Second phases.

10/5/52

Plant irradiated strains on serum-motility agar

1. SW671 /i,12. (Insuff. antibody to prevent slow mass diff.) No buds or rapid swarms. Growth out not entirely even, but still i.

2. SW673 = para B/o sport b+. /b. See 975. After 24-48h., four buds/plate.
Test: b, enx, 1,2, i : 4/4 inagglutinable. Purify 1 as SW676
may show trace reaction with b. (pur? or phase variation?) = z_{33}

3. SW670 = typhi H901 x abony b enx /b. 4 buds. 3/4 still react strongly c b (= 3-1)
#4 inaggl. = 3-2. Streak out, reisolate, and retest on b agar.
#1 very weak b.
#2 inaggl. but.
10/22: /b slow spread.
rough ← T.O.
j ?= z_{33} by slide test 3/5/53

4 SW672/b. (= typhimurium x abony) → 4 days : non b, i, 1,2, enx
10/22 /b slow spread
SW435 itself is relatively poorly motile! (auxotrophy? 435. moves very slowly through
623,435/"I" - apparent buds still i. (exp. transduces) i but 1,2 phase appears
LT2 & LT22 → swarms 1,2

5 SW703-4. 704/b (spicer absorbed) → 1,2
703 k, r, a, gm → inaggl. Repeat
✓ 703/b inaggl. b, i, 1,2, 1,7, enx. swarmed immediately on b agar
see over ← 703 itself = b. Streak out: occasional more opaque colony, non-b = 976-5A (glu + lac −)

6. SW618. Swarmed /b → still b! (Ineffective serum?)
↘ slow spread /b → b

7. 623/i 12h. slow spr.
612/b " " " → b
623/i → slow spread → i
(over)

LT22/i 3 isolated buds (perhaps owing to slowness of main strain)
SW569/i no sw. ↘ later heavy swarm.
↓
slow spread

Note: "b" serum inhibits SW546 (phase II) and therefore contains 1,2.
So Seeliger's "b" standard serum? Check SW435/"I" OK! ∴ alt. phase of SW609... may be a third "j" phase

Check Species sera

- 618 swarmed on i, not on b.

974D5 b - sw. i not b
i : sw. b not i

SW435 is relatively immotile compared to LT22. This may relate to behaviour of SW672

703 (purif.) /b → 1, 2 (3 bands)

SW 672 /b (spun) ⟶ 1, 2 (very infrequently)

a) Rechecked single colonies: some seem to react both 1, 2 and ex

Regrow: 1, 2 only

Second phases

~~991~~ 976

1 SW671/i, 12 No.

2 SW673/b → agglut. SW676

3 SW670/b → " ; very weak b. save

4. SW672/b(1,2) → agglut. cf. SW435 - poorly motile as tested and gave no bands /i
/b → 1, 2

5. SW703 orig. → contaminant, isolated later s serum = 976-5A
SW703 pur → 1, 2 Separate phases
SW704 → 1, 2 " "

6. SW618 No

7. SW623 No ("sw" = i)

8. SW612/b "

9. SW569/i " → d (1/2)? ~~but may be migrant rough~~ Not distinguishable from typhi. Vaw phage ± entam?

10. LT-22/i isolated bands 1, 2.

Unstable transduction

10/13/52.

SW 684 is Bal +/-. Transmotilyze à PLT22: ca 50-100 tracks and swarms. Pick more or less well separated buds, streak on

A. EMB Bal & look for a superimposed transduction. 15 swarms 0 = 1 trans & stationary
All swarms were substantially pure + or - 12 -, 2 + 1 mixed but no U.

B. SW666 + ~~FA 437~~ FA (SW684) only 7 papillae.
6 gave -, pure + 1 had +, (IV)? ? Restreak

(Cf. 986D - This one not saved) ⇐ this has phage - mottled appearance, not +/-

C

New transducing phages;
R forms.

10.../52

1. R isolated from aged broths of eastbourne, Boyd 1104 typhimurium. SW688.
688 is sensitive to φ2715 (Desrondneau), 688S original, appears resistant although this is described as an O phage!! It is highly lytic on R but may it transduce to S?

SW688 is resist. to PLT7, PLT22 S is sens. See over →

2. LT-10, LT-1 a) aged b) grown with Boyd 4. No phage activity in heated supernates either on original parent or Boyd 4. LT-10 in penicillin 1 unit, no plaquing in ~~streak~~ streak-out.

3. Cross brush LT-1 to 10 /SW688 LT10? on SW688. SW688 shows
θ-10. discolored plaques, ~~lysis~~ with θ10 (spun)

4. θ-10 / H901, thompson, aberny 1, gall, montevideo, heidelberg, stanley, altendorf
SW666, eastbourne. No action - probably too dry.

LT-1 to 10 all R.

5. SW ~~##~~ 665 self plaq. Cultivate, ✓ broth.

6. Test misc φ on SW435, 666. (Select for R?)

	435	666
HP18		
PLT 22		S
7	S	SR
LP 30		
LP 36	S	S
φ2715	S..	
HP22		
S21φ	1 pl.	plaques.
HP20		
HP13		

streak out survivors,
pick to broth: none were rough!

SUMMARY

θ-10 (thompson φ) has a component v. weakly active on SW688 (rough). wait for Spicer to build it up

ditto PLT10 - need to find other hosts to build it up

"O" phages attack rough phases. θ-10, PLT-10 inactive on bovis morbificans strains here. Wait for Boyd's material.

	φ2715-43	942B
Boyd 1104	R	R
" SW688	S	S±
Typhi 1+90	S	S
~~SW540~~	~~S~~	
LT-1	S	S
thompson	R	R
marbendes	R	R

These are not strictly R or S phages.

SW-688 on motility agar - see 974DD
motile selection appears "smooth" - test i phage
still resistant to PLT22. (Check somatic antigen.)

of available material, abony (B enx-b) and typhimurium (B i-1,2) seem the most appropriate to study phase variation, as FA and sera are available.

In previous experiments, SW672 was made by SW435 ⨯ abony^I. In preliminary tests, SW672 appeared monophasic b. SW435 however is itself suspicious in its motility behavior; in addition sera previously used may have acted as b-1,2.

① Test SW670, 672 in b agar.

② Repeat, but ~~LT~~ LT2 ⨯ abony^I and ^II. (Use LT2 to improve possibility of progeny tests).

+anti i
A LT-2 FA14 → b ⟷ 1,2 SW699
B LT-2 FA15 → enx ⟷ i SW698
C LT-2^II FA14
D LT-2^II FA15

NOTE: i-enx is found only in group C (bareilly) but i is otherwise a rare kind in general.

③ Try enteritidis as recipient for transductions.

④ See 971D SW666+ FA15 (abony^II) 17 swarms: all b, not enx
✓ Gal−.

~~Purify and test vs. b.~~ Purify 4. Test all directly on b(1,2) agar.
4: ~~8~~ → inagglutinable (sl. rx 1,2 ?)
~~6: ✓ inagglutinable cf. SW678~~
small buds from #4, 6. rich, 2d plate is mainly grown. Pick center (general)

⑤ Phases 1 and 2 of SW703.

979-5A = FA23 ⨯ SW666

979-5B = FA24 ⨯ SW666 Several thousand (earliest swarms) test pooled from various spots / b.

15 spots (probably tests >100 swarms): none swarmed directly out on b agar

3 gave what may be buds. pick these: 5B1-3 : still b. Reincubate

Repeat 5A + B: number of swarms is phenomenal.
Test Cal + transduction?

---

streak out pooled swarms on EMB Cal: all –

Also, test on b serum: no swarm from A or B

5B: 27 single colonies: all b, none 1, 2. (2: may show equivocal reaction in 1, 2)
+ 8 added (sw981) all b → Recheck all b. or maybe sl. rough

5A: ~~20~~/20 b.

10/26 Repeat ⓒ deleted FA...
1/100 no tracks or swarms

10/27 FA 1/10

---

6 ~~FA~~ (sw~~5~~88) → sw666 6 1, 2 FA streak: –
BADS 2 mixed
more or less well isolated swarmer 14 b
streak out on EMB Cal.
b, 1, 2 – both Cal – sw699, 901.

Note sw533 (= 703?) → 534 → ~~588~~

But ~~533~~ 703 behaves differently from 588. Recover NZ culture of 533

Recap + summary — Phase variation

10/28/57

anti-i

② A. LT-2^{I} × ← abony^{I} gave 1 swarm SW699 b → 1,2 $\xrightarrow{\text{slow}}$ b (Hemagar)

B. LT-2^{I} × ← abony^{II} gave " SW698 enx → i → enx

C and D not successful in initial trial (LT-2^{II} × ←)

④ FA15 (abony^{II}) → × SW666 17/17 b.

⑤ A. FA23 (SW703') → × SW666 15/15 b on bagar. Very high rate!

In second run, streak out pool (>>100 swarms) and test colonies.
20/20 (b). Pool does not migrate on b agar.

B. FA24 (SW703") → × SW666. spots of pool b on bagar
27/27 (b) Pool does not migrate on b agar. ~~b~~ at furthest.

⑥ FA (SW588 — B.A.D.S.) → × SW666 14 b pur. + save SW699
2 mixed
6 1,2 " " SW901

979DD 6: FA18 (LT-2^{II}) → × SW666 18/18 b no alt. phase could be selected

Consideration of ②, ④... leads to hypothesis of phase variation:

~~i~~ i^{*}; 1,2 ⟷ i ; 1,2* where * is a locus-fixed activation (or suppression).

$\dot{A}_1^{i}\ A_2^{1,2}$ ⟷ $A_1^{i}\ \dot{A}_2^{1,2}$

④, ⑤B, 979DD-6, and previous expts also cryptically lead to hypothesis that $\hat{A}^{1,2}$ cannot be expressed in monophasic strains. Apparent paradox:

SW703 =? SW533 $\xrightarrow{K}$ 534 $\xrightarrow{sp.}$ 588

↓→ b only ↓→ 1,2 ; b

∴ SW534 may have a unique $A^{1,2}$. Also SW546 = SW857.

(over)

11/2

LT-1^{I} reacts both 1 and 2

LT-1^{II} " prudens 2

699 " " b

abony^{1} " " b

abony^{2} " " eux

E abony 1 FA22

F " 1 FA18

G " 2 FA22

H " 2 FA18

11/3 Repeat. LT-2^{I}. Rx prudens 1.

A LT-2^{I} F-H 14

B I 15

C II 14 Bud: ? aggl. ✓ single colonies → 1,2 + (?) → aggl. (abcdienxgm 1,2)

D II 15 ✓ lysogenic for LT2; loc-. Save as 979-C for later identification. May be poorly motile. (Last weekend of Boston Trip)

E abony 1 22 —

F 1 18 2 b

G 2 22 2/5 i? + 3/3 b 1: i → enx. PLT22^{R}SW924 pur: 2: i " " 3: i " " 4: i " " 5: i " " } 4. S.

H 2 18 6/6 b ~~3/6 b 1,2?~~ 1/1 b 2/2 b (enx?)

No clear-cut buds. E-H mostly b, although G-H were originally purify single colonies, and test alt. phases enx.

C moi ✓ b++ enx±)

1-2 plates each. all negative except as indicated

A-D in i, 1, 2 serum; E-H in b-ser.

serum probably more antibody than b.

J abony 1+2 × FA 22A
i : 12

Numerous swarms (10-20/plate).

Of #1-16, purif. 11 are i
5 are b (plated by HLB)
later, i isolates from three unpure kinds.
17-33 (not purif.) all but 18 and 29

Confusing variants probably wrote H.

appear i. # 29 i+1,2?? 18 b?
29: < 3 colr A : i
1 colr B : i b±?

18: single colonies < A i+++ 12+3
B i± 12+?

K. × FA 18
i : 12

possibly over-inoculated.
i+1? /4 plates. (Repeat!)

Note: FA 18 × 66 gave only a few swarms. Make new preparation FA 18 A (see below).

L. SW 698, 699 × FA 14, 15 i, enx or b, 12 ~~Neither~~ 1 plate each. No swarms many (parental specific overgrowth)

K				S	
1.	1,2 ↔ b	Lp^R			i : i
2.	1,2 → b	Lp^S	SW 932	18A i	18B enx++
				18A' (enx)?	18B' enx++?
				29A i	29B i
				29A' (i and V. wrk. 12.)	29B' enx

S. Conclude: 18A i : enx, 18b : enx : (b)?
4/cols of 18A are enx+++ 1,2 - 1/5 i+++ 12±
/5A 29B i : enx

11/30/57.. 29A = 34 is i. Repeat 34/i: enx!
.. rolling 29A' = i (v. weak).
.. odd hue.

#14 appears agglutinable. All others are i.
See over for /i..

Repurify 18A before further tests are made. 18B may be preserved contam. from abony. 18A' is enx, but also shows trace 1,2? /Lp^R 18A "as 18.

29B as 29

all 34 are Lp^R!

There's no clear cut 1,2 × from FA 22.

979-AA Note: phase variation in S. abony. Stock culture labelled abony II, isolated recently from single colony is about 20% b! Many individual colonies react both b, enx. ∴ the phase of FA 14-15 and cells abony' and 2 cannot be regarded as under control. This suggests enx → b. Not known whether b → enx likewise. In a parallel check on LT-2 II (from Salmi susp.) 6/10 were strong 1,2 4/10 were scarcely agglutinable (i?): regrow these 4 (see over).

Reisolate phase 2 cells for new FA pops

LT-2 II

4: These colonies when regrown give only trace agglutination in 12 (sm? i?)

→ In broth, #4 is very sluggish motility, again only trace 1, 2.

#5 (strong 1, 2) very mot.

most cells immobile; some quite active. Reselect as motile again then gives a strong 1, 2.

Streak out present suspensions of LT-2, above. (H.L.B. tests)

b ↔ enx frequent

above I: b: 7 enx: 3

above II: enx: 2 b, enx: 2 (see above)

HLB

LT-2 I: 16: i 1, 2: 1 ? 5 (both or weak?)

→ 18 i 1: 1, 2 others R. retest

LT-2 II: 0: i 1, 2: 20 weak 1, 2: 3.

Thus, FA 22 - FA 18 are fairly reliable, but

FA 14 - 15 are not, but nearly equivalent.

9795 second phase: select m/i serum. Test swarms: (i b 12 enx) all isolates swarming on i agar.

Streak out pool of 9795, pick colonies test Lp (2 2)^s: undetermined proportion of Lp^s. Purify and test SW 941 as Lp^s; i:

979K...

12/1/52

K. FA18B → abony 1,2: doubtful outgrowths, very late: ~~3~~ not Salm, 4.

4	1,2 → [redacted] b
5	i → enx
6	1,2 → b
7	1,2 → b

all Vp H.S.

11/30. FA18C, D. 12/1. 3 on C 0 on D. 5, 6, 7.
12/2 ladd. c. 1 D 8, 9

(15 colonies of #5 tested all i.

12/4. FA18D (canc) → abony 1,2. 5/4 plates.

8.	1,2 → b
9.	1,2 → b
10.	1,2 → b
11.	1,2 "
12.	1,2 "
13.	1,2 "
14.	1,2 "

12/6. (15. 16.) inagglut. Less s.c. notably medium. (occurred v. late)
react v. weakly with b: presumed "j" phases.

8/3/53 see 1025 J14 is (i [redacted] enx)
K1[redacted] enx:
K1[redacted] enx:

J: 12/1/52 FA22 → abony. Crowded swarmers. Pickpool and steals (for ij s).
(Previous isolates Vp^a - not suspecting as separate isolation was delayed)

typhimurium — x absorp two step

979LM

Selection is c b.12 serum.

L. FA22 → SW932 [FA18 → absorp] Phase 1,2

M. " " " Phase b.

= ants FA22 (but excess) and appear = cells.

Swarms are fairly heavy in both L and M but appear considerably more numerous in M. Phase control not yet established. Isolate 4 each.

(not 1,2 mutually)

L 1	i →	1,2
2	i	"
3	i	"
4	i	"

M 1	i →	1,2
2	i	"
3	i	"
4	i	"

Lp tests? all appear +. save L1 = SW 973

Thus, i: 1,2 can be reconstituted from i: 1,2 → b: env in two steps.

J. abony 1+2 × FA22A

33 swarms. #34 = 29A All are i except #34, inagglutinable.
Select second phases ⓘ anti-i serum. All 34 are Lp^R.

18, 29 straightened out.

In view of variability of abony (I = 7/10 b II = 20% b), make purposeful mixture of phases for these experiments. LT-2 I : II :

count 33.

Second phases of #1-34 (14, [redacted] not included) are all enx.

In later test, #14 = i. Second phase: + 1/1

∴ 32/32 are i → enx.

Probably roughness conferred same of above. 33/33

K. 16 swarms total: 1 cont. (not Salmonella)
2 inagglut (weak b? ≈33?)
12 1,2 → b
1 i → enx

Summary: In this experiment:

Input / output	i - 1.2	i - 1.2
i → enx	33	1
b ← 1.2	0	12

add 9796: total: 38 1
0 12

There can be no question of a significant difference in the FA of the two phases of LT-2

Input phases:
① 18 i : 1 i,2
② 20 1,2 : 0 i.

Estimate purity ca. 95%, but maybe slightly altered from inoculum used for FA

Note: no i : 1,2 or
b : enx
∴ no linkage

Selection of O- and H

10/23/52.

A. SW 603 $^{G+H-}$ + SW680 $^{G-H+}$

~~B SW603 + SW 618 $^{G+H+}$~~

C. SW666 $^{G-H-}$ SW618 $^{G+H+}$

B. SW603 $^{G+H-}$ + SW666 $^{G-H-}$

D. SW618 $^{G+H+}$ 10: SW666 $^{G-H-}$:1

Work ē D (10+ : 1−)

Prepare broth mix cultures as indicated.
Except possibly in C, (+ > −) initial ratios are maintained in a passage through Penassay (rough check estimate).

① Motility agar center still 10:1
periphery pure + (perhaps slight increase −).

② In soft agar - gelatin tubes: Incr top and recover after 24 hours
− glucose ca. 10:1
+ glucose + ca. 30 : 1 −

Should use fully non-nutrient medium.

③ In broth + b serum .02 ml/5
a. inoc from top of broth
b. sediment lightly − top of broth
perhaps allowed to grow too long.

Heavy agglutination.
ca 5:1 ?
ca 10:1
Repeat ē growth 11:35 − 3^{30}
(recovery)

E. Owing to its resistance, the SW543 line is committed for selection by χ phage. In pilot experiments, make up mixtures of SW666 and SW703 $^{H-}$, and determine what proportion of /χ are the pre-introduced Gal−

But SW703 showed virtually no response to χ! of SW533
Phage n.g? Repeat ē χ/SW592 : now allow susceptible (incl SW537)
∴ 592 −1 has lost some activity
Repeat ē new χ

9803: short serum passage:

unsedimented: ca 6:1 +:−

briefly sedimented: ca 20:1 +:−

control: too dense to read, but very low − apparent.

∴ brief sedimentation appears to be harmful.

Repeat i second pass:

control is another tube from original 980 mix culture serum (ca 1:300) mix i pour into serum tube after light sedimentation. Compare no, light, strong sedimentation.

ca 20:1 heavy sed.

180:1 control

D4 ½% agar. ± glucose

Broz: washed cells, 980D sec. passage:

	Val ratio +	−
+ glucose	100	<1
− glucose	100	<1

∴ this method is ineffective for concentrating O forms.

Methods for concentrating O forms.

10/29/52

1. SW666 has a selective disadvantage in broth passage with SW618
2. Dilution by migration through agar (± gelatin ± glucose) does not conc. the O forms
3. Asher's method may be slightly efficacious — needs to be worked up.
4. X phage not yet properly tested. Of 588 as H^+G^+ + H^-G^+ 666. start fresh inocula

---

F. SW603 (H^-G^+) and SW680 (H^+G^-). Wash, conc. to ca 10^{10}/ml.

~~Mix 100600 : 1680~~ 100680 : 1603 Add b serum to titer of 1/100.

~~Let stop in ca 1½% salt. Put at room temper. 6:25 –~~ N.G.

Repeat in original broth (mixed & dilution in saline). (poor agglutination)

Initial	ca 100– : 1+
4½ h. no centr.	ca 100 : 1
" heavy centr.	>> 100 : 1

Ineffective selection.

Both cultures here labelled 603 agglutinates in b. (Mixup?) Test slants

G. X selection. Make up SW666 (H^-G^-) 1 : 100 SW588 (H^+G^+).

Cross-brush with X SW592 on EMB Gal, Lac. Compare ratio before and after. Note: SW666 is lysogenic for SW588 : grows plaques on 588

	Gal+	Gal–	
X(Lac) [dry agar, poor phage action]	H+	H–	ca 1 : 1
X(Gal) marked phage action		ca 3 : 1	
		ca 1 : 1	
Initial		> 100 : 1	
Final & phage		100 : 1	

∴ X phage probably is effective to extent of 100 : 1. But how can it be used to study flares?

* recovery result may be influenced by lysogenicity. Should start out from area of mixture arrays from phage action.

In some ~~cases~~ control tests,

SW666 reacted weakly i b when grown in broth,

but not the saline suspensions from agar.

Retest both cultures viaz from → v + but very weakly b not i

	Orx	Water
A slant	±	−
B broth culture as check	−	−
C saline susp	−	−
D motility plate (negative)	++	++, not d, i, 1, 2

microscopically: nonmotile

Limit test from cells on D gave only ± b − d, i

603 broth also v b.

This may be related to the "weak b" reactions seen in earlier experiments.

Restreak D, spot on motility agar.

Conc O [illegible]

11/4 G. χ selection 666 + 588. Initial ca 100+ : 1− 2:30

6:30 Control "

χ ca 50− : 1+ ∴ ca 5000 fold selection!

4 Gal+ tested: still $H^{1,2}$: all are now X^R (slightly rough?)
Restreak and retest.

F. SW603 ($H^- G^+$) + SW904 ($H^+ G^-$)

roomtemp. { + i serum (.2 1/2) Initial 4:30 8:30 lt. cont. heavy cont. } ca 50− : 1+ each despite heavy agglutination! (why?)

11/8. F2. a 904 1:1 603 — 6:30 PM

b 904 100:1 603 — add .2 ml i serum 1/10 spec.

9 AM. a: heavy aggl moder. turb. — Too much serum? O-reaction or nonspec. aggl?

b: heavy agglut., lightly turbid supern.

603 "spont aggl?" — not as heavy

904 heavy aggl.

These stocks not entirely suitable

Quantitative comparisons of tracks, swarms and Cal+ transduction.

Mix .2ml SW666 & .2ml FA24 ('/10 or '/100 in broth)
Incr areas of con & ea. 0.01 ml each Count swarms + tracks overnight.

	.01 ml	EMB Cal	total tracks		total swarms	
	x			3 days		24h
30° 1:100	7		0	0 ✓	2	3 ✓ (...)
1:10			15		3	
37° 1:100	5		3		0	
1:10	5		20		5	

all b

b+ 37° T. may not have developed sufficiently

each swarm had a distinct flare, several hundred microcolonies. Picks of one distinct to repeat effort at isolating O-forms: both only 1 does not have an adjacent track

→ 15 colonies from regions of flare, all b++.
(Total now 60).

% 30° 1:100 Strep?

		Cal+	
1:10	5	2	Note non-linear response also seen in previous experiment
1:100	5	2	

Background papillae & satellite effect! Restreak these → possible slow Lac fermenter. ✓ in ferm tube.

See 979 BB

10/31/52.

According to stock list,

SW533 —x→ SW534 → SW588
‖
SW703

However, SW703 → x only b to SW666

SW534, SW588 } → x 1,2. Also, SW546 → x 1,2.

In view of change of phase of SW534 cf. 533 and alteration in the behavior of its 1,2 antigen question arises as to identity of 534 and possibility of its derivation from SW546.

Possible differentials:

ARE ?:	533 = ~~7~~03	546 = 857	not "706"
	IV V ~~XII~~	IV V XII	IV V ~~XII~~

When were cultures received? 703, 70[illegible], 706 are already listed in stock list there is 857 is not ↓ but nowhere mentioned

· "533" recorded as misc. Bordy A and Z.

	Xyl (F.T.)	Rhamn		Inos		P942B	P942C	P666/588	P2714-43	EMB Mtl
SW546	+ less gas	—	✓	—	✓	+	++	+	++	++
SW588	+	—	✓	—	✓	+	++	+	~~±~~ +±	++
SW942		—		—						
SW703	+	+	✓	+	✓	±	÷	—	+	+++
706		+ 709		+						

✓ 48 hours

Most differences are slight but all tend in same direction.
SW588, 534 must derive from SW546.

Note: 534 mot + SW666 on motility gives swarms; parents alone do not.
Streakout: 2 were Gal+ 1,2! (Anomaly in direction of transduction)

(see over)

Retests & PRE original stocks (via N Z index)

	Rhamnose	Phage A1	A2	A3	
#3	+	R	S	S	
#4	+	R	R	R	
#6 ?	−				Salmonella did not grow out
#157	−	S	S	S	
SW534	−	S	S	S	
942	−				
807	+				

857 = culture out of Edwards' slant

546 = culture maintained here, presumably identical ± above.

546 and 534 should be compared with PRE #6, which has evidently become contaminated here. It appears almost certain, however, that 546 = 857 → 534.

Note 3/1/53 SW703 contam. (see 976-5) now proves to be −1,2, and may be ± Edw 157. This would explain origin of SW534 from stock culture of 703. Some of contam. ??

Additional phage tests

11/1/52

Single cross streaks on EMB Lac. S. typhimurium

→ ↓	Boyd 4	4R	LT1	2	3	4	5	6	7	8	9	10	PT22 22	FA26 466/588
Boyd 4	Boy												+	
Boyd 4R			–									–	–	
LT 1	–		~~X~~									–	+	±
2	–		–									–	+	
3	–	+	–		maybe suppl.							–	+	
4	– ~~++~~		++									–	+	
5	–		–									–	+	
6	–		–									–	+	few pl.
7	–		–									–	+	
8	–		–									–	+	
9	–		–									–	±	
10	–	±	–									X	+	v.f. pl.

∴ ~~FA~~ LT4/LT1 LT3/Boyd R should be grown out. Mox mix. to broth

---

✓ in homologous pairs: PLT4/1 → overgrown lysis on LT1. FA 27

PLT3/688 → full lysis on SW688. FA 28

build up again; needs to be "adapted" ← FA26/LT6 → ~~no lysis of LT~~ scattered plaques on LT6, 588.

↓ sens.	FA27	FA28
LT1	+	–
2	±	–
6	+	–
7	–	–
10	–	–
22	–	–
Boyd 4	–	+ (maybe res. R?)
688	–	++
666	–	–
909	–	–
Dov. mot. 1	–	–
Lindau	–	–

Transmotil.

	26	27	FA9	28
906	–		+++	
908			Hazy? T+S	
909	–	–		–
666	2 Tr., neg.	–		–

11/13	27	28
LT-1	+	–
912	+ (lysin?)	÷
688	–	++
688 Mot	–	++

[of FA28 prior Boyd 4 > 688 / 688 mot +]

titres of FA27/28 may be inadequate

FA28 is certainly a "rough" phage

Misc. phage tests

11/12. Prepare Boyd A1, A3 (grown on 912). Test on Boris-mod. strains 1-8 (species): no action. Or on 912, no action on 6887. Same results obtained with the bacterial cultures in lysogenicity tests. (Write Boyd for his boris-mod. indicators.)

	A1	A2	A3	
703	~~703~~ −	−	−	later +
666	~~66~~ −	−	−	
905	−	−	−	
546	.. −	..	..	(inadequate cells)
534	++	++	++	

None of these phages appeared to transduce ~~S. para~~ 666.
Recompare 534 i cultures from NZ of available "S. paratyphi B".

	A1	2	3
LT2	R	S	S
7	Rs	S	S
22	R	R	R
927		R±	RS±Id?
abony		R	R
coli 1		R	R
2		R	R
3		R	R
(Lac-) 4		S	S
5		R	R
eastbourne	R	R	R
stanley	R	R ±	R
typhi 757	R	R	R
~~22~~			
558	S	S	S
sendai	R	R	R
duby	↓	↓	↓
montevideo			
florida			

Include LT2 ± UVLT2/A2, A3.
558 seems to be most satisfactory general indicator. A1-3 not advantageous for other Salmonellae (cf. abony).
A2-3/ S. coli 4 ?

		+ SW435 EMB Gal	+ SW666 Gal.	+ SW666 Mot
1st preps.	A1	2	0	−
	A2	5		−
	A3	5		−

φSW666 transducing ability
misc.

11/1/52

SW666 seems lysogenic for SW588. See 983 for tests
φ666/588 = FA26

FA26 + SW666. No swarms. Numerous tracks: 18, 10 tracks
Retest, of 37° and 30°

FA26 + SW906 (nonmotile): 1 track, no sw.
FA26 + SW534 several tracks no swarms.
FA26 + SW666 2 plates < 1: 2 (1?) swarms. both b, Gal −, 35 tracks
+1
0 swarms, 29 tracks.

2 s. 64 swarms

n EMB Gal − 0.1 ml → no papillae

SW534 + 666 → 2 swarms, Gal+, 1, 2 ! (i.e. SW666 → 534 !) Gal plaque !!
cell mix

θ-20 + SW666 - mot. and Gal : No +, T, or S

see over for host range of FA26:

FA 26 tests for lysis (~~[illegible]~~ EMBLac

Resistant: London (self-lyc.), master., duby, eastb. stanley florida
poona, senft., sundai

E coli 1,2,3,5, ~~[illegible]~~ Boyd 4, SW704, LT-2, abony.
(9790-1-5) SW905-908, SW666.

sensitive gallinarum 774 and SW558 → A few plaques on "coli 4."
very faintly

b - agglutinability of SW-666

985-

Cultures sitting on desk
appeared to show b agglutination (Shiele tests at 1:100 serum)

Streak out: numerous rough colonies. These are non-specifically agglutinated from colonies. How explain specif. broth?

Prepare both cultures from isolated R and S colonies.

		LP22	FA-mot	
1	stable	R		
2	sed.	R	–	
3	sed.	R	–	SW910
4	stable	S	++	

Resuspension of #1 streaked out gave two kinds of colonies, rough and very rough.
" VR" gave stable susp. "R" gave aggl. growth in broth, but mixture of R, VR colonies.
Select in broth, restreak.

985-1-

		FA9	FA26	md. cols.	broth on EMB
1	stable	R	R	VR	R
2	aggl.	R	R	R	VR
3	stable	R	R	VR	R
4	aggl.	R	R	R	VR
	666	S	R		
	526	S	S		

Restreak (transparent) each of two types now appear stable
These cultures are not specifically agglutinable i b!
Repeat #1 = SW909
Assume that "b-agglutination" is spurious.

Lysogenicity and b/i transductions

Nov. 5, 1952

Summary of transductions/ratios. — × SW66

974 E1	SW904	25 i		LP22 (FA9) 21 R	7/S
		2 b		1 R	1 S
974 DD5b	SW623 (12B)	11 i		10 R	1 S (SW904)
		1 b			1 S ? (SW696)
5A		10 i			
D5 *		1 b 19 i	8 b		
974-12		4 i	2 b (18 i — ?)		
974 D1	SW682 FA16	2 i + 7 i	1 b		
D2	685	4 i	2 b (colonies from pool)		
974 ..	PLT22/2	5 i	2 b		

Note heterogenicity	other data
* 12:5	
* 19:8	35 i : 2 b
31 13	7/. 12, 12B ×

Bruce's data:

971 D	?	32 b : 10 i !	(but may be phase 2 FA)
(588 →×)	979-6	31 b : 13 1,2	
573 →×		18 b : 2 i	
546 →×		5 b : 1 1,2	
971 A	(dublin)	5/10 b	
A3	attendorf	10/10 b !	
A6	(entutibis)	6/11 b	D6 1/3 b
A8	sandiego	13/15 b	

Contrast typhimur!

but check rates of phage

There is some indication of differences in the transducibility of b/i in different cases.

Collect additnl. data

A. 27A × (FA from purified phage 1) ~~20~~ (plates kept room temp overnight prior to incub.)

FA9: ! 19 b : 1 i
19 S 1 S
A2 A1

Swarms relatively close together

B. 21 × 666.

N pilus:
9 : i 2 : b 1 b+i

FA9: $8^R : 1^S$ 2^R 1^R
B1 B2 B3

Swarms very crowded!
but all reactions clear cut except as indicated. (But FA21 ~~stay~~ !):

→	↓ LT-2	FA 9	FA 22	≙
A1	−	S	S±	
A2	−	S	S±	
B1	++	R	R	
B2	−	S	S±	
B3	++	R	R	

C. Lysogenicity:

→	LT2 ↓	SW666 ↓
SW697	+++	±
904	−	−
LT-22	+++	±

∴ SW697 has become lysogenic, presumably the basis of resistance

D. Streak out clyte area SW616/FA9. Test single colonies: ~~9~~ $9^R : 12^S$
(should use instead the stationary part of a × SW616!) Restreak 1 and retest
1 rough 2 new sens. 1 $FA9^R$, not lysog. may be rough.
Repeat plating 666 + FA22, FA10 on EMB Cal. (complete lysogen; transduc?)
Cassid. Cal+ from FA10, no visible ϕ act.; FA22: 2 Cal+/plate ca 300 plaque-forming units.

Note: A was treated with PLT-22, B with FA21 (adapted to typhimur. and para B resp.
but both were tested with FA9. Also test c PLT-22

! → EXPS with FA21 doubtful unless marked st.! Re-streak FA21!
A+B all Cal−, as is FA2/cart. A pres. OK.

E FA22 or. → ×666 37°

F FA 22A → 666 37°

G. FA 21ST → 666 37 Uniform outgrowth. Sterility of FA21? Reduce to: 1st expt no repeat!

H. 22A R.T., then 37

I 21ST R.T., then 37. 12 swarms mor PII.

J

G. 22 sw. 4b 18i (#i-4=b) FA9: G(b): 4^R. i: 12^R 6^S Total: 16^R. 6^S

I 12: 1b 11i all ^{R} 12^R

F 11 sw: all b. ! : all ^{S} all ^{S}

E 24 sw: 23b: 1i ! b: all ^{S} i: all ^{S} all ^{S}

34b: 1i for FA22

5b: 29i for FA21

No obvious correlation s, i, lysog. except that FA (thymus) → > b, ^{S}

FA 21 → > i, ^{R}

No drastic temper. effect either.

K. 21 → ×603 (to refute sterility problem) finally! 16 swarms: all bal+ ←

2b 1b+i 14i

all ^{R} 5 FA9^S 9 FA9^R

in full agreement with G-I above.

			9R	9S
Totals: FA21: (count b+i as 1 each)	b	8	0
	i	32	11

FA	AEF		9S	9R
22		b	~~5~~ 3	0
		i	2	0

Does frequency of i relate to host adaptation of the phage or to the previous selection for an i transduction? See 993

P- FA10, streak out Gal+ papillae ~~(only 2 papillae)~~.
a) papillae: 20: all plus +
b) recover Gal- assoc. : papillae
c) pick inter-papillae areas: (visibly phage free : always possibility of invisible plaques)

1 PLT22 × 666 (only 2 papillae)

E: cross heads LT2 /PLT22 and 666 /FA9. (1) (2)
streak out and test colonies for lysogenicity on LT2

1. 10/12 are lysogenic 2. 1/12.
Pertinent to isolate = 986~~E~~1, 986~~E~~2
R = lysogenic S = sens.

a-b: D1: 1^{R}-a both band/a ^{S}. D2: →

c: D1: $18^{S} : 3^{R}$ D2: $16^{S} : 3^{R}$

a	b
−	
+	+
+	−
+	−
−	−
+	
−	+
+	
−	
+	
−	
+	−
+	
+	
+	
−	
+	−
+	−
−	−
+	−

13+ 7- 2+ 8-

11/23

a	b
+	−
−	−
−	−
+	−
−	−
+	−
−	+
−	−
+	+
−	
+	+

5+ 6- 3+ 7-

c: 25 all − !

(over)

No obvious correlation a and b, the latter appear to be random.
Note: Gal+ are $13^{R+} : 7^{R-}$
D2 Gal- (adding b+c) are $24^{S} : 5^{R}$

13	7	Gal+
5	24	Gal-
Lys	non lys	

Sufficiency of phage?

(over)

a/b comparison

	Lp+	Lp-	
Cal+ [a]	18	13	31
Cal- [b]	5	15	20
	23	28	51

a/b+c1

a/b+c1+c2

D3 - Lp+? colony is 4/23. Apparently throws stable Lp(+?) more frequently than D2
- most regular colonies Lp~~+~~ -
  irregular " Lp+. save as D3

SW905-6-7-8

As received from W. Hirsch

905	b (1,2)	Rapidly motile	} in first selection
906	b	Non "	Mailed slime wall, especially
907	b+1,2	" "	908, also 905...
908	0	" "	} Isolate "pure" b phases 905-906

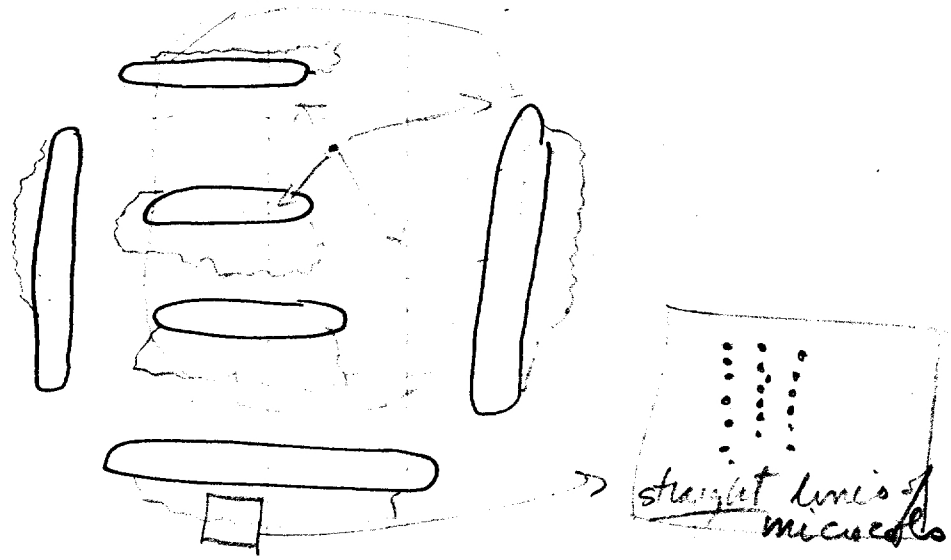
907: 3 single colonies: pred. 1,2, also b! Restreak

7/7 colonies mat i b as well as 1,2. Pick #4, least reaction i b ⇔ b,1,2 as SW907 (1,2). Either very high mutation rate or

Note. Contaminant spread on 908 plate. As it did so, there was a filmy, limited spread from edges of non motile 908:

A).

One repetition,
SW 908 and SW 554 responded very strongly;
SW666 and 550 gave no appreciable spreading.
Swarming is confined to the surface.



Reisolate the contaminant for further study: inhibited on NA (gent. wash) and EMB., but apparent pure culture in broth. It is a spore former, not actively motile. The outgrowths are not the spore terms but not actively motile.

B Phage tests: all 4: FA 26^R; faint secs. to FA 9.

908: gave a spont H^+, no plate / 3·4 (no flare).
+ FA 9 gave a good yield of offtarts and swarms, occ. flares in these

SW905: b ⇒ 1,2 ⇒ b ca 20% per streaking! Most colonies grossly mixed!

a) note very high rate of phase variation.

C b) FA21 → 908 ; numerous T+s. (tracks unusually long + prolific).

14: 6 & 1,2 variants (ca ½ each predominate!)

2: 1,2 + 6? (or i?) Restrict these for further test: definite 0, no variants.

phage responsive to FA9 are indistinct. However, 10 (or more) appear to be still sensitive to FA9.

L

O-form incorrect

4/1/53.

		re SSagan		
(1)	SW616 + SW967.	Controls — .	(x)	4 extinct tailless. (1 Gal -)

O-form crosses

ca 11/12/53.

666 × 550
666 × 551
666 × 554
550 × 551
551 × 554
551 × 554

No motile. sw550 sends innumerable very short tails into agar. (cf. sw

906 × 550
906 × 551
906 × 554
906 × 666
906 × 534
906 × 909
534 × 909
909 × 550
909 × 551
909 × 554

1st trial: bad agar: ruled repeating

A sendai 771 x (a,15) abony² (15) 11/9. 2 buds 4/11 3d bud (2d plate)
not saved 1. eux → a
2. eux → a
SW925 Rev...not tested
sl. rough?
11.5 3 eux → a
12/13 4 a (not transd.)

Purify swarms
Isolate single colony
+ select on homologous
serum for alt phase
B x (a,15) abony' (14) 1 bud — not transd.

C. eastbourne 770 x (eux 15) abony² 1 "bud"? — eux+. not transd.

D x (eux 15) abony'

J x ——— FA22 typhimur. → 1 "bud": weak eux Rev: eux++ i +?
maybe mixed: streak out and look for i No.
12/12 eux+.

E SW557 (typhi 901) x (d) SW588 (FA25) 2 plates, 1 bud each •
save #1 = SW930 1. 1,2 → − 3. 1,2 → − [illegible]
2. 1,2 → −
Purify and test alt. ph. single cols 1,2 not d. monophasic 1,2.
Control: [illegible] 546 /1,2 → no mot.
F SW [illegible] 546 x (1,2) FA typhimurium FA22 [illegible] buds: [illegible] all i
L G 546 x ← FA11 3 buds → b
H 546 x ← FA [illegible] 15 1 b !
2 b !
3 eux
} Does this mean transfer of b or of expressivity of b in 546?
~~See H, supports latter~~

Note 546 on motility agar shows no flare.

Flares (end tracks)

Resumé

A. SW534 ← FA22. 30 tracks: 2 swarms (no flares obvious)
534 maybe heterog. rough: neurolate smooth.

B. FA21 → SW666.

Tracks	Swarms
~~5~~ 13	4
28	6

Picks free tracks. 5/7 are Lp+

C. Stuck out from non-methylated regions: 3/11 are Lp+.

11/20../52
See 989

test pleasantly Lp's (same as SW)

F. FA22 → ×546 11/11 : all i 1-4 tested as anti i : i : —

11/24, repeat. very numerous swarms 16/16 all i. Purify and test /i ; Lp = 9
In first few hours, none swarm. 8/16 are sensitive to P22.
Overnight : no swarm. (#1,3,4,6,13,14,15,16) Save F1 as SW-934

G. FA11 (SW618 b:-) → ×546 3/3 : b: — (all Lp^R)

T.O. 9916-1A eventually isolated. Save for z33 test. ✓ prob. z33 by slide test
9916-1 = SW139 Lp^R

H. FA15 (b:"enx") → ×546 1.-2. b : —
3. enx ⇔ 1,2 = SW926

H3 tested after several passages and 3 single colony purifications.
In first tests, enx phase also reacted with 1,2., but purified version
was pure enx on slide agglutination.

H1 eventually gave "inagglutinable" H1A. Save for z33 test. ✓ z33 3/6/53

H1 → Lp^S, H2 Lp^R. Save H1 = SW937 H2 n.s.

pass SS 3/5/53 → both 1,2 T.O.

K. FA18 → ×546 (cf. F) K1 ~~pan~~ agglut? reacts b, enx, ... ? save.
K2, K4 still 1,2 K3, K5 "inagglutinable" but slow 1,2.? 3/5

C. 546/1,2 control 2 "buds" < 1. 1,2 / 2. inagglut. (cf. K3,5) save! C2 3/5/53 inagg. pass SS

M. 926/1,2-enx". 1 "bud" - still 1,2. Not ~~tested~~. n.s ✓ still inagg. save

L. FA22 → SW926/1,2; enx → Many T+S. 8/8 i → enx
3/8 are Lp^S. n.s. others Save #1 as SW933

N FA22 → ×937 numerous sw. Both 12 ; all i : —. ✓ Lp: #3-5 others: K. do not save

P FA18D → ×937 (rare). ~~Hopeless~~. 14/4 plates agglut! Reselect as b.enx
(med control as SW937): 2 grew more than others, but aggl. Select for motility again.
Q = 937/b control: ~~dodged sw~~ ~~overnight~~ 1 sw/plate after 36 h. T.O. 6 added; also inagglut : aggl. (weak 6?) cf. H1A!

(see over for conclusions).

a. N25 → 157 presumably a spontaneous mutation
(b) (1,2) see Edwards 1946. b → 1, 2.

b. #157 carries a allele at H_1 as shown both by ×546 and 546 × experiments

c. The H_2 allele is either null or the "j" phase that occasionally crops up.

N25 = SW942 should behave as $H_1^{\ b}\ H_2^{\ °}$

a) b: homologue of H_1

b) $H_2^{1,2}$ × 942 should → $H_1^{\ b}\ H_2^{\ 1,2}$

see 1000.

See 989E.

E. 546 × typhi H901 537 : 3 ~~#~~ Purify: single colonies pure 1,2.
(1,2), (d?) on 1,2 again:
1 1,2 → — SW930
2 1,2 → —
3 1,2 → —

A 546 FA25 × abony[1] 11/19: 4 tubes: – all 1,2
1 1,2 → enx 5 12 → enx
2 " 6 "
3 " 7 "
4 "

B 546 FA25 × abony[2] 11/15: 3 tubes – all 1,2
1. 1,2 → enx —3s.c.→ 1,2 [redacted]
2. 12 → enx
3. 12 → enx
4 "
5 "
6 "

all are 1,2 except A7, save as SW938

Study B1 in greater detail:
1,2 phase does not agglutinate (slides) in enx
enx phase shows same (1,2).

~~C. 546 /1,2 control~~ (see 979 for abony[1] ≟ abony[2])
~~D 18 × 546 /1,2.~~

Host adaptation of PLT22 - ...

b/i determination

993

SW686 unfortunately is Lp+

A FA31 (= FA9/LT-2I). × 666 Many T+S.
2i; 11b.
In cross-back tests (~~no plaque count~~) this phage appears to have retained its specificity for ~~prototrophs~~. Ca. half the bursts are phage-plqued. Phage character should not be determined s prior streaking out of the culture.
This result would indicate ~~that~~ host adaptation of the phage does not determine b/i ratio.

B. FA21 × ~~928~~ 928 Fairly distinct swarms: 4b: 16i

C. FA22 × 928 Many T+S (confluent). Pick plaques. (1 lytic area noted in swarm!
(Pick and test for Lp^s or Ph. mutant.) ⇒ no evidence of turbid clear area may have been single staking infection.
Swarm not too well separated. 14:b 1:i 2:b+i. Read total as 16b:3i

∴ lysogenicity of transducee does not influence b/i ratios.
SW686 is unfortunately not feasible for FA (Lp+); this remains the basis for a final test of mechanism of ratio. In addition, other i's from FA22 × 666 should be examined. See 973C3

4/24

D1	FA 36	A	×	666	T+S	9i : 9b	sic!
2		B	×	666	T+S	7i : 7b	"
3		C	×	666	No	7i : 3b	
4		D	×	666	No.	6i : 2b	

(SW935 / SW936)

Test 36A × 666, 36B × 666 for Lp^s i (vague opportunity for reinfection during isolation).
A 6/8 are Lp^s ~~some~~ 1s
B 3/7 are Lp^s " "
n.s.

E FA12 × 666 Relat. few swarms, but 9/9 i

(over)

11/28. 628 → x 666   7i 4b 1b,i

8i : 5b

This appears to be quite distinct from 623

11/28 FA12 → x ✓ 5i : 0b
FA12B → x 12i : 2b

Using pools from
12 → x 15i 5b
12 → x 14i 6b
12B → x 16i 4b

poor agreement with other data

625 → x 7i 3bi 3b

(swarmers must have been confused, from overtoft agar)

680 → x 14i 6b 1bi

15i 7b.

note: ~~sup~~ suspicious run of b's as lined up on plate:

i	i
i	i
i	i
i	i
i	b
i	b+i
i	~~b~~ i
	b
	b
	b
	b
	i
	b

Possibility that there are hidden cues for b/i on the swarm plate?

Rx verified!

b:i determinations summaries

12/5/52 → × 666.

Need more data on parity. B adaptation.

		i	b	
A. FA31 (= FA/LT-2^I) → ×		2i	: 11b	
B. FA21 → × 928		16i	: 4b	
	cum. total	60 (13)	12 (11)	
C 22 → × 928		3i	: 16b	
	cum. total	7	80	
D 1 36A (SW935)		9i	: 9	
2 36B		7	7	
3 36C		7	3	
4 36D (SW936)		6	2	
E (SW623) FA12 → × 666		9	0	cum tot 61i:4b
" "		5	0	
12B "		12	2	
pools, colonies: (HLB)	12	15	5	} discrepancy?
	12B	14	6	
	12B	16	4	
625		10	6	(3 were mixed)
626 FA37		49	21	(+16 mixed)
628		8	5	
680		15	7	1 b+i } heterogeneity?
addnl (HLB scored)		22	5	
		63	7	
		100	19	
18D		–	24	
18 see 971 D 18.		5	52	

see 973 C3

Number of classes is doubtful.

SW935 ca. 50% each
623 61i:4b
680 100i:19b (heter?)
626

?? Selection discriminating b:i?
Difference might be controlled by selective effects of the linked markers! For further study, more efficient scoring is essential!

11/25 FA10 titrated as 2150/ml* on SW666. 2 plates each

A. (.05 ml FA10 → SW666)

B. SW609 (= Gal⁺ H⁺) 177; smeared

C SW609 + SW666

D .05ml + SW609 + SW666.

Control on φ³ of 109 Lewis!

24 hours. Counts in C appear > D. D noticeably but not greatly ahead of A

11/5:

		controls H⁺	transduc H⁻		
Totals: type	Lp⁺	53	18	3	18
	Lp⁻	43	43	43	3

After 24 hrs. Rate of lysogenization fairly low.

This experiment would be improved if lysogenization were limited by known factors (insufficiency of phage)

D:

	Lp	H⁺	H⁻	Not	2	3
C 1	−	+	+	+		
A 2	+	−	−	−		
C 3	4 −		+	+		
C 4	+	−	+	?		
D 5	−	+		−		
C 6	−	+		+		
C 7	−	+		+		
A 8	44 +	−	−	?		
C 9	−	+		+		
C 10	−	+	+	?		
C 11	−	+		+		
C 12	−	+		+		
C 13	44 −	+		+		
C 14	−	+		+		
C 15	−	+		+		
C 16	4 +	+	+	+		
C 17	−	+		+		
C 18	−	+		+		
C 19	−	+		+		
C 20	−	+		+		
C 21	−	+		+		
C 22	−	+		+		
23	+	−	−	?		
24	+	+	+	+		
C 25	−	+		+		
C 26	−	+		+		
C 27	−	+		+		
B 28	+	−	+	?		
C 29	−	+		+		
C 30	−	+		+		

	Lp	H⁺	H⁻	Not	2	3
C 31	−	+		+		
C 32	−	+		+		
A 33	+	−	−	−		
C 34	−	+		+		
C 35	−	+		+		
A 36	+	−		−		
C 37	−	+		+		
C 38	−	+		+		
C 39	−	+		+		
C 40	−	+		+		
A 41	+	−	+	−		
C 42	−	+		+		
D 43	−	−		−		
C 44	−	+		+		
C 45	−	+		+		
A 46	+	−		−		
A 47	+	−		−		
D 48	−	−		−		
A 49	+	−		−		
C 50	−	+		+		
A 51	+	−		−		
A 52	+	−		−		
C 53	−	+		+		
C 54	−	+		+		
D 55	−	−		−		
C 56	−	+		+		
C 57	−	+		+		
C 58	−	+		+		
C 59	−	+		+		
C 60	−	+		+		
A 61	+	−		−		
A 62	+	−		−		
A 63	+	−		−		
A 64	+	−		−		
A 65	+	−		−		
66	+	+		+		
C 67	−	+		+		

			.1	.01
FA10	SW666		215	18
EM/Gal	SW928		360	29

FA22 .1ml 928
.01ml 978

A. Goldstein strains

995

Received 11/2[illegible]/57.

W2148 = "MSFB"

very rough, apparently homogeneous on EMBLac; MH. grows well on D/0).

2149 = Harvard (Goldstein)

dimorphic, especially on EMBMH.

A: smaller, darker (lytic ?) [illegible] colony

B: gummy, [illegible]

poor growth when streaked on D/0).

11/24. Separate A and B for further test — character persists on EMB MH.

Replica plates: A: no growth on D(0) or EMS Lac in 48h. Eventual reversions ?

B: all colonies grow (ca 50 tested).

For further work, need to run down requirements of A.

In subsequent plating of B, ca 2/300 auxotrophs. Pick as 995B 1-2

4/14/53 EMP

Subtypes W2149 observed

homogeneity W2148

Both R to 2-2

Srp with W1926 (F-) and W-1177 (F+)

on $Smal_{sm}$ + Lac_{sm}

Dienes' L forms

Acc 1319 8/56.

11/16. Brought back from Boston slants of "Proteus 52" and Salmonella typhimurium "3". In addition, a vial of L-growth of the Proteus (badly shaken in flight.

(A) Plant in Penassay ~~agar~~ broth; no growth here for two weeks. 11/30 - 12/1 surface film noted in 1 of 3 tubes.

12/1. Transfer to fresh tubes: Penassay; Penassay + horse serum 10%; Penassay + cotton; motility agar; motility agar + SW66: Good growth in 2-3 days, especially on penassay broth. Very poor growth on agar. Note a curious film of material on the surface of L-form growth: fatty material? in phase microscope.

(B) (1) Proteus 52 heavily inoculated in agar layer with ca 1000 u penicillin ca 11/18

(2) ca 11/21 cut out block and smear over surface of fresh N.A. + penicillin plate. Both show L-type colonies especially in depths of agar.

(3) Transfer to broth / agar slant + horse serum + penicillin.

12/15: Dense growth on agar over liquid! No bacteria noted.

12/16 Reinoculate to wet slant & horse serum + penicillin

12/17 a Broth; motility agar: typical motile bacteria

b Agar + serum: mostly bacteria; some granules + fragmentation; rare thin hyphae in microcolonies.

c Penicillin serum agar: no marked growth.

12/19

(C) Transfer cultures of 12/3... to v. small inoculum.

P24

1. Broth	0	−
2. " + antifoam		−
3 " + collodion (boiled)		++
4 " + mineral oil		−
5 D/nic)		−
6 D/nic) + a.f.		−
7 " + oil		−
8 Mot agar tube		+++
9 " 1/2 & penassay		+
10 " 1/6 " "		−

23 11 Streak out on ~~mot~~ motil. agar.

12/28:

Motility agar, in tubes seems best. Emulsified inoculum, however, does not grow well. Heavy inoculum grows well in broth

Proteus 52 pour quite well in D(o) ? impossible ?

12/20 996 B: grew very well on motility + ~~pen~~ icillin; no swarming.
Microscopic examination: large spheres + small granules.

D Salm SW 996: smaller sph + gran. } on Motley medium.
EP SW 997: like B

Test for recovery of bacteria; try to float B on broth.
12/28 ~~C~~ E all gave turbid growth. Carry on penicillin broth. Transplant
D to second penicillin - mot agar.

E gives faint limited turbidity in penicillin broth. Microscopically,
only swollen forms were seen. Titrate on EMB lac and plain, broth
penicillin.
D gave clear broth. Plate out E ↗ Broth ± 500 u/ml penicillin

E: 10^{-1}, 10^{-3} ml: Crowded on EMB Lac 10^{-5} ca 100 (size v. variable)
24h: 10^{-7} 0 on EMB.
Pen assay Turbid ± penicillin 10^{-1}, 10^{-3}, 10^{-5}.
10^{-9} clear 10^{-7} - penicillin turbid
+ penicillin turbid but settles granularly.
These are generally more turbid than above inoculum.

Microscopically
a. Inoculum spheres and granules
b. 10^{-7} - penic. motile bact
c 10^{-7} + penic " "
d. 10^{-1} + pen swollen bacteria and mar - spheres
e $^{-1}$ - pen. spheres and " "

note large grey inocula → deformed bact
smaller " → normal bact!

try 1 ml inocula

(over)

c8, stored on motility agar in frig., and carried back and forth to Chamblee gave a diffuse growth in broth 3/10/53. Resembles Sarcina ⁘ ⁘ under microscope. Presumably contaminant — fails to grow on EMB lactose

11/14/53 D - (Saline) has shown no turbidity or growth of any kind

E. ②. Had been turbid throughout medium; has now also developed a film on surface. Transplant to fresh penicillin broth + penicillin ± collodion = E3 a, b.

F. Centrifuge E②. Assay supernatant and filtrate of same for bacteria on EMB Lac. Resuspend sediment in 1 ml (ca 5×) H_2O — forms a gelatinous mass. Under scope, huge clumps of L-type 

Put on plain agar 1:15.

after ca 6-8 hours, small microcolonies of plump L-types appear in conjunction with clumps. Bacilliform could not have been present in comparable numbers but detailed origin was not discernible.

filtrate: sterile on broth and on plate. ~~Unfiltered~~ Unfiltered sup & had ca 10^4 colony formers/ml. (1000×10^1 but 0×10^{-3}!)

Osmotic Shocks

998

12/4/52 Osmotic Shocks.

A. add .25 ml PLT22 (961.4) in broth to .75 ml saturated NaCl.
Add 10 ml H_2O. Add 2 ml broth.

Est titer $\frac{1}{4 \times 12} \times 4 \times 10^{10}$
= ca 10^9

B. as A, but add NaCl last.

Assay plagues; .01 ml samples on sw666 / motility: too crowded.
.1 ml on sw435. < 50 on either! see 999
for host adaptation peculiarities.

A: plague: 17×10^7 B: 33×10^7
525×10^6

No marked effect of shock on plague titre

~~Repeat 12/9 using diluted PLT~~ 22. Dilute in sat'd saline
~~to an estimated titre of~~ of Diluted ca 40:1

Assay for plagues ~~on sw666~~;
for prototrophs on sw435 D60)
Salt " " EMBSal

use NaCl in more dilution?

A. no salt
B. salt added to diluted
C. salt added, then diluted 10:1 for shock.

(counts

A 45×10^7 B 53×10^7 C 138×10^6

The shock has had only a negligible effect, $pS \ll 1$.
Set aside until more details on conditions are learned.

assay on sw435:	SalEMB	A	B	C
.1 ml	D10)	3	0	3
		8	1	3

435 has been behaving peculiarly lately.
Probably now largely Hayes!

UV - FA21; FA12

December 4, 1952.

Preliminary experiments

Assay FA21.

SW666 21×10^8 219×10^7 $= 2.2 \times 10^9$ LT2 1×10^7 !

(also 18D = 192×10^7)

Place 1ml FA in Petri dish. Expose to UV 120 seconds.

12/5/52 ~~Add 4ml H~~ ~~and recover~~. Assay survival. ~~and t~~

~~SW666~~ (SW666): 15×10^7 Plaques smaller?

Control: 128 $\times 10^7$ SW666 253 $\times 10^5$ LT2

... $\times 10^5$ ∴ 22B may be ill adapted to LT2. (to cells n.g.)

Recheck; FA/435 D/O). FA22 .1ml 325

FA21 " 0

FA21UV " 2 Resistant thereplagues.

Swarm induction

FA21: .001 samples:

Σ	#	#swarms
	6	0
6	6	1
8	2	2
9	3	3
4	1	4
23	18	$= 1\frac{5}{18} =$ ca. 1.3

999: .001ml samples:

No zeros. Estimate ca. 5 / sample but not properly countable! Cannot count tracks either. Tracks also still prominent.

Relative activity: $\frac{1.3 \text{ transductions}}{128 \times 10^7 \times 10^{-3}} = \frac{1.3}{1.3 \times 10^6} = 10^{-6}$ per phage.

(For future: Use 120 × use doses., more dilute inocula.)

FA12 maybe more satisfactory.

b:i ratios:

	control (FA21)			999		
	b	i	mix	b	i	mix
	4	13		4	18	2

999B

12/7 Pool FA12, 12B for further studies.

Assay at UV = 0, 120, 240. Plate .001 ml samples on SW666; motility.
1 ml / 10 ml plate on SW666

A	0	666	swarms too numerous	3.55×10^5 / LT2	1.05×10^7 / 666
B	120	"			1.85×10^6
C	240	"			4.30×10^5
D	0	918	15 sw / 9x, 101		
E	120	"	18 sw / 10x, 101		

More dilute platings needed.

		Phage / 666.	Gal+ / 666.
12/8.	UV 0	[redacted], 87,100	
	360	130×10^5 — $\times 10^4$	

Swarms: 0: ".101" ca 5/ea.
".0002" 28 (+2 or 3?) / 19 [redacted] $\frac{1}{6} \times 10^{-3}$
".00004" 3/20 $\frac{1}{36} \times 10^{-3}$ =

Fortuitous, 1 plate shows 29 T 0 Sw
9 T 3 sw (denser agar)

UV 360: .001 ca 2-3/ea. 27/10
31/10
31/10

.0002 14/10 + 13 trailers discrete. plaquing notable!

est. ca 50% reduction in Fla+ FA action. Cf. Gal+ ratio.

Note, UV 360 has phage "activity" of 1.3×10^7, Fla+ of [redacted] 1.4×6000
6×1000
= ca 84000 /ml.
= ca 1/2000 phage particles!

Phage inactivation considerably faster than FA? Still larger doses needed for specific inactivation studies.

12/8/52 ① UV·0 φ/666 87; 100 × 10^7 Gal+/666 ·Incl: 376
FA12
② 360 130 × 10^5 280

SWARMS. Samples of 1/10 .01ml , × 1/6 , × 1/6 .
A B C

FA/φ = ca 10^{-5} sw = .4×10^{-5} Gal+

1A. >> ca 5/
B. ca 28 /19
C. ca 3 /20 < 29 trailers 0 sw
later, about 11 additional sw. appeared. 9 · 3 " (denser agar)
* The 3 early are 3/3 i. The later swarms are 7/11 b!

FA/φ = ca 1/1300 sw = 1/5000 Gal+

2A 27/10 31/10 31/10 10/10 i
B 14/10 : 12i : 2b (+13 trailers) discrete plaquing (predicted ca 300 plaques/bundle)

12/9. ③ 600 sec. ~~(add 2 ml for evap.)~~
mic evap. noted (ca .5ml recovered)

φ: 172 × 10^3 ; 13×10^4 0×10^5
Gal+: ca 1500 on SW666 ; 28 on SW928
per .1ml (count ¼)
Swarms: .001 ml samples: (12 hours): 19/23
ca 1/sample
trailers still evident even on soft agar.

∴ FA/φ = 1000/172×10^3 = 1/172 !

$$\therefore \frac{Gal^+}{\varphi} = \frac{15\times10^3}{172\times10^3} = ca\ .1$$ see E

Non-confluent plaquing is obvious on this plate.

---

* C1B: 26 i /2b includes 3i/1b classed as "small swarms"
C1C: early 3i /0b 1–3
← delayed 4i /7b! 4–7, 8–14 motility of these should be compared!
C2A: 10/10 tested : i hold remainder
C3: 10 tested: 3b, 7i Interpretation?
see 1001

*Evidence for selective differentials of b, i presumably owing to linked factors rather than the alleles themselves.

UV - activation of lysogens? 999D

12/10 1. UV = 0 2 UV = 120. (dil 1:1 prior!)

A	35	A	54
B	99	B	(155)

A = 666 B = 928
.05 ml samples.

12/12: no FA: 0

~~This dose partially inactivated: FA/666 activated FA/928.~~

ca same activation at this dose

Linearity of FA, UV = 0.

Calf	666	928
.1	534	16
.05	291	7
.02	113	2
.01	52	2

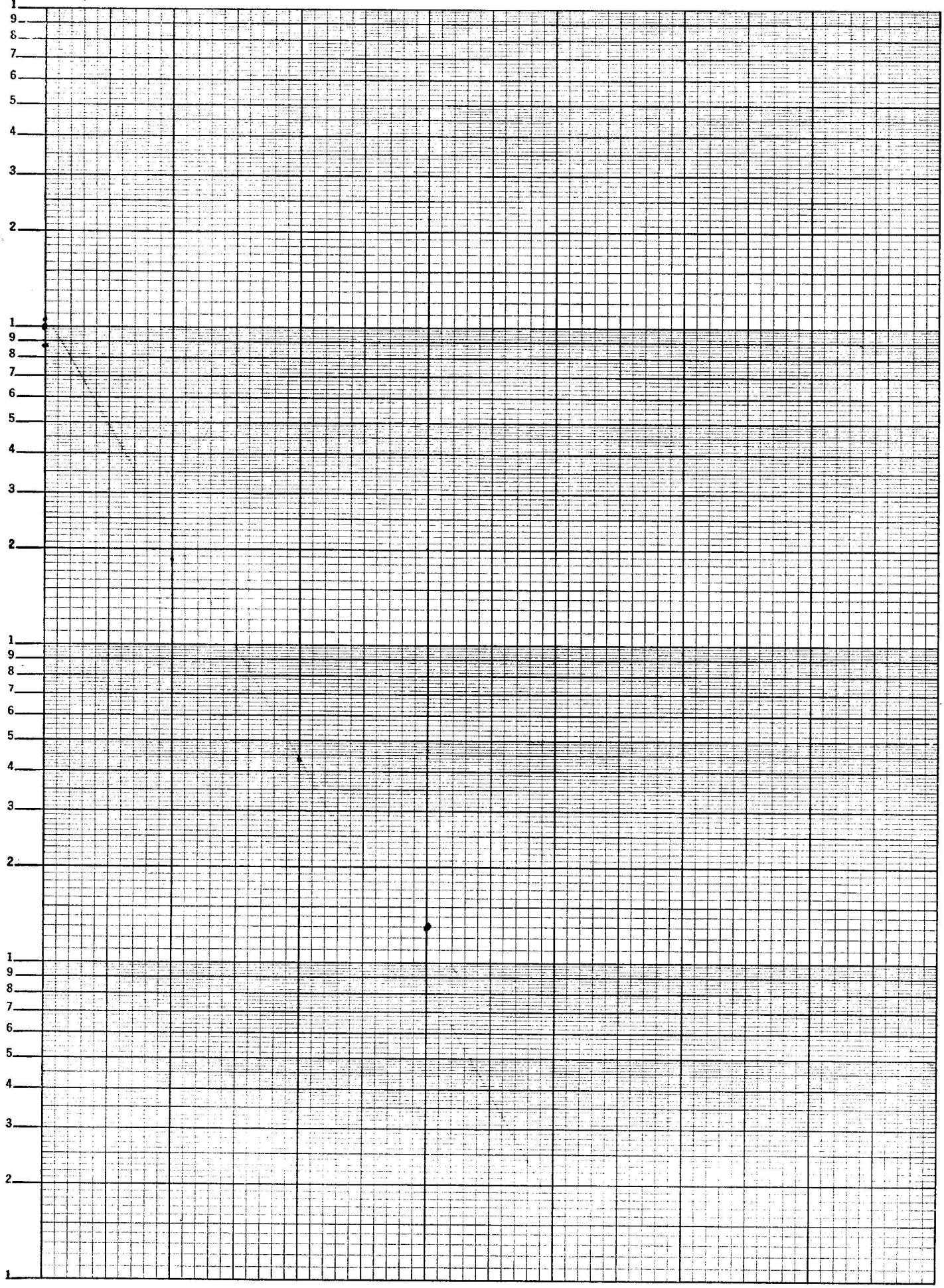
satisfactory linearity but of 999C1 (somewhat less)

" ditto, but why lower efficiency? of.

different batches of 666 cells may account for < heritable differences.

FA12/666

999



10^9

KRUFFEL & ESSER CO., N. Y. NO. 358-81
Semi-Logarithmic, 4 Cycles X 10 to the Inch, 5th lines accented.
MADE IN U.S.A.

120 180 360

999E

Limiting inactivation of FA ~~12~~ 12. UV 15 minutes 50 cm.

① Dilute 2ml FA12 with 2ml H_2O. "

② " FA22 " . "

③ " FA12 UV 5 mins ca 15 cm distance.

12/12.

		SW866		SW928 Gal+	
E1:	$\frac{1}{2} \times .02$:	Gal+ 65	φ 100	11	∴ ca = plaques and transductions.
	$\frac{1}{2} \times .1$:	Gal+ 380	φ 360+	68	
now E10			.05 φ	91	Pick transductions away from plaques.

E2: Residual phage uncountable at 10^{-1}.

E3 φ16 / .1ml 16 Gal+ (streak out 12)

E2-0	SW435	.01 ml:	18
		.05	71
E2		.01	3
		.05	14

Repeat c LT22!

note shine on D(0) at RT!

ca 5-fold decrement in FA.

E1-3 (1-20 are E1 21-32 E3).

#3 lysogenic: (scattered plaques)

19, 20, 21 lysogenic

all others are non-lysogenic; sensitive to FA9.

These are presumably fortuitous. Lysogenization does not occur typically c transduction by mediated vents.

Restreak all on EMB Gal. All pure + # 19, 20, 21 show some colonies s sheen (λ^+/λ^s?) Restreak: 1 normal of 20, 21

of E3 #21 showed poor evidence of lysis in streaking.

Spot pur. test of 1-20, none were lysogenic.

Aug 1966

SW 942 (b: —) homologies

1000

12/7/52 Edwards N25 = S. paratyphi B java, source of SW857, recd. Prepare FA 42 (FA9/942)

12/8 A. FA25 (SW588) × 942 V. Numerous swarms. Isolate and test 4: 1,2: — (stable > 3 days as 1,2)

~~#1,2, 3are Lp+~~ save #4 = SW945

~~10/9~~ ~~B. FA18 × 942 → b: 1, 2~~

~~C FA15 × 942 → b. enx~~

~~D FA42 × 944 (+ enx) → b enx~~

~~E FA42 × 946 (+1,2 enx) → b e. not studied.~~

F 18D × ~~H~~ SW609 (H_2?)/b. 8? sw / 3 plates ÷ 8, either b or maggl. Not ident. 1,2 of # × 546 but may be due to much greater rate of potential phase variation in the latter.

B. FA15 × SW945 1(1,2)

C. FA15 × SW942 1(b) → 1 sw. 1,2 (see!) 1 bud maggl?

D. FA15 × SW609 1(b) 1 sw : maggl. 1v motil 3/5/53 : 233 T.O.

E FA15 × SW 857 ~~1,2~~ (1,2) (note rarity of enx in pure.)

2 buds. both still 1,2

4-5 plates each as indic..

C 1: stable 1,2: —) saved 2: not but maggl.) E

This expt. gave nothing except ?? C9, recurrent 1,2 from SW942?

D ① still b.

B no buds
D only 1 maggl bud.

B ① still 1,2 partly rough ?
② " " colony reacts ⑥ and 1,2 ?
Re-select 1,2 ÷ no swarming. T.O.
several maggluninable