

10

Mp: Selected groups 2T/7
Unselected 0/10

hardly a dramatic result though warranting more extensive study.

Reexamine 232.

1) A3a - act. swarm i residual Fig probable
4) B2b " " " "
2) B6a act. sw. " " "
3) A5b also has numerous motiles (c. 20% or more) swarms.

attendances are not unreadable
(most have no evident on casual examination
some a few str.)

Try. B5a, hes ca. $10^4/10^4$
B6b $10^4/10^3$
~~B~~
C2a $/10^5$

MAR 1 2 1955
No trails.

MAR 1 2 1955 single clones from residual drops of clones A3a B2b B6a and A5b are being tested for motility and saved in selection. also stab originals as 1237-A.

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

MAR 12 1955

Isolates Flat and ~ from 1-4.

Attended on NSA, test on 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~~ 0+: 25-

2 ~~4~~ 7+: 27-

can be used for flare production
in unselected motile lines
diff may depend on selective residue.

~~Rotate 4.~~ pool +,- to stab

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

MAR 13 1955

2411/47 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 ggar.
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: apont 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 + 1sw.
			58w/13/79/100
1233	1/34/47	Single small trails; a few other clusters. c. 2^6	1sw, c, T
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 micr/osc. 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 1 2 1955

The principal point is perhaps best met by experiments like 1234, plating fairly early. A correlation 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 and (or pluricatentation) 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 v Salmonella.

DATE: REF:

1	2	3	4	5	6	7	8	9	10

a) 3/14/55 Collected ca (100) from 1234, 1236 preps for comparison + plated in MGA. (app. left out at RT so result is misleading. 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)
Froze 1:40 Dool. (late) 3:20 – 3:50 A) 200/2 ml ~~B) 50 (1)'s~~ but lost by error.

Running out of gelatin!

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

	Est mac.	C	T	Sw.	Cols.	Σ
SIC (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 c̄ 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 (~.25 ml).
~~½-1 ml of samples.)~~

	NSB	Clusters	Cols.	T.	Sw.	Σ
Est input 50 cells	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
sw diffuse
" " ; TRAILS flourish!

Basic agar must have been unusually stiff. Swarms in NSB$_0$ v. compact also. Thus incidence and quality of trails increases with decreasing agar concentration!

---

also D) Fuse 12^{30} collect to 2^{30} (1). P. drops to 3PM Incubate to ?5^{30} PM. (Some refrig. to 12:15 PM Thurs. for plating:

E) plated 12:15 Thurs.

C: 13 blank 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 blank, 5 clones.

1238 C1 – aerphoto.

T_1 c. 400 ohms

$T_{...}$ << 50 ~~ohms~~

DATE: Mar 17, 1955. Thurs REF:

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

1238 pepn. Freeze traps 12N. Spotted out 1:40 Clones picked and incubated c. 2^30 – 6PM (3½ 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 straight. 1 contam, 12 blank, 16 clones add. 2 swarmed < No def. colonies; 2 large plaques / " " (vague sev. initials; 1 plaque

all singles. no trails

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

1. all singles
2 " "
3
4 " "
5 " "
→ (6) at least 11 trails, 1 major. * see photog. > 110 cols! / T2 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:

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

1. MGA (100). 4 swarms, 56 singles (remnants of yesterdays MGA + Ref. equally tiff)
12 2's
8 3's
5 short trails c 6 clusters

2. MGA (200) (somewhat loose, – of 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) 6 clusters
26 singles
5 3's

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

P c G mels
incubate 3[15] 6 6[00]

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) 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.

19 plates photo

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 LT 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 + ; 15 ; 23 ; 16 + ; 33 ; 13 (incl 2'), 60; 11; 14;

(Gry)

b) in W, ~~agar~~ gelatin agar. (a sloppy - gelatin must try again!). 9 blanks 10 clones.

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 f. + 8 cols.
8 12 " t. 18 cols
9 1 linear t 15 cols
(10.)
~~Fr~~. 7 tails . 0 cols!

these tails off. linear

Conclusions 1938-40.

MAR 21 1955

among motile initials plated

1. Incidence of trails/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!

Butterfield Doubtless spent this week cleaning up away from lab.

APR 3 1955

What happened last week? N.G.

① Acet/gelatin

② No guiding what to do next on chains!

③ Change medium < D(o) - flattens out too far (wets glass - how counteract?)
necessary 10% - poor growth.
Acet - swells in H_2O seemed limited. Metal poisoning ↑?. Try pure water.

problem: don't want to follow mid. pedigree more than 3-4 generations but minimum size drops allow too many cells ~~at~~ ultimately. Should have 1. inal clones of about 300 cells. Try partly exhausted medium.

④ Serum effect

i /TML of course diff. owing to H_2 1, 2

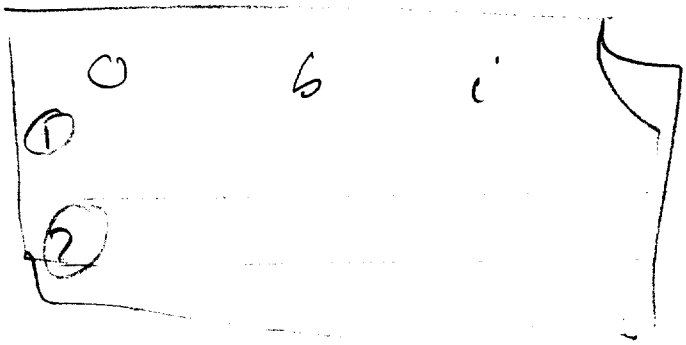
b < 1237-2(H_1^b). at first almost completely inhibited, but some probably inhibits by anti i at 1:100. with overnight growth, see active motility and agglutinated clumps - May still be worth trying at 1:100. (serum titer ca 10⁴).

APR 1 1955

Seven effects

① 1237-2 = b ② TM2 = i

1. Try against sevens 1/200 in both.



0 cells almost completely ruled by b sevens, partly by i

i cells partly ruled by i or b (1, 2 cays?)

Plumb
Trains
Pos. Eff

37-X866

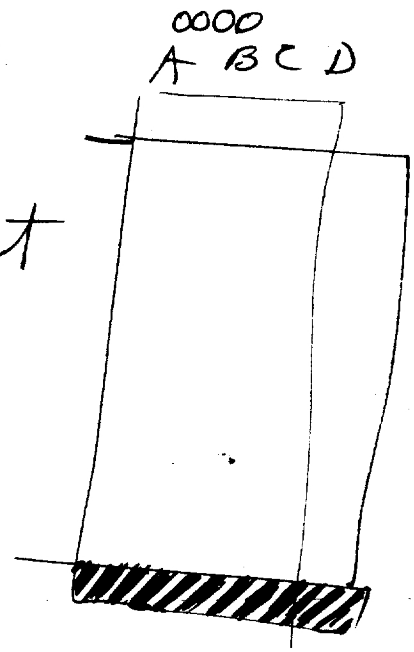
1241 (E)
MAR 31 1955

Inc. Centr. Decant.

New preps. 1/2 : 1/2 10AM — 11:45 — 12^10 Refr.

E F

Collect in 10% broth ; D/0).



Resetops 2^45 (51) by 2^55 spot at

E) 22 deposited to 3^05 A↓, B.

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

to 3^25

Stet R.T.

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

F 1–4 (often (2)) [illegible]

spreads [illegible]

Viscosity?

9^30 AM Poisoning to [illegible]

little growth.

→ Sc866

1241

Misc.

3/28 NC

3/29. 3) 10% broth dilution. A) Permassay 40 ①'s, same split, 1-4. No stuff in many channel, not closely examined (too many cells, >10%.) sent 3 serum-clones A < ⊕ 1 ⊕ 2 C / ⊕.

save for later check of identity + homogeneity.

B < ⊕ 1 ⊕ 2

3/30 cf. 10%, 100% Permassay: middle tubes

To 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 (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° 20 mins., the sediment and decant. Numerous fine granules still present).

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).

B

3 - 0's 2: about 100 Fla⁻. 5 had 1+/c. 100, of which 4 isolated to broth for plating clones. 3 (4?) had 2+ (* C1, 2,3), each isol.

C14 had 29+/1000 C13 23/1000 C6 12/-00. (These 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).

APR 6 1955

DATE: REF:

Note: OCG picked in sequence, but this was randomized for plating. Examine 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% WS3.

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

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

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

29 + c. 20 trails, few 1's.

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

36: Prob cart.; No colonies — cart.

39 sw + c. 100 singles + short clusters.

MGA:

	3	ca 60	no T	2 2's.		
30	7	6 1's				
	15	c. 60 1's				
	19	c. 45 1's,				
note contained mannose 6 yr?	21	6 1's	8 c's (3-6)	1 T^{35}	1 T^{10} (closely).	
	27	Imagi trail +	9 c's (3-7)	+ 6 1-2's.		
40	37	1 T	;8 1's	3 2-3's.		
	43	111's	2 2's			

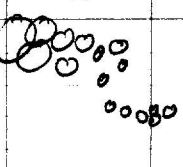
MGA 60% 40% WS3

	4.	ca 20-30	profuse trails	~~sw~~ 50	1's.	somewhat detached
	10	>100	all 1's	24, 10, 24, 23, 14, 6, 15, 10, 19, 24, 30, 47, 13, 11, 100, 150, 6, [redacted], 23, [redacted], 14, 15, 16 and others. (colonies per trail.)		
50	(11)	save for plat.		37-1's	5 c (<10)	21 T's.
	18	9 T's;	38 1's			
	18	seems somewhat tighter aga,	12 T;	3 clusters (3-7);	38 1's	

1242A

DATE: REF:

1 2 3 4 5 6 7 8 9 10

20 1T () ; 60± 1's

22 (partly clogged) 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, 761's.

34 #5 1's

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

20

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

42 14T's 331's (rough counts).

and in photos 11, 18, 30.

30

Test "swarms" in B bac in order

9, 13, 24, 29, 33, (3), 39 ~~plates~~ ✓

APR 9 1955

Note BADS letter — #11 illustrates close & 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 "mop trails"

50

37 → X 666

1242
+ B

APR 5 1955

1:1

① New prep. A flask aerated SW666 × — FA37 } ~~[illegible]~~ Rotate 10^{20} to 11^{35}.
B non aerated " × — }

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

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

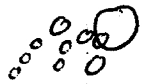
②. Freeze drops A - 12^{30}

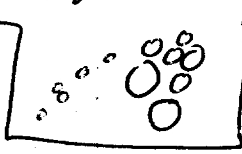
see 1242

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 thing of 8 mau 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? scor.

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

(024)

C – ~30 1's no T

C – 0

C – ~40 1's. no T.

Terminal ⊕ have poor chance of starting a longish trail.
Medium OK.

~5 – 1 } 20 small 1's + ant swarm
2 } ditto
3 " "
4. " "
5 40 1's " "
6 " " " "
7 " "
8 0 " "
9 30 1's " "
10 30 1's " "

(small angles prob. also ant.

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

1242B

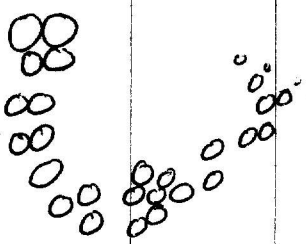
DATE: APR 7 1955 REF:

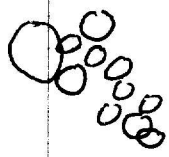
1 2 3 4 5 6 7 8 9 10

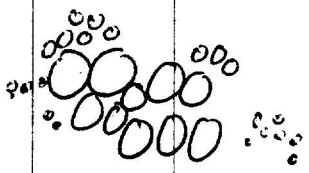
B6: 7 1's 6 clusters 3 trails

Σ 16

Input:



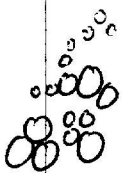




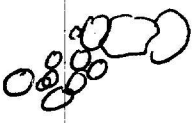
def. linear prob linear ? linear

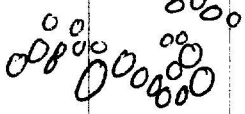
B13 16 1's 1 globular cluster

3 linear clusters (4 B);



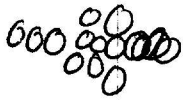
and





Σ 25

prob uni clusters



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

B14 See photog. Most trails linear in exput.

21 1's 12 T's 5 C.

But not C13 nebula!

DATE: APR 6 1955 REF:

	1	2	3	4	5	6	7	8	9	10

3; 40 – 6 PM Trap 1000 D / 1 m². Plate 0.1 ml sampler 25 ml total volume.

				T	C	I's	SW	S
– 1	MGA			88	12	9	5	114
2	MGA		0.03 ml					
– (3)	10 ml	Penassay		129	0	8	12	149
4	"	H₂O						
– 5	"	Baling						
– 10	"	NSB						
7	5 "	NSB	} misinoculated?					
8	7.5 "	"						
9	12.5 "	"						
10	15 "	"						

3 gave best development of trails. Used 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 agar is showing almost 100% trails! all with photo's

St. room trip to bring out swarms more sharply.

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

1244A

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	
			2²	0		20	
* 2				1		43	
			1²⁻³	0		78	
				0		62	
				2 linear		43	
3				0		59	
			3	0		67	
4				2 ~~m~~ U.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	.
			1³	0		44	
13				0		0	.
				0		6	
14				1		20	
				1 extrus.		17	
15						0	.
						0	.
16				6 m.s. - 1 linear		53	
				5 " ; 1 glob.		41	
17						0	.
			1²			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: 12/3/3 (Lt. value 3-25) REF:

1	2	3	4	5	6	7	8	9	10

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 mins.
for large.

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

f8 1/20 +X.

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

Same stops

EMB Le plate (margins etc. for tiny?) 1/20 f4.5

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

1238 B1 mailed.

small plates ~~same setup~~ with lens # 3, no ext tube.
1238C1 . . .
1244A1 (4), A2 (4), (mailed).
1242 C13

4, 9. warm solutions!

1244'

DATE: 4-9. APR 9 1955 REF:

1	2	3	4	5	6	7	8	9	10

Photography 35mm Leica setup again as yesterday

Large plates ~~at~~ no front lens.

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

1243 1-6 1243 ; 3,4,5,6,7,2 f8 1/20 sec.

7 λ-6A6

7

HF 7-6

negs came out beautifully

D19 clear 8 minutes

EMB f3.5 2744-X last + 2769

EMB overexposed

1426A + 6.

~~1243-6 again~~

1238B1

1242C -0

-0

-40

-40

-60

-60

small plates f1 3 hrs.

1241-1 (4)

-2 (4)

1242A 11, 30, 18 (2, 1)

1242 C6, C13, C14

1231B

Resume 1242 - 44.

APR 9 1955

1242

A) clones from single initials.

18 empty

7 had swarms (diffused) + trails [^{The} 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.

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

Terminals

3 0's. 3 0⁺ 5 1⁺ 3 2⁺ and

groups of 29, 23, 12 (C6) (c13) (c14) from 3 others. Plant these as groups or clone in MGA60. From singles, No clear trails from clones. Groups:

~~C13~~ 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 Pen assay or 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 MOB } 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 mine.

"We have never obs. E cells in > 1 subline ~~[illegible]~~ ~~observation~~". How many E clones have been similar

B claims one case of E at n_{22} only 1?

Need myom data on E+E or E+S in 1 clone

style; numerical calculations; first conclusions first.

Where are pedigrees?

No time now to clean up pedigrees.