July 8, 1955. Resume

SRP tests on named cultures.

① Frederiq 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 Emerging

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

for first 1500, mostly only 1177 as parent.

→ × SW666

chains

DATE: Jan 11, 1955. REF: 216/217

Preliminary trials FA22 → × SW666 (TA42) No notebook sum. Recall that this shows low transductant titer (phage compatibility?)

FA37; and SW686(induced lysate) → × SW666

Practice runs to help recognize what to look for.
(18) isolates. 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.

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

Card

Effect of volume on stasis, etc. 1214

DATE: REF:

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

cells are 3/4. Sw666

No Tor's!

Recalibrate.

11-14 = .1ml FA37/1ml Sw666 (old; dil. 1:1 c broth) and 3 decade dilutions in Sw666! Use flat drops, ~~agar~~ loop D.

1/24		Motagar		(4.4% agar	NM fall through agar	1 or 2 s/2
	11		0^5 15^2 $2^{3.5}$			0
	12		0^6 $1T^1$ $2^{7.1}$			0
	13		0^8		Swarmers will be ill defined.	0
	14		0^8			

/8.

JAN 24 1955 Plates Reincubated 4hr 5 plates each of 9, 10, x ca. 10 spots each (100) only 2 swarmers (late) no trails

Note, however, numerous poorly defined clusters under surface growth. Incidence should be compared c Sw666 s phage and prolonged incubation!

A24 .5 broth tubes of .4% agar c ~~dilution H~~ (.1ml FA37 + 1ml Sw666 (old)). Spot loop D.

FA37 → SW666
quantitation: ratios of Trails: Swarms

1214

DATE: JAN 19 1955 REF:

Old susp. SW666 (Ref.).

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

2 .01 "

5P19 3 .001 "

10 .0001 "

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

~~Use fresh SW666 Redo tube #4 and compare with 10⁻³ dilutions of tube #1~~ (=5)
(diffuse ie number of cells.

Use 1/2 ×10⁻³ as standard level.

~~5 = 5×10⁻⁴ ml FA37 + 1ml (fresh) SW666~~ (in 0.1ml)

6 = [(.1ml SW666 + .1ml FA) ×10⁻²] .1ml + 1ml broth.

5 = [(.1ml broth + .1ml FA37) × 10⁻²] .1ml + 1ml SW666

allow 6 to stand 20 minutes for adsorption.

1/21 7 ≐ 5.
50 8 ≐ 6

In re chemis

JAN 19 1955

1. Assume affirms that isolated motiles do not form ~~chemos~~ 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 cezar.

Tests: ① What is ratio of trails to swarms?

What is ratio of 1- catenates to swarms?

poly- "

② Get a 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 dye enough)

Try 37-xSW666. Y dilutes è cells, broth, phenol.

Linkage. (Salvarula)

JAN 19 1955

JAN 1 1955

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

2. From Grace's letter, H, linked Fla⁻ are:

SL13 = SW1048 para A.*

SL28 = SW1092 = Heidelberg

SW543-666 PB

* SW544 = Schuetze O

SW553 - dublin

* SW966 (Ithaca es b)

* rather poor; others are monophasic except SW1092.

3. Test spring (1130 ff.) to load for linkage & complementary crosses as bases for pedigree analyses and thereby get into chemistry.

Recollection of some trials with SW1092 but cannot promptly find the notes on it. Should repeat to evaluate suitability for linkage study (& Dino). ↓ of Alda Buontini.

DATE: 1/21/55 REF:

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

A. 0 swarms, smeared no obvious T.

B " "

D 3 swarms. ~~0, 0, 0~~ 0^{12}; $(^{13}IS\ IT)$; ^{14}IS; ^{15}IT

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

maybe too fluid!

C 0^{15} IS; IT

6: A 6cm plate 0^4 (IS, T) (~~2~~ 2T, S) (IS) (IT) 8

B 0^3 IS^6 IT IS^1 10

C 10cm 0^4 IS^3 IS IT^1 IT^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 calm data?

DATE: 11/22/55 REF:

A22

7: A (pre "dud" plat) 0 10

B	0 9		47-0	37-0	
C	0 10		2-T	2-T	
D	29	(1T)			
E	89	1T		39	
8A	09				
B	0 10	1S	multiple Haves	41-0	
C	0 11	1S		5-S	
D	09	2S 1T		1-T	
E	0 11	1S		47	

again: note 8>7; reduced incidence of yesterday's dependence of Trails but low number res is disturbing. Cf. earlier data.

Note: present expt entails large fluid volume + potential strains might proliferate without ever entering aga. of effect of inoculum volume

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

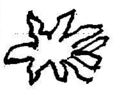
DATE: 1/20/55 REF:

1 2 3 4 5 6 7 8 9 10

① Plate ca 15 SW666 per plate motility agar poured. c/s underlayer of NSA. In each case, discrete colonies with no doublets or spread arise formed.

1/21/55

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

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

11.0/55

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 identity / multiplication. Present data do not define where units 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 2 4 1955

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

—x967 gives great excess of trails.

See 1033 segregation.

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

—xSW1048 120T:10S.

See 999 T/S ratio of UV treated FA12 UV$_0$:

29T : 0 swarms!

9T : 3 swarms!

"dimorphism".

Has SW666 changed? Or is FA12 ≠ FA37?

of SW543?

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

Use SW666 trail!

991: FA21 —x SW666 41T:10S 5/7 bp+

FA22 —x SW534 30T:2S

JAN 25 1955

Salmonella Comment.

Spent the last couple of days reviewing notes on Salmonella.

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

1. Monophasics
2. H_1 duplications
3. F/a⁻ mapping
4. Specificity of transduction (by localization)
5. phase variation; exhaustion;
6. other phages as → x
7. Pullorum
8. Kauffmann-Lederberg c
9. Misc H_1 → x monophasics (z_{28}; lw;)
10. backcross data
11. lysogeny - protection (coli proved better)
12. heterogenotes (SW 686?)
13. paralysis.
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 ō 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!]

.05 ml FA22 (ca 10^{10}) gave ca 500 papillae! (973)

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

BADS claim: 1 ml FA = 5×10^6 T ! and ½ as many swarms!
5×10^{10}(?)

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

JAN 2 6 1955

Note - BAOS remarked that

534 - ~~x~~ 553 gave T >> S (1T/17,100 particles!)

LT2 - x541 many T, S. (claims 2×10^{-3} T/cell!
4×10^{-4} S/cell)

Try unequally!

compare plant yields. Can other makers for 553/541? See notes. Ought to use 553 to demonstrate traits from isolated tubules!

(consult Andy or Clive)

JAN 26 1955

M S comments.

Sw 54/1 x — TM2

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

∴ delay.

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

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

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

5a earlier expts 9 ≠ 6; later 24 isolates : 1 n.g. 1 sec

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

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

30:3 (E) 44:3 (E)

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

∴ E's catenate also.

6c how can n ≷ 10

7c ! ! critical

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

DATE: Jan 27, 1955 REF:

1	2	3	4	5	6	7	8	9	10

p26 FA22 ~~10^-x 1ml~~ .1ml + 1ml SW967. decimal dilutions in SW967.

loop D. ~~#1 ... sec tails / spot~~ #2: ~~...~~ / spots: $0^4 1^2$, strokes $0^1 1^1 3^1$

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

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

~~FA~~ 50A

B ① : 11 spots $S+3T^2$ 1^2 ca 5^1 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.

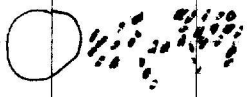
1216
b

DATE: REF:

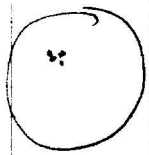
	1	2	3	4	5	6	7	8	9	10

A29.C

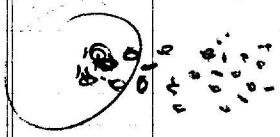
1 Motility agar standard
2 " .30% agar 2 brown
3 " .35 blue
4 " .40 red
5 " .5 gum
6 " .6 yellow
7 " .40 no gelatin.

C7:  clusters (& 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. interspersed of small and large colonies suggests minor branching. In general has appearance of concurrent clusters rather than single trail. A few possible initial branches but hard to distinguish from numbers of swarms.

C3 swarms have more linear, or multiform appearance. appear more rounded than 4.

1216
C

DATE: REF:

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

C1 Trails/Mostly, 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 small microcolonies also shows up pretty well also. Considerable question on uniformity 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 poly c.	
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	

spore smear.

Total: 1 sw am.
68 40 T
126 spts.

some definitely near linear:

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

(over)

Exp. 63 48 10 2 1

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

12162

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

E same field as D, 2 spots on agar c/s gelatean:

s = 1, 2

c = 3, 4

1. 4T+/30 v. thin agar

2. 6T+/18. somewhat fuzzed but discrete, not channeled.

3. 3T' 1T2/18 more "linear" (more overgrowth, tighter gradient)

4 9T'/24. ; 1 "cloud" - remnants

8a - percentage of chains.

JAN 2 7 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 ≐

11/5-1 E cells.

10c I have seen groups but would interpret them differently

10d Spread.

Discussion - growing point.

Bruce found 3/15 motiles → trails

What are the physiological controls?

—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)

A B

Isolate		# of Fla+/-	Trails from clone G	T	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.	✓	~~0~~ pigment	
F-6		22/.4	✓	pigment	F1234E34 22: (10+12) 13g OT
H-6	a	6/4	•		H124 7 1: not how many grew? several some 42
	b	1/.4	•		H1245 1: 0
A-5		1/.4	✓	●	B4 1: ng
B-5		4/.4	✓	0 pigment	B3 4: 3/4g OT
C-5		4/.4	✓	long pigment trail	c4 4: 1/4g !? T grew
D-5		13/.4	✓	pigment trail or clusters	D1234 13: 8/13g. not T ! ? grew
E-5		n.g.			
F-5		0/.4			
G-5		0/.5			
G-6		7/.4	✓	? scattered cells	G34 7: (5+2) 4/7g. no T.

• not planted (picked reservoir by mistake)

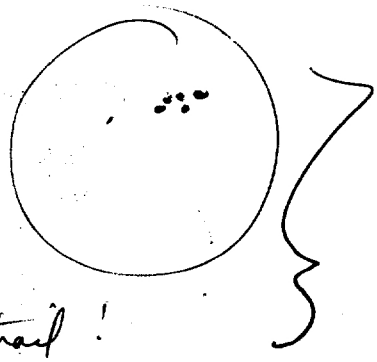
Breeding of trails in column A vs B probably reflects chemotactic stimulus from neighboring Fla⁻. Compare circulation with and without added SW 666 (for Fla⁻ males)

Recovery of cells ≐ 30/53 > 50%!

(om)

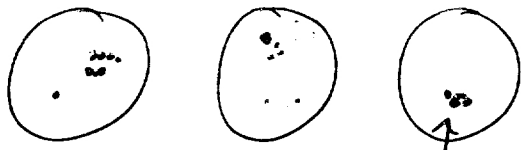
Pneumo expts had been indecisive. Thought it better now to transplant directly to determine ① how many trails per initial and ② role of factor.

C1: 1 gen
5 colonies
blow again!



note C5 also had long tail!

D1234 8 gen most 0, also



went downwards definitely

1218

FEB 8 1955

226

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

add droplets to spots
M motility agar c̄ SW666

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

No def. advantage of plating c̄ SW666!

(crossing?!)

	spots	T.	
G1	6	0	swarm
G3 } G4 }	4	0	1 has 8 scattered subsurface colonies
A2	2		
6	2		
B1	2	all 0	
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 in 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

(invaded)

H1, 2, 3, 4, 5, 6 — ca ½ each 0

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

35 + 28 + 38 + 24 + 32

"157 s.c.i"

This proves a poor method indeed, only 1 clear track.

Misc: Serratia from D [illegible] 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. Put expt i Cla 1mg/ml n.g. ditto Clam (chloroacetamide). Both sterilized in autoclave; former buffered i NaHCO3.

2/8/55: 100% sols Cla, Clam; 20% Clh (EtOH). – ml/10ml NA plate.

Cla: .2ml heavy growth
.4ml spaces, no pop.
1 ml NG

Clam .2 small background Cl H .5ml: n.g.
numerous papillae + colonies
.4 few colonies in heavy bunch. 1ml: n.g.

1 n.g.

W2754

Remitate.

Isolate 1218B 1 from Clam .4

Recodated and found to be:

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

See letter to Lichstein FEB 12 1955

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

(over)

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

.1% – 1% formal n.v.g. ~~S. typhi — OK~~

S. schottm ± – ~~test~~

S. typhi – only weak ±) not sharp enough

difference. Suggest DCG worked out.

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

Chloroacetate soln. – is probably decomposed on autoclaving while ClH was not evenly mixed – gave peculiar periphery mounds.

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

APR 22 1955 Send Ewing

APR 26 1955

W 2745

1218C1 = aerial cont

1218C2 = Johnson's strain 80K

3/28/75

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	~680	~784	~732	2.4×10^5
5	298	267	282	2.3×10^5
6	80	71	75	2.5×10^5
7	29	28	28	2.3×10^5
8	8	8	8	2.7×10^5

2.4×10^5 bacteria / 1 mg wet weight

no definite coloration yet (2 days)

replicate onto EMB lac for proportion of "coli"

Total	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)