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 Evening

? were Shapiro's, other strains (W1028 str.) ever tested ?

for first 1500, mostly only 1177 as parent.

→ X SW666 1212

chains

DATE: Jan 11, 1955. REF: ~~214~~ 217

Preliminary trials FA22 → X SW666 (TM2) No motiles seen. Recall that this shows low transduction titer (phage compatibility?)

FA37, and SW686 (induced lysate) → X SW666

Practice run 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.

Cont'd. 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.

Effect of volume on streaks, etc. 1214

DATE: REF:

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

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

cells are 36h. SW666

No T or S!

Recalibrate.

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

1/24 Mot agar

11	0^5	$1S^2$	2^2T^1	/8.
12	0^6	$1T^1$	$2^?T^1$	
13	0^8			
14	0^8			

(4.4% agar NM falls through agar 1 or 2 S/2

swarms would be ill defined. 0 0 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

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

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

FA 37 → SW666 quantitative : ratios of Trails: Swarms

1214

DATE: JAN 19 1955 REF:

Old susp. SW666 (Refr.).

A. 1 1ml SW666 + .1 ml FA37 . Test loopfuls (Dine's loops) on motility agar. Preliminary test to define range for counting T+S, and distributions of T.

2 .01 "

5P19 3 .001 "

4 .0001 "

Plates somewhat smeared.

JAN 20 1955 : 1: Each spot swarmed.

2: 7 swarms / 7 spots

3 1 swarm / 7 spots } useful range?

4 No swarms: contain? }

wait for trails.

~~c fresh SW666 { 10AM Redo tube #4 and compare with 10^{-3} dilution of tube #1 (=5)~~

~~(difference is number of cells.~~

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

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

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

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

allow 6 to stand 20 minutes for adsorption.

1/21 7 ≐ 5.

8 ≐ 6

In re chains

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 cegar.

Tests: ① What is ratio of trails to swarms?

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

② Get a ~~swarm of~~ 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 dry enough)

Try 37-XSW666. If diluted c cells, broth, phenol.

Linkage. (Salmonella)

JAN 19 1955

JAN 1 1955

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

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

SL13 = SW1048 para A.* SL28 = SW1092 = Heidelberg

SW543-666 PB * SW544 = Schuetze O

SW553 - dublin * 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 (c Sino), cf Alek Bernstein.

DATE: 1/21/55 REF:

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

A. 0 swarms, smeared no obvious T. ✓

B " " ✓

D 3 swarms, ~~0, 0, 0~~ O^{12}; $(1S\,1T)^{13}$; $1S^{14}$; $1T^{15}$ –

Note: D13 showed numerous flares; motile cells obviously entered at various points.

maybe too fluid!

C O^{15} 1S; 1T ✓ (Re ✓ A2 = 1)

6: A 6 cm plate O^4 (1S, T) (~~2S~~ 2T, S) (1S) (1T) 8 ✓

B O^3 $1S^6$ $1T\,1S^1$ 10 ✓

C 10 cm O^4 $1S^3$ $1S\,1T^1$ $1T^5$ ✓

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

	1	2	3	4	5	6	7	8	9	10
7:	A (pre "dried" plate)			0^{10}						
	B			0^{9}			47-O	37-O		
	C			0^{10}			2-T	2-T		
	D			8^{9}	(1T)					
	E			8^{9}	1T			39		
8A				0^{9}						
	B			0^{10}	1S	multiple lawns		41-O		
	C			0^{11}	1S			5-S		
	D			0^{9}	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 (Lino's) whose volume, full, is about .00351. loop D, full, is about .00234; flat = .00073; retention .00022., delivering from flat ∴ ≐ .00051 Call this 5×10^{-4} ml, and use about 10x conc of phage over previous expts.

Trails data: misc.

1215

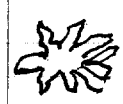
DATE: 1/20/55 REF:

	1	2	3	4	5	6	7	8	9	10

(1) Plate ca 15 SW666 per plate motility agar, pour. C/S underlayer of NSA. In each case, discrete colonies with no doublets or spread are found.

1/21/55

Add FA37 0.1 ml to 1 plate: colonies still discrete 1/25 ✓ A22.

A25 No swarms. colonies becoming more radiate; initially rocy 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 us, note we can detect division but not (directly) multiplication. Present data do not define where events are between gene and final effect.

TRAILS: Grant.

JAN 2 4 1955

901 FA24 — x 666 23T: 6S

975 FA22 — x SW666 12T/15.

∴ Hasum SW666 x— FA12 38T: 3S

x— FA22 42T: 3S

x— FA24

Review data on trails

JAN 24 1955

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

—x 967 gives great excess of trails.

See 1033 Segregation.

Note "C" T not very numerous of S. "Arb or i seems numerous swarms, no trails.

—x SW1048 120T: 10S.

See 999 T/S ratio of UV treated FA12

UV₀ :
29T : 0 swarms!
9T : 3 swarms!

"denser Ogar".

Has SW666 changed? Or is FA12 ≠ FA37? of SW543?

i UV, remarked that 13T: 14S! difference?

Use SW666 trail!

99n: 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 duplication
(3) Fla^- mapping
(4) Specificity of transduction (Lp localization)
(5) Phase variation; exhaustion;
(6) Other phages as $\rightarrow x$

(7) Pullorum
(8) Kunzendorf c
(11) lysogeny-protection (coli proved better).
(12) heterogenotes (SW 686?)

(9) Misc $H_1 \rightarrow x$ monophasic (z_{28}; lw;)
(10) backcross data
(13) paralytics.
(14) especially chains.

Some of these are partly tackled

There is ~~th~~ 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 c̄ pedigree data.

Trails: incidence

JAN 25 1955

, request comment that —x967 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!]

.05ml 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: $\frac{1\text{ml FA}}{5\times10^{10(?)}} = 5\times10^{6}$ T ! and ½ as many swarms!

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

JAN 2 6 1955

Note - BADS remarked that

~~[illegible]~~

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

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

Try usually!

compare quant yields. Any other markers for SW541? See notes. Ought to use 553 to demonstrate tracks from isolated motiles!

(consult Andy or Clive)

JAN 26 1955

MS Comments.

SW 541 X — TM2

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

∴ delay.

4b Trouble shooting (squirt oil at them.) 100 much interpretation.

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

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

5a earlier expts a ≯ 6; later 24 isolates: 1 n.g. 1 sw

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

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

$\underbrace{30:3}_{E}$ $\underbrace{44:3}_{E}$

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

∴ E's calculate also.

6c how can n ≈ 10

7e !! critical

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

→ x SW967 pul.

1216
a

DATE: Jan 27, 1955 REF:

p26 FA22 ~~[illegible]~~ .1 ml + 1 ml SW967. decimal dilutions in SW967.

loop D. ~~#1 [illegible] occ. trails / spot [illegible]~~ #2: ~~[illegible]~~ / spots: $0^4\ 1^2$
strokes $0^1\ 1^1\ 3^1$

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

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

~~FA~~50A

B ①: 11 spots $S+3T^2\ 1^2$ ca $5^4\ 2^3$

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.

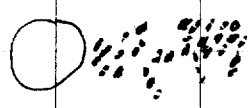
1216
b

DATE: REF:

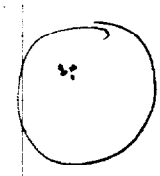
1	2	3	4	5	6	7	8	9	10

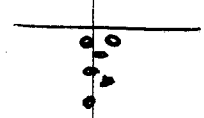
A29.C

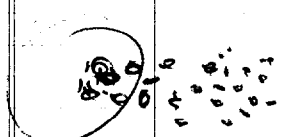
1	Motility agar standard		
2	" "	.30% agar	2 brown
3	"	.35	blue
4	"	.40	red
5	"	.5	green
6	"	.6	yellow
7	"	.40	no gelatin.

c7:  cluster (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 had to interpret owing to numbers of swarmers.

c3 swarmers have more linear, or multilinear appearance. appear more selected than 4.

1216
C

DATE: REF:

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 uniliniarity of trails but will have to be settled by more precise methods. It is very difficult to say whether one trail or two.

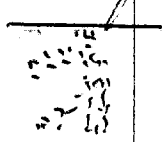
D. distribution of T's:

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 sw arm.
68 T
126 spots.

some definitely non linear:



(over)

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

Exp. 63 48 10 2 1

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

12/6d

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

E same dil as D, spots on agar i/s gelation:

$\dot{s} = 1, 2$

$\dot{c} = 3, 4$

1. $4T^{+}/30$ v. thin agar

2. $6T^{+}/18$. somewhat fuzzed but clustered, not channed.

3. $3T^{1}\ 1T^{2}/18$ more "linear" (more overgrowth, tighter gradient)

4 $9T^{1}/24$. ; 1 "cloud" - remembrate

8a - persistence 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 several.

Discussion - growing point.

Bruce found 3/15 motiles → trails

What are the oligocatenate 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, as well as mass transfer from residual clone. (A) (B)

Isolate		# of Fla+/-	A Trails from clone G	T	B pltd. Growth, trails from singlechains.	
.4= 10^4 A-6		0/.4	✓	0		
B-6		1/.4			B4 1:	1g OT
C-6		0/.3+	✓	0		
D-6		0/5.	✓	0		
E-6		0/4.	✓	~~[illegible]~~		
F-6		22/.4	✓	profuse	F1234E34 22: (10+12)	13g OT
H-6	a	6/.4	•		H124 7 6:	no T how many grow? several same 4 L
	b	1/.4	•		H~~124~~5 1:	0
A-5		1/.4	✓	[illegible]	H4 1:	ng
B-5		4/.4	✓	0 plus profuse trail	B3 4:	3/4g OT
C-5		4/.4	✓		c4 4:	1/4g 1? T seeow
D-5		13/.4	✓	1 profuse trail or clusters	D1234 13:	8/13g. no T 1? seeow
E-5		n.g.				
F-5		0/.4				
G-5		0/.5				
G-6		7/.4	✓	0? scattered cols.	G34 7: (5+2)	4/7g. no T.

• not planted (picked ~~[illegible]~~ reservoir by mistake)

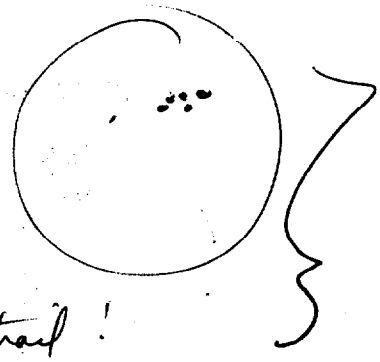
Incidence of trails in column A vs B probably reflects chemotactic stimulus from neighboring Fla⁻. Compare inoculations with and without added SW666 (for Fla⁻ marker)
Recovery of cells ≐ 30/53 > 50%!

(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.

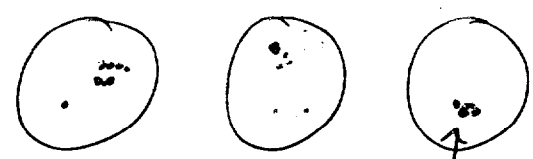
C 1/: 1 gen

5 colonies below agar!



note C 5 also had longer trail!

D 1234 8 gen most 0, also



went downwards definitely

1218

FEB 8 1955

226

Plate subclonal cells i SW666:
5 = group of 5 Fla+ 1-3 i SW666; 4-6 s
4-6: 2 prolific T 4 3 1 T.

add droplets to spots
on motility agar i
SW666

No def. advantage of
plating i 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 on 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

(inward)

H1		
2		
3	ca 4 each	0
4		
5		
6		

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

35 + 28 + 38 + 24 + 32

"157 s.c.i" #

This process poor method indeed, only 1 chart each.

Misc: Serratia from D Stone A 1218
Cla^R for Lichstein B.

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 autostoved; former buffered c̄ $NaHCO_3$.

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

24h. ClA: .2ml heavy growth ClAm .2 dense background ClH .5ml: n.g.
.4ml spaiser, no pop. numerous papillae + outgrowths 1ml: n.g.
1 ml NG .4 few colonies in heavy brush.

1 n.g.

Remirbate.

Isolate 1218B 1 from ClAm .4

Reisolated and formed to be:

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

W2754
See letter to Lichstein FEB 12 1955
See also notes on papers by Grey, ES Penfold + Harden.
(over)

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

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

S. wichita = –

S. typhi – only weak =) not sharp enough

difference. Suggest DCG work it 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 – grew from periphery inwards.

Might try lower concentrations or purer material when F-G screening method is worked out.

APR 22 1955 Send Ewing

APR 20 1955

W2745

1218C1 = aerial cont

1218C2 = Johnson's strain 80K

3/28/-5

DATE: REF:

wet weight : 310 mg

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

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

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

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

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

dilute 1:10 = 30 γ/ml ; plate 12 γ #5 plate 0.04 cc onto NSA

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

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

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

No.	after 2 days 30°C	37°C	average	#/mg
1	too crowded, i.e. nearly confluent			
2	– Not counted			calc
3				calc
4	~880	~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	fem	non	% fem	
29	11	18	11/29	40%
68	19	49	19/68	28%
8	2	6	2/8	25%
28	4	24	4/28	14%
133	36			27%
225	61	164	27%	
455	97	358	22%	
813	194			24%

lac + (E. coli) 24% of population

no definite coloration yet (4 days)