Second phases.

10/5/52

Plant indicated 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 spont 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 × abony b enx /b. 4 buds. 3/4 still react strongly i b (= 3-1)
#4 inaggl. (= 3-2!) Streak out, reisolate, and retest on b agar.
#1 very weak b.
#2 inagglut.
10/22: /b slow spread.
rough ← T.O.
j ?← z_{33} by slide test 3/5/53

4 SW672/b. (= typhimurium × abony) → 4 days: non b, i, 1, 2, enx
10/22 /b slow spread
SW435 itself is relatively poorly motile! (anxotrophy? test transducers) 435. moves very slowly through i but 1, 2 phase appears
623, 435/"I" – apparent buds still i.
LT2, LT22 → swarm 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. Streakout: 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 stock) → later heavy swarm.
SW569/i no sw.
↓
slow spread

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

Check Spicer's sera

618 swarmed on i, not on b.

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

SW 435 is relatively immotile compared to LT22. This may relate to behavior of SW672

703 (purif.) /b → 1,2 (3 buds)

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

a) Recheck single colonies: some seem to react both 1,2 and enx

Regrow: 1,2 only

Second phases

~~981~~
976

1 SW671/i, 12 No.

2 SW673/b → inagglut. SW676

3 SW670/b → " ; very weak b. same

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

5. SW703 orig. → contaminant, isolated later & 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 incipient rough~~
Not distinguishable from typhi. How prove contam?

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

Unstable transduction

977

10/13/52. SW 684 is Gal +/-. Transmotilize & PLT22: ca 50-100 tracks and swarms. Pick more or less well separated buds, streak on

A. EMB Gal to 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 + ~~FA937~~ FA(SW684) only 7 papillae.
6 gave -, pure + 1 had +, (1v)? Restreak
(Cf. 986D - This one not saved) → this has phage-mottled appearance, not +/-

New transducing phages;
R forms.

10.../52

1. R isolated from aged broths of eastbourne, Boyd 1104 typhimurium ... SW688.
688 is sensitive to φ2715 (Desroulneau), 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 ~~[illegible]~~ streak-out.

3. Cross brush LT-1 to 10 / SW688 LT10? on SW688. SW688 shows discolored plaques, no lysis with θ 10 (Spicer)
θ-10.

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

LT-1 to 10 all R.

5. SW ~~[illegible]~~ 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

Litto 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.

	φ 2715-43	942B
Boyd 1184	R	R
" SW688	S	S±
Typhi 1+90	S	S
~~SW540~~	~~S~~	
LT-1	S	S
thompson	R	R
montevideo	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.)

Phase variation.

of available material, abony (enx-b) and typhimurium (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 on b agar.

② Repeat, but ~~LT~~ LT2 ←× abony' 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 (bariensis) but i is rather a rare kind in general.

③ Try entritidis as recipient for transductions.

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

~~Purify and test vs. 1b.~~ Purify 4. Test all directly on b(1,2) agar.
4: ~~b~~ 6: ✓ ~~magglutinable~~ → magglutinable (sl. rx 1,2 ?) cf. SW678 small beds from #4, 6. pick, 2d plate is heavily grown. Pick center (guernish)

⑤ Phases 1 and 2 of SW703.

979-5A = FA23 → × SW666

979-5B = FA24 → × SW666 several thousand tranks (swarms) test pooled tran various spots / b.

15 spots (probably tests >100 swarms): none swarmed directly out on b agar
3 gave what may be beds. pick these: 5B1-3 : still b. Reincubate

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

---

streak out pooled swarms on EMB Gal: 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 on 1, 2)
+ 8 addnl. all b
(see 981)
> Recheck all b. or may be sl. rough

5A: 20/20 b.

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

10/27 FA 1/10

---

6 FA (SW588) → SW666 6 1, 2 FA strike: ✓
BADS 2 mixed
more or less well isolated swarms 14 b
streak out on EMB Gal.
b, 1, 2 both Gal – SW699, 901.

Note SW533 (= 703?) $\xrightarrow{\chi}$ 534 $\xrightarrow{sp.}$ 588
But ~~533~~ 703 behaves differently from 588. Recover NZ culture of 533

Recap + summary Phase variation 979

10/28/57

inanti-i

(2) A. LT-2^{I} x— abony^{I} gave 1 swarm SW699 b → 1,2 $\xrightarrow{\text{slow}}$ b (thin agar)

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

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

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

(5) A. FA23 (SW703') → x SW666 15/15 b on b agar. 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") → x SW666. spots of pool b on b agar
27/27 (b) Pool does not migrate on b agar. Rx b at furthest ext.

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

979DD6: FA18 (LT·2^{II}) → x SW666 18/18 b no alt. phase could be selected

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

$i^*; 1,2 \leftrightarrow i; 1,2^*$ where * is a locus-fixed activation (or suppression).

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

(4), (5B), 974DD-6, and previous expts also i typhi lead to hypothesis that $A^{1,2}$ A^{enx} can not be expressed in monophasic strains. Apparent paradox:

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

x → b only x → 1,2; b

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

(over)

11/2

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

LT-1^{II} " predom 2

699 " " b

abmy^{1} " " b

abmy^{2} " " enx

E abmy 1 FA22

F " 1 FA18

G " 2 FA22

H " 2 FA18

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

A LT-2^{I} FA14

B I 15

C II 14 1 bud: ? aggl. ✓ single colonies → 1,2 + (?) → maggl. (abcdienx gm r 1,2
✓ lysogenic for LT2! Loc-. Save as 979-C for later identification. May be poorly motile.
(Last weekend of Boston trip)

D II 15

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 " "
} n.s.

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

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

(moi ✓ b++ enx±)

semm probably more anti enx than b.

1-2 plates each. all negative except as indicated
A-D in i, 1,2 semm; E-H in b-enx.

J abony^{1+2} ← FA 22A
i : 12

Numerous swarms
(10-20/plate).

of #1-16, purif. 11 are i
5 are b (pulsed by HLB)
later, i isolated from these impure buds.
17-33 (not pur.) all but 18 and 29

Confusing reactions probably weak H.

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

K. ← FA 18
i : 12

possibly over-inoculated.
1+1? 14 plates. (Repeat!)

Note: FA 18 → 666 gave only a few swarms. Make new preparation FA 18 A (see below).

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

L. SW 698, 699 ← FA 14, 15 / i, enx or b, 12 ~~Neither~~ 1 plate each. No swarms on any (parental spread overgrew)

(K) 1. 1,2 → b Lp^R
2. 1,2 → b Lp^S SW 932

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

11/30/52.. 29A = 34 is i. Repeat 34/i: enx!
∴ nothing 29A' = i (v. weak).
∴ odd hue.
#14 appears inagglutinable. All others are i.
Thus no clear cut 1,2 → from FA 22. See over for /i..

(J)

18A i 18B enx^{++} 1 = /i
18A' ( ) enx ✓? 18B' enx^{++} ?
29A i 29B i
29A' (i and v. wk. 12.) 29B' enx

Repurify 18A before further tests are made. 18B may be presumed contam. from abony. 18A' is enx, but also shows trace 1,2? Keep "18A" as 18.
29B as 29 all 34 are Lp^R!

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^1 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 saline 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 preps

LT-2 II

4: These colonies when regrown give only trace agglutination in 12 (sim? 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 on motility agar then gives a strong 1,2.

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

b ⇔ enx frequent

abony I: b:7 enx:3

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

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

→ 18i 1:1,2 others R.

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

Thus, FA22 - FA18 are fairly reliable, but

FA14-15 are not, but nearly equivalent.

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

streak out pool of 9795, pick colonies test Lp(22)s: undetermined proportion of Lps. Purify and test SW941 as Lps; i:

979K...

12/1/52

K. FA18B → x 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 Lp^R n.s.

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

(15 colonies of #5 tested all i.)

8. 1,2 → b
9. 1,2 → b

12/4. FA18D (conc) → x abony^{1,2}: 5/4 plates.

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

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

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

J: 12/1/52 FA22 → x abony. Crowded swarms. Pick pool and streak (for ip^S).
(Previous isolates Lp^R – not surprising as separate isolation was delayed)

typhimurium -x abony two step 979LM

Solutions c̄ b.12 serum.

L. FA22 -x SW932 [FA18 -x abony] Phase 1,2

M. " " " Phase b.

= amts FA22 (but excess) and approx = cells.

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

(not 1,2 - mitotally)

L 1	i → 1,2		M 1	i → 1,2	
2	i	"	2	i	"
3	i	"	3	i	"
4	i	"	4	i	"

1p tests? all appear +. save L1 = SW943

Thus, i:1,2 can be reconstituted from i:1,2 -x b:enx in two steps.

979
JK.
Summary

J. abony 1+2 × FA22A

33 swarms. #34 = 29A All are i except #34, inagglutinable.
Select second phases i 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 : H.L.B.
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 as above. 33/33

K. 16 swarms total: 1 cont. (not Salmonella)
2 inagglut (viable? ≈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 may be 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. SW603 $^{G+H-}$ + SW680 $^{G-H+}$

~~B SW603 + SW618 $^{G+H+}$~~

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

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

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

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

Work in D (10+ : 1−)

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 ca 5:1 ?
b. sediment lightly − top of broth ca 10:1
perhaps allowed to grow too long. Repeat in growth 11:35 − 3^{30} (recover)

Heavy agglutination.

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

But SW703 showed virtually no response to X! of SW533
Phage n.g? Repeat in X/SW592 : now all are susceptible (incl SW537)
∴ 942 − 1 has lost some activity
Repeat in new X

9803: short serum passage:

unsedimented: ca 6:1 +:−

sedimented briefly: 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) more i previous serum tube after light sedimentation. Compare no, light, strong sedimentation.

ca 20:1 heavy sed.

180:1 control

D4 ½% agar. ± glucose

Val ratio

	+	−
Inoc: washed cells, 9800 sec. passage:		
+ 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. Archer's method may be slightly efficacious — needs to be worked-up.
4. X phage not yet properly tested. Cf 588 as H^+G^+ + H^-G^+ 666. start fresh inoculum

---

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.

~~Set up in ca 1½ % salt. Kept at room temper. 5:25 –~~ n.g.

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

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

But culture here labelled 603 agglutinates in b. (Mix up?) Test slants

Ineffective selection.

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: grow plaque on 588

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

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

* recover result may be influenced by lysogenicity. Should streak out firm area of mixture away from phage action.

In some ~~con~~ control tests,

SW666 reacted weakly i b when grown from broth,

but not the saline suspension from agar.

Retest broth cultures incr from → ✓+ but very weakly b not i

	Drx	Water
A slant	±	–
B broth culture on deck	–	–
C saline susp	–	–
D motility plate (negative)	++	++, not d, i, 1, 2

microscopically: nonmotile

direct test from cells on D gave wk ± b – d, i

603 broth also ✓ b.

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

Restreak D, spot on motility agar.

Enc O. forms

980

11/4 G. X solution 666+588.

Initial ca 100+:1−
2:30

6:30 Control "
X ca 50−:1+ ∴ ca 5000 fold solution!

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

F. SW603 ($H^- G^+$) + SW904 ($H^+ \overset{i}{G}^-$)

Initial 4:30
room temp. { + i serum (.2%/2) 8:30 } ca 50−:1+
lt. cont.
heavy cont.

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.
b: heavy agglut., lightly turbid supern.
603 "spont aggl?" – not as heavy
904 heavy aggl.

Too much serum? —
O-nactean or
nonspec. aggl?

these stocks not entirely suitable

Quantitative comparisons of tracks, swarms and Gal+ transduction.

Mix .2ml SW666 & .2ml FA24 (1/10 or 1/100 in broth)
Moz areas ~~of~~ & ca. 0.01 ml each Count swarms + tracks overnight.

	.01 ml x	EMB Gal pap.	total tracks	3 days	total swarms	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

T. may not have developed reincubate

each swarm had a distinct flare, several hundred microcolonies. Picks where distinct to repeat effort at isolating O-forms: but only 1 does not have an adjacent track → 15 colonies from region of flare, all b++. (Total now 60).

of 30° 1:100 sign p

		Gal+
1:10	5	2
1:100	5	2

} Note non-linear response also seen in previous experiment

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

See 979 B

Pedigree of SW534 .. 548 etc. 546 982

10/31/52.

According to stock list,

SW533 $\xrightarrow{x}$ SW534 $\longrightarrow$ SW588
||
SW703

However, SW703 $\rightarrow$ x only b to SW666

SW534 } $\rightarrow$ x 1,2. Also, SW546 $\rightarrow$ x 1,2.
SW588 }

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:

PRE: ?	533 = 703	546 = 857	note
	IV V XII	IV V XII	"706" IV V XII

When were cultures received? 703, 704, 706 are already listed in stock list thesis
857 is not
but nowhere mentioned

· "533" recorded as susc. Bordg A and C.

	Xyl (F.T.)	Rhamn	Inos	φ942B	φ942C	φ666/588	φ2714-43	EMB MtL
SW546	+ less gas	− ✓	− ✓	+	++	+	++	++
SW588	+	− ✓	− ✓	+	++	+	+±	++
SW942		−	−					
SW703	+	+ ✓	+ ✓	±	÷	−	+	+++
706		+ no g	+					

✓ 48 hours

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

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

(see over)

Retests c̄ PRE original stocks (via NZ 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 c̄ 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. Source of contam. ??

Additional phage tests

11/1/52

Simple cross strains on EMB Lac. S. typhimurium

→ ↓	Boyd 4	4R	LT1	2	3	4	5	6	7	8	9	10	PT22 22	FA26 4666 588
Boyd 4	Boy												+	
Boyd 4R			−									−	−	
LT 1	−		~~+~~									−	+	±
2	−		−									−	+	
3	−	+	−		maybe suf pl.							−	+	
4	− ~~++~~		++									−	+	
5	−		−									−	+	
6	−		−									−	+	few pl.
7	−		−									−	+	
8	−		−									−	+	
9	−		−									−	±	
10	−	±	−									X	+	v.f. pl.

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

---

✓ on homologous parents: 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.

φ sens.

	FA27	FA28
LT1	+	−
2	±	−
6	+	−
7	−	−
10	−	−
22	−	−
Boyd 4	−	+ (maybe res. R?)
688	−	++
666	−	−
909	−	−
Dov. morb. 1	−	−
Landau	−	−

Transmotil.

	26	27	FA9	28
906	−		+++	
908			nearly T+S	
909	−	−		−
666	2 Tr., no s.	−		−

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

of FA28 ; pr. Boyd 4 S
688
688 mot

titres of FA27/28 may be inadequate

FA 28 is certainly a "rough" phage

Misc. phage tests

11/12. Prepare Boyd A1, A3 (grown on 912). Test on Boris-morbificans 1-8 (Spicer): no action. OK on 912, no action on 688M. Same results obtained with the bacterial cultures in lysogenicity tests. (Write Boyd for his boris-morb. 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 c̄ 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 ±1	RS ±1 d?
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 ±S	R
typhi 757	R	R	R
~~[illegible]~~			
558	S	S	S
senftenberg	R	R	R
derby	↓	↓	↓
montevideo			
florida			

Recheck LT2 ± UPLT2 / A2, A3.
558 seems to be most satisfactory general indicator. A1-3 not advantageous for other Salmonellas (cf. abony).
A2-3 / S. coli 4 ?

1st preps.		+ SW435 EMB Gal	+ SW666 Gal.	+ SW666 Mot
	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 (mimotic): 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
in EMBGal − 0.1 ml → no papillae

SW534/+666 → 2 swarms, Gal+, 1, 2 ! (i.e. SW666 → 534.!) 0.1 ml/plate

θ-20 + SW666 - not. und Gal : No +, T, or S

see over for host range of FA26:

FA 26 tests for lysis ([illegible] EMBLac

Resistant: london (self. lys.?), martw., duby, eastb. stanley florida
poona, senft., sundai

E coli 1, 2, 3, 5, ~~[illegible]~~ Boyd 4, SW704, LT-2, abony.
(979G 1-5) SW905-908, SW666.

sensitive gallinaeum 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 (slide tests at 1:100 serum)

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

Prepare broth cultures from isolated R and S colonies.

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

Suspension 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.

Retest in broth, restreak.

		FA9	FA26	ind. cols.	brush on EMB
985-1- 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		
546	S	S		

On restreak (tran agar) 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 transduction

986.

Nov. 5, 1952

Summary of transduction ratios. — x SW666

974E1	SW904	25 i	LP22(FA9) 21R	4 S
		2 b	1R	1 S
974DD5b	SW623 (12B)	11 i	10R	1 S (SW904)
		1 b		1 S ? (SW696)
5A		10 i		
D5 *		1 b 19 i 8 b		993E 9i 0b
974-12		4i 2b (18i — ?)		

Note heterogeneity	other data
* 12:5	
* 19:8	35i:2b
31 13	cf. 12, 12B x

974D1	SW682 FA16	2i + 7i 1b	
D2	685	4i 2b (colonies from pool)	
974..	PLT22/2	5i 2b	

Bruce's data:

971 D	?	32b : 10i !	(but maybe phase 2 FA)
(588 — x)	979-6	31b : 13 1,2	
573 — x		18b : 2i	
546 — x		5b : 1 1,2	
971 A2 (dublin)		5/10 b	
A3 altendorf		10/10 b !	
A6 (entuticlis)		6/11 b	D6 1/3 b
A8 sandiego		13/15 b	

Contrast typhimur!

but check ratio of phase

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

Collect addnl. data

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

! 19 b : 1 i — Swarms relatively close together

FA9: 19^S 1^S
A2 A1

B. 21 → 666. — swarms very crowded!

12 pulsed: — but all reactions clear cut except as indicated. (But FA21 stop?):

9 : i 2 : b 1 b+i

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

→	↓ LT-2	FA9	FA22
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 lytic area SW616/FA9. Test single colonies: 9^R : 12^S
(should use instead the stationary part of a → SW666!) Restreak ↑ and retest

1 rough 2 now sens. 1 $FA9^R$, not lysog. may be rough.

Repeat plating 666 + FA22, FA10 on EMB Gal. (correlate lysogen; transduc?)

Consid. Gal+ from FA10, no visible φ act.; FA22: 2 Gal+/plate ca 300 plaques.

C. 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-strike FA21!

A + B all Gal −, as is FA21 cont. A pres. OK.

E FA22 or. → × 666 37°

F FA 22A → × 666 37°

G. FA 21ST → × 666 37 Uniform outgrowth. Sterility of FA21? Recheck: 1st exp no repeat!

H. 22A R.T., then 37

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

J

G. 22 sw. 4b 18i (#1-4 = b) FA9: G(b): 4R. i 12R 6S Total: 16R: 6S

I 12: 1b 11i : all R 12R

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 b, i, lysog. except that FA (tymus) → > b, S

FA 21 → > i, R

No obvious temper. effect either.

K. 21 → × 603 (to refute sterility problem finally!) 17 swarms: all Gal+ ✓

2b 1b+i 14i

all R 5 FA9S 9 FA9R

in full agreement with G-I above.

Totals: FA21: (count b+i as 1 each)

	9R	9S
b	8	0
i	32	11

FA 22 A,E,F

	9S	9R
b	~~34~~ 53	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

986 D

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

1 PLT22 → 666 (only 2 papillae)

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

1. 10/12 are lysogenic 2. 1/12.

Restreak to isolate = 986E1, 986E2

R = lysogenic S = sens.

a–b: D1: 1^R–a both b and 1 a ^{S}. D2: →

c: D1: $18^S : 3^R$ D2: $16^S : 3^R$ –

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	nonlys	

sufficiency of phage?

(over)

a	b
–	
+	+
+	–
+	–
–	–
+	
–	+
+	
–	
+	
–	
+	–
+	
+	
+	
–	
+	–
+	–
–	–
+	–
13+ 7–	2+ 8–

11/23

a	b
+	–
–	–
–	–
+	–
–	–
+	–
–	+
–	–
+	+
–	
+	+
5+ 6–	3+ 7–

c: 25 all – !

(over)

a/b comparison

	Lp^+	Lp^s	
Gal+ a	18	13	31
Gal- b	5	15	20
	23	28	51

a/b+c₁

a/b+c₁+c₂

D3 - Lp v? colony in 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 "	Marked shine wall, especially
907	b+1,2	" "	908, also 905...
908	0	" "	

Isolate "pure" b phases 905-906

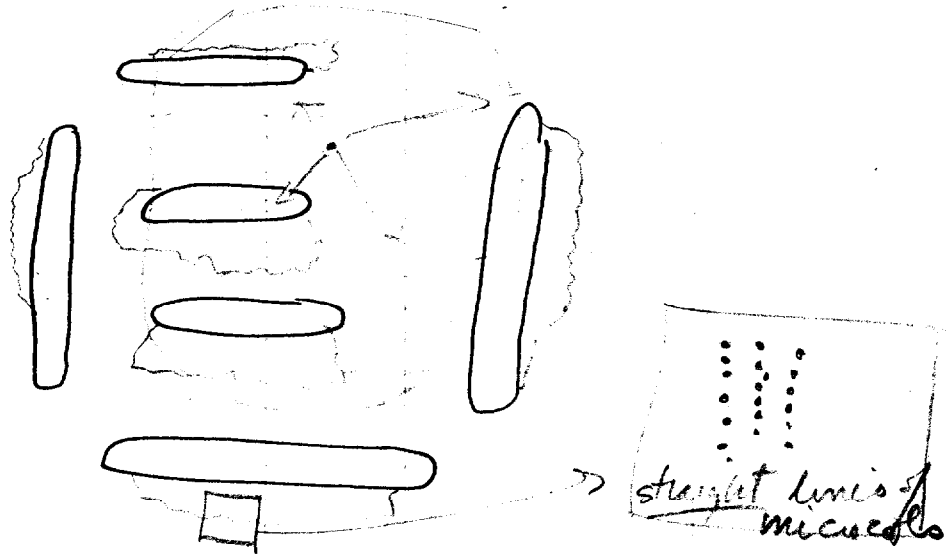
907: 3 single colonies: pred. 1,2, also b! Restrain

7/7 colonies react c ~~1,2~~ b as well as 1,2. Pick #4, least reaction c b as SW907 (1,2). Either very high mutation rate or b ↔ b,1,2

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

A).

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



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

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

908: gave a spont H^+, one plate / 3·4. (no flare).
+ FA9 gave a good yield of of tracks and swarms, occ. flares in these

C

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 crosses

988

4/1/53.

on S Sagar

(1) SW616 + SW967. Controls — . (x) 4 extra trailers.
(Gal⁻)

O-form crosses

11/12/53.

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

No motile. sw550 sends innumerable very short trails 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

Transductions to sendai, eastbourne, ... 989

A sendai 771 x—a,15 abony² (15) 11/9. 2 buds 11/11 3d bud (2d plate)

not saved 1. eux → a SW925 Reversed not tested

2. eux → a sl. rough?

n.s 3 eux → a

11/13 4 a (not transd.)

Purify swarms
Isolate single colony
+ select on homologous
serum for alt phase

B x—a,15 abony¹ (14) 1 bud — not transd.

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

D x—eux 15 abony¹

J x—FA22 typhimur. → 1 "bud": weak eux Re ✓: eux++ i +?
maybe mixed: struck 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 → - 11/13
2. 1,2 → -
Purify and test alt. ph. Single cols 1,2 not d. monophasic 1,2.
Control: 546 /1,2 → no mot.

F SW 546 x—1,2 FA typhimurium FA22 4 buds: all i

G 546 x— FA11 3 buds → b

H 546 x— FA 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 (and tracks) 990

Resumé

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

B. FA21 → SW666.

Tracks	Swarms
~~15~~ 13	4
28	6

Pick free tracks. 5/7 are Lp+

C. Strish out from non-motilized regions: 3/11 are Lp+.

11/20../52
See 989

test phasicity Lp^s (same as SW.) F. FA22 —× 546 11/11 : all i 1–4 tested on 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.
(# 1,3,4,6, 13,14,15,16) Save F1 as SW-934
Overnight : no swarm.

G. FA11 (SW618 b:-) —× 546 3/3 : b : — (all Lp^R)
T.O. 991G1A eventually isolated. Save for z33 test. ✓ prob. z33 by slide test
991G1 = SW939 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 inversion was pure enx on slide agglutination.
H1 eventually gave "inagglutinable" H1A. Save for z33 test. ✓ z33 3/6/53
H1 is 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 ? pontagglut? 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
still inagg. save

M. 926/1,2-enx " . 1 "bud" - still 1,2. No [illegible]. n.s.

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

N FA22 —× 937 numerous sw. Pch 12 : all i : —. ✓ Lp: #3.5 others: R. do not save
P FA18D —× 937 (conc). ~~[illegible] plates~~. 14/4 plates inagglut! Reselect on b. enx
(mid control on SW937): 2 grew more than others, but inaggl. Select in motility agar.
6 addl; also inagglut : inaggl. (weak b?) cf. H1A!
Q = 937/b control: ~~[illegible] swarms overnight~~ 1 sw/plate after 36h. t.o.
(see over for conclusions).

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

b. #157 carries an 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 $H_1^{\ b}\ H_2^{\ o}$

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 → x typhi H901 537 : 3 (1,2), (d?) Purify : single colonies pure 1,2. In 1,2 again:

1 1,2 → — SW 930
2 1,2 → —
3 1,2 → —

A 546 FA25 → x abony¹ 11/19 : 4 buds: — all 1,2

1 1,2 → eux 5 12 → eux
2 " 6 "
3 " 7 "
4 "

B 546 FA 75 → x abony² 11/11: 3 buds — all 1,2

1. 1,2 → eux $\xrightarrow{3\,s.c.}$ 1,2 [redacted]
2. 12 → eux
3. 12 → eux
4 "
5 "
6 "

all are Lp except A7, save as SW 938

Study B1 in quiter detail: ←
1,2 phase does not agglutinate (stiles) in eux
eux phase shows some (1,2).

~~C. 546 / 1,2 control~~ (see 979 for abony¹ ≟ abony²)

~~D 18 → x 546 / 1,2.~~

Host adaptation of PLT22 ...

b/i determination

993

SW686 unfortunately is Lp+

A. FA31 (= FA9/LT-2^I). → 666. Many T+S.
2i; 11b.
In cross-bursch tests (no plaque count) this phage appears to have retained its specificity for paratyphi B. Ca. half the bursches are phage-plaqued. 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 Fairly distinct swarms: 4b: 16i

C. FA22 → 928 Many T+S (confluent). Pick flares. (1 lytic area noted in swarm! (Pick and test for Lp^s or Ph. mutant.) ⇒ no evidence of either. Clear area may have been simple staling inhibition.
Sw. Not too well separated. 14:b 1:i 2:b+i. Read total as 16b:3i

∴ lysogenicity of transducer 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

11/24

D1	FA36 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
B 3/7 are Lp^s

~~same 1s~~
~~" "~~
~~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

(swarms must have been confused, from oversoft 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 determination
Summaries

12/5/52 → × 666.

		i	b	
A. FA31 (= FA/LT-2^{I}) → ×		2i	: 11b	Need more data on parity. B adaptation
B. FA21 → × 928		16i	: 4b	
	cum. total	60 (13)	12 (11)	
C 22 → × 928		3i	: 16b	
	cum. total	7	80	
D1 36A (SW935)		9i	: 9	
see 973 C3 2 36B		7	7	
3 36C		7	3	
4 36D (SW936)		6	2	
E (SW623) FA12 → × 666		9	0	cum tot 61^{i} : 4^{b}
" "		5	0	
12B "		12	2	
pools, colonies : (HLB)	12	15	5	} discrepancy?
	12	14	6	
	12B	16	4	
625		10	6	(3 were mixed)
626 FA37		49	21	(+ 16 mixed)
628		8	5	
680 addnl (HLB scored)		15	7	
		22	5	1 b+i } heterogeneity?
		63	7	
		100	19	
18D 18 see 971D18.		[illegible]	24	
		5	52	

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 efforts 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 φ⁵ of 109 Lewis!

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

11/5:

		control H⁺	transduc H⁻		
Totals: type	Lp⁺	~~8~~ 3	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/172	H⁺	H⁻	Not	2	3
C 1	−	+	+	?	+	
C 2	+	−	−	−	C	
C 3	4 −	+		+	+	A
C 4	4 +	−	+	?	−	C
D 5	−	+		−	+	C
C 6	−	+		+	+	A
C 7	−	+		+	+	C
A 8	44 +	−	−	?	−	C
C 9	−	+		+	+	C
C 10	−	+	+	?	+	C
C 11	−	+		+	+	A
C 12	−	+		+	+	C
C 13	44 −	+		+	+	D
C 14	−	+		+	+	C
C 15	−	+		+	+	C
C (16)	4 +	+	+	(+)	+	
C 17	−	+		+	+	
C 18	−	+		+	+	D
C 19	−	+		+	+	
C 20	−	+		+	+	C
C 21	−	+		+	+	A
C 22	−	+		+	+	A
(5) 23	+	−		?	+	E
(24)	+	+	+	(+)	+	C
C 25	−	+		+	+	D
C 26	−	+		+	+	C
C 27	−	+		+	+	C
C 28	+	−	+	?	−	C
C 29	−	+		+	+	C
C 30	−	+		+	+	C

	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	−	+		+	+	
C 41	+	−	+	−	−	
C 42	−	+		+	+	
D 43	−	−		?	−	
C 44	−	+		+	+	
C 45	−	+		+	+	
C 46	+	−		−	−	
C 47	−	+		+	+	
C 48	−	+		+	+	
C 49	+	−		−	−	
D 50	−	+		+	+	
A 51	+	−		−	−	
A 52	+	−		−	−	
E 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 11/24/57.

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?) ~~or gummy~~ colony

B: gummy, ~~poor~~

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 auxotrophic. Pick as 995B 1-2

4/14/53 EML

Subtypes W2149 observed

homogeneity W2148

Both R to λ-2

Srp with W1956 (F-) and W-1177 (F+)

on Smal$_{sm}$ + Slac sm.

Dieries' L forms

see 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 + SW666: Good growth in 2-3 days, especially on penassay broth. Very poor growth on agar. Note a crinkled 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 Remoculate to wet slant c̄ horse serum + penicillin

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

b Agar + serum: mostly bacteria; some granules + fragmentation; refractile spheres in microcols.

c Penicillin serum agar: no marked growth.

12/19

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

p20

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

12/23 11 Streaks out on motil. agar.

12/27: Motility agar, in tubes seems best. Emulsified inoculum, however, does not grow well. Heavy inoculum grows well in broth

Proteus 52 pure quite well in D(0) ? improve i mi ?

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

D Salm SW 4/6: smaller sph + gran. } on Mot Pen medium.
EP ~ SW 4/7: 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)
24 h.: 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 quickly.

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 near - spheres
e -1 - pen. spheres and " "

note lagging inoculum → deformed bact
swollen " → normal bact!

try 1 ml inoculum

(over)

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

1/14/53 D - (Salm.) 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 supernant and filtrate of same for bacteria on EMB Lac. Resuspe sediment in 1 ml (ca 5x) H_2O — forms a gelatinous mass. Underscope, large clumps of L-type [illegible]
Put on plain agar 1:15.

After ca 6-7 hours, small microcolonies of plump Proteus appear in conjunction with clumps. Bacilliforms could not have been present in comparable numbers but detailed origin was not discernible.

filtrate: strike up broth and on plate. ~~Unfilted~~ Unfiltered sup't had ca 10^4 colony formers/ml. (1000×10^1 but 0×10^{-3}!)

Osmotic shock

12/4/52 Osmotic Shock.

A. add .25 ml PLT22 (966.4) 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 plaques; .01 ml samples on SW666 / motility: too crowded.
.1 ml on SW435. < {0 on either! see 999 for host adaptation peculiarities.

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

No marked effect of shock on plaque titre

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

Assay for plaques ~~[illegible]~~;
for prototrophs on SW435 D(0)
Gal+ " " EMBGal

use NaCl more dilution?

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

φ counts A 45×10^7 B 53×10^7 C 138×10^6

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

		A	B	C
assay on SW435:	Gal EMB	3	0	3
.1 ml	D(0)	8	1	3

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

UV - FA21; FA12

December 4, 1952.
Preliminary experiments
Assay FA21.

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

(also 18D = 192×10^7)

Place 1ml FA in Petri dish. Expose to UV 120 seconds.
12/5/52 ~~Add 4ml H2O to wash and recover~~. Assay survival. ~~and~~
~~on 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. (or cells n.g.)
Restrick these plaques.

Recheck, FA/435 D(0). FA22 .1ml 325
FA21 " 0
FA21UV " 2

swarm induction

FA21: .001 samples:

Σ	#	# swarms
	6	0
6	6	1
~~8~~	~~4~~ 2	2
~~9~~	3	3
4	1	4
~~24~~ 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 × nsec. doses., more dilute monila.)

FA12 may be more satisfactory.

b:i ratios:

control (FA21)

b	i	mix
4	13	

999

b	i	mix
4	18	2

12/7 Pool FA12, 12B for further studies.
Assay at UV = 0, 120, 240. Plate .001 ml samples
1 ml / 10 ml plate on SW666. on SW666; motility.

A	0	666	swarms ~~[illegible]~~ too numerous	355×10^5 / LT2	105×10^7 / 666
B	120	"			185×10^6
C	240	"			430×10^5
D	0	978	15 sw / 9 x .001		
E	120	"	18 sw / 10 x .001		

More dilute platings needed.

12/8. UV 0 — Phage / 666. ~~[illegible]~~, 87, 100 — Gal+ / 666.

360 130×10^5 — $\times 10^4$

Swarms: 0: ".001" ca 5/ea.
".0002" 28 (+2 or 3?) / 19 ~~[illegible]~~ $\frac{1}{6} \times 10^{-3}$
".00004" 3/20 $\frac{1}{36} \times 10^{-3}$ =

For tracks, 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 tracks 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 ~~[illegible]~~ 1.4×6000
~~6 x 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 $\frac{Gal+}{666}$ .1 ml: 376
FA12
② 360 130 × 10^5 280

SWARMS. Samples of 1/10 .01 ml A, × $\frac{1}{6}$ B, × $\frac{1}{6}$ C.

$\frac{FA}{\varphi}$ = ca 10^{-5} sw = .4×10^{-5} Gal+

1A. >> ca 5/
B. ca 28/19
C. ca 3/20 < 29 tracks 0 sw; 9 " 3 " (denser agar)

later, about 11 additional sw. appeared.
* The 3 early are 3/3 i. The later swarms are 7/11 b!

$\frac{FA}{\varphi}$ = ca $\frac{1}{1300}$ sw = $\frac{1}{5000}$ Gal+

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

12/9. ③ 600 sec. ~~[illegible]~~ some evap. noted (ca .5 ml recovered)

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

$\therefore \frac{FA}{\varphi} = \frac{1000}{172 \times 10^3} = \frac{1}{172}$!

$\therefore \frac{Gal^+}{\varphi} = \frac{15 \times 10^3}{172 \times 10^3}$ = 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 lysogenic? 999D

12/10 1. UV = 0 2 UV = 120. (dil 1:1 prior!)
A ~~[illegible]~~ 35 A 54
B 99 B (155)

A = 666 B = 928
.05 ml samples.

12/12: ~~[illegible]~~ no FA: 0

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

ca same activation at this dose

Linearity of FA, UV = 0.

Gal+	666	928
.1	534	16
.05	291	7
.02	113	2
.01	52	2
		"

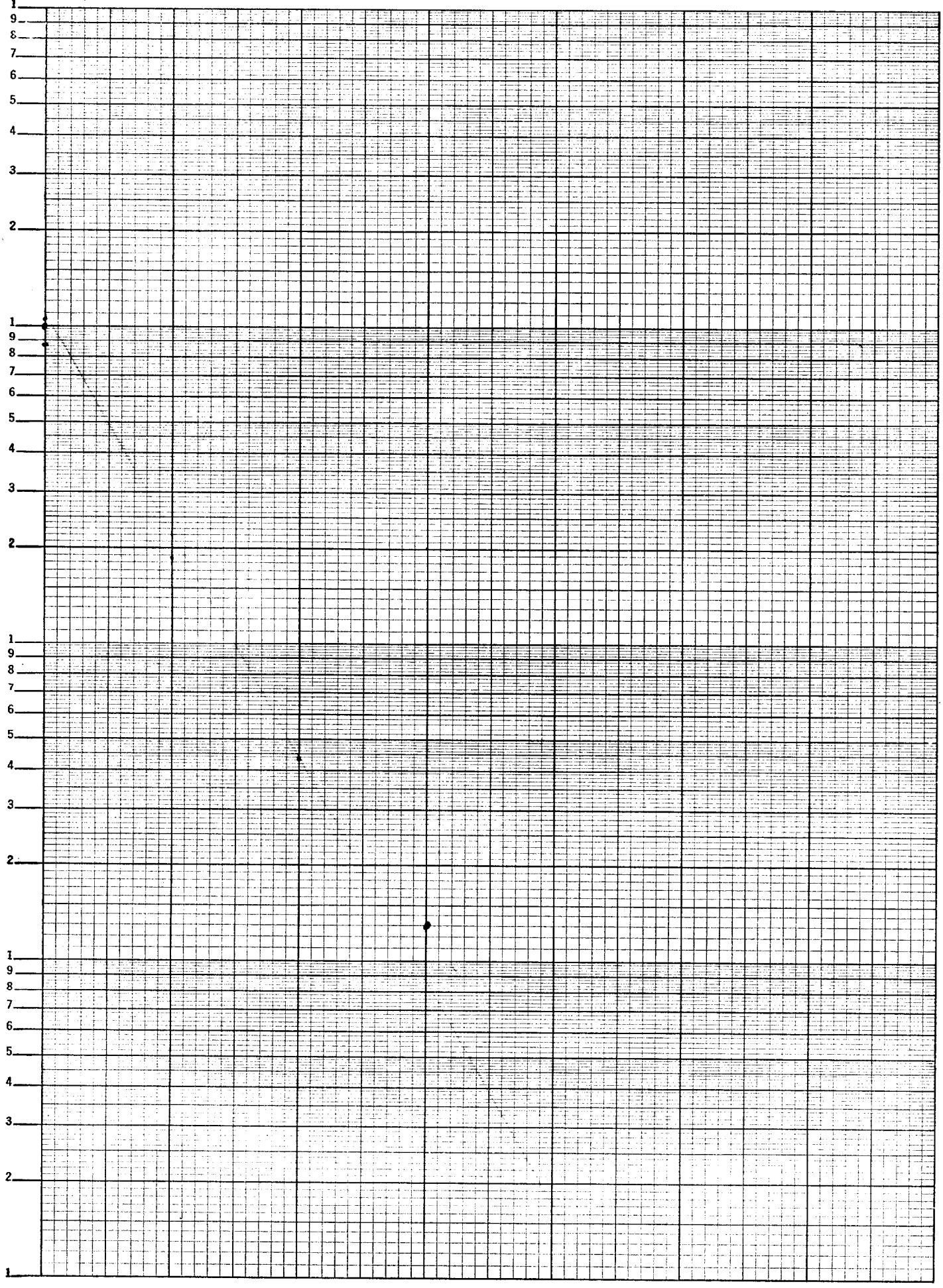
satisfactory linearity but cf. 999C1 (somewhat less)

ditto, but why lower efficiency? Cf.

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

FA12/666

999



10^9

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

180 360

Limiting inactivation of FA ~~21~~ 12. UV 15 minutes 50 cm.

(1) Dilute 2 ml FA12 with 2 ml H_2O. "

(2) " FA22 " . "

(3) " FA12 UV 5 mins ca 15 cm distance.

12/12.

		SW666			SW928 Gal+	
E1:	½ x .02 :	Gal+ 65	φ	100	11	∴ ca = plaques and transductions.
	½ x .1 :	Gal+ 380	φ	360+	68	Pick transductions away from plaques.
no UV	E/0		.05	[illegible]	91	

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

E3 φ 16 / .1 ml 16 Gal+ (struck out 12)

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

Repeat c̄ 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 c̄ transduction by inadvertent venis.

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

In a poor prel. test of 1-20, none were lysogenic.

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

Ale 1006

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. FA 25 → 942 (SW588) V. Numerous swarms. Isolate and test 4: 1,2: —
(stable > 3 days on 1,2)
#1,2, 3 are Lp+
save #4 = SW945

~~10/9 B. FA18 → 942 → b: 1,2~~

~~C. FA15 → 942 → b. enx~~

~~D. FA42 → 941 / i enx → b. enx~~

~~E. FA42 → 926 (#1,2 enx) → b. enx not mixed.~~

F 18D → SW609 (H_2?)/b. 8? sw / 3 plates : 8, either b or maggl. Not i or 1,2
cf. → 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 (sic!) 1 bud maggl?
D. FA15 → SW609 1(b) 1 sw: maggl. (v motil) 3/5/53: Z33 T.O.
E. FA15 → SW 857 (1,2) (note rarity of enx in pur.)
2 buds. both still 1,2

4-5 plates each as indic.

C 1: stable 1,2: —
2: mot but maggl.) saved

E

This expt. gave nothing except ?? C1, 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 (b) and 1,2 ?
Re-select m 1,2: no swarming. T.O.
several inagglutinable