Discrepancies in b/i ratios.

999C1C .. seems to point to a bias among i : b transductions correlated with earliness of swarms.

Hyp. 1. i's start earlier for unknown reasons, but move at same rate as b

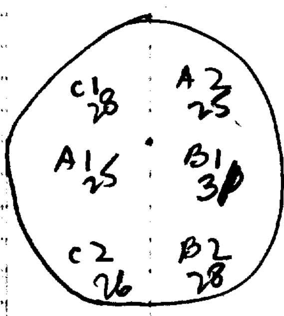
2. transductions start at the same time, but there are differences in effective motility

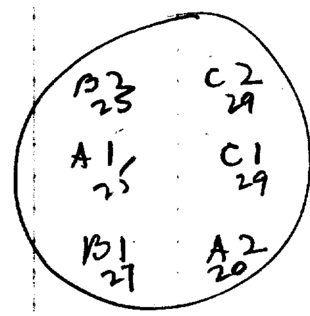
2B. Followed by selection which results in comparable rates!

Repick unpurified sweep of 991C1C:

			Lysogenicity/LT2.
1001 A 1-3	= C1C early swarms,	i	all +
B 4-7	= C1C delayed	i	all +
C 8-14	= C1C delayed	b.	#1? 2,3,4,7 + (5,6 −) of the [illegible]

From suspension inoculum compare movement on mot. agar 2PM − 8PM c duplicate plates, 2 from each.





− diam in mm

A	25-25	25-20
B	31-27	28-25
C	28-29	26-29

<u>If anything</u>, B, C > A rather than the converse! These were isolated from a different plate, and any difference may be irrelevant to the present issue. Hyp. 2 may be excluded, but 1 vs. 2B cannot now be determined.

But see also 1030.

Prepared by M.L. Morse
ca 12/5/52 Label as lw.

922 Lw 1 } had same Fla,+ activity i SW666.
927 Lw 1 }
928 Lw 1 no Fla,+ as expected. ~~same cleavage~~ same changes.

Titrate 928 on SW666, 922 and 927 on LT-2.

922 < 10^7

927 < 10^7

928 ~~412×10^7~~ 4×10^9 titer. 10^{10} by loop dil.

unfortunately, few suitable

					Gal+/666 /ml	Swarms
12/12	927 lw 2.	94×10^6 /LT2				
	999–16 = SW944	lw. MLM.	ca 10^{10};	40×10^8	625	+++
	999–24					
	999–66		< 10^6 but many minute plaques!			
	SW686	lw. "	ca 10^{10}	30×10^8	397	+++

Retitrate it on SW950.

10^{-7}	A. SW927	No UV			
10^{-8}	B	25 s	200×10^5		
10	C	40 s	100×10^7	(927 lw 3)	Respectable lysis!
1/8.	D No UV as A		10^5		
	E} No UV Sup't and sed of 24-48h. culture		10^4 ±		plates smeared;
	F} Try higher doses		3×10^5		assays ± 100x

Serum inactivation

12/12/52 Shaw's anti FA serum.

add .1 ml to 10 ml ca 10^8 PLT22 in broth. Incub. at 37° 10 minutes.

Assay on LT-2.

	control	serum
3	++	82
5	182	3
6	21	

poor counts

∴ ca 2 decades in 10 m. at 1:100

∴ k = ca 50/minute.

Do a more careful test in this range:

Quantity of serum needed to prevent plaquing (and presumably, secondary lysogenization).

12/19. Mix ca .1 ml 2×10^6 PLT22 + .1 ml 10^{10} LT-2 + 10 ml ~~saline~~ broth

After 15 minutes add to equal volume of various serum dilutions, plate on EMB Lac.

1	1:1	Plaques erratic, small, may be reduced in number.
2	1:5	ca 10^{3+}
4	1:10	"
8	1:20	"
16	1:40	"
32	1:80	"
64	1:160	"
128	1:320	"
256	1:640	"
0	0	ca 10^{3+}

Note: clear plaques noted: 3 on "0"
1 on "2"
no others!

Very high serum conc. will be needed to prevent cross-infection!

Copper / Weed

Dec. 15, 1952

12/14. Inoc ca 5×10^7 cells in 10 ml D(o) + Cu. A = W1485 B = E coli B

A

	$CuSO_4$	A		a (colonies)	b	c	B		a	b	c
1	0	+++	+++	+++			+++		+++	+++	
2	5×10^{-6}M	+	++	+++	+++ 10% min?		+	++	+++	+++	
3	10^{-5}M	±	++	+++	+++		±	++	+++	+++ / min!	
4	2×10^{-5}	±	+	+++	occ min?		±	+	+++	+++	
5	10^{-4}	±	+	+++	+++		±	+	+++	+++	

6 PM. 1st reading 9:30 AM 12/15.

a) Streak out on N.A. 9:30 AM. Reincubate.

b) 1:40 PM

c) 6 PM 12/16.

Restreaks 1004 Ab2-4, Bb3 — normal size colonies in both.

c: few smaller colonies in B5, A2, B2, A3, A4. Restreak → (normal size

12/18. Repeat expt. in T(o) as above

B

	$CuSO_4$	1485 48h (colonies)				B			
1	0	1	++ +++	++	8	++ ++		++
2	5×10^{-6}	2	+ ++	++	9	+ ++		++
3	10^{-5}	3	− +	few ++	10	± ++		++
4	2×10^{-5}	4	− −	−	11	− −		−
5	10^{-4}	5	− −	++	12	− −		−
6	D(o) 10^{-5}	6	+±		13	+		
7	D(o)	7	+++		14	+++		

No dwarf colonies from any plating!

(D/o) "citrate? – detoxifies Cu)

Streak out at ca 28h. no scv seen! Restreak 48h.

C.

Recheck W1939:

12/18 AM

	T(o) colonies		T(typ)		D(G)		D(typ)		Pen NB
1939	1	+	5 ++	+++	9	+	13 ++	++ 17	
1939A	2	+	6 ++	+++	10	+	14 ++	++ 18	+++
2042	3 ++	+++	7 ++	+++	11	+++	15 ++	++ 19	
2090	4 +	++	8 +	++	12 +	++	16 +	++ 20	+++

~~front 28h~~ Note almost = growth 18, 20 on Pen assay. Also Nutr Broth from Pen assay; streak out. Note lack of stim. by tryptophane for 2090. 2090 seems to do relatively much better in broth than on agar!

(over)

Try B-12 in broth, agar. No effect.
$Na_2S_2O_3$
2090 – [illegible] sl. less turbid than the others in [illegible]assay.

cf. aerated and unaerated [illegible]assay 2090, 1939A: 2090 Aer: increased in density at same slow rate as unaer. but reached higher final dens.

---

despite poor growth use inoculum from C2 for MNQ expt.
in T(0) **4P19** E coli B + W1939A, inoc. per Colwell
D) 25, 12, 6, 3 ppm MNQ to T(0) liquid.

		"1939A" turbidity overnight	36h.	"B" turbidity.
MNQ	0	+++		++
PPM	3	+++		++
	6	+++		— ++ normal colonies only.
	12	+++		— ++
	25	— n.gr.		— +

12/21. Restreaks. A25, B
A25: sterile
B6,12 normal ++ only
B25 some minute colonies
c̄ numerous ++. Restreak these.
all normal type!

∴ these expts. fail to confirm Weed, Colwell re isolation of scv

---

As received from Colwell (1st and 2d time) W1939A is a mixture of Mal+ and Mal-. Both are highly stable, but Mal- after purif. did give a few papillae → slow and +. (Mal+) + W2090 did not give normal Mal-.

Stocks for phase control.

S. Sendai SW771 is being considered for ~~ph~~ studies on determination of phase. However, it appears to be unduly rough. Attempt to isolate a smoother strain.

All isolates, incl. from lyophil, are at least partly rough in habit on nutrient agar, making H-scoring difficult. In addition, H- is rapidly attenuated on solid medium. Ditto for SW925.

∴ look at other Salmonella diphasics

see 1024, 1035

Multiplicity reactivation

FA12: ca 10^9 999 E3: "160/ml". Does this depend on multiplicity?

SW666 ~~[illegible]~~ 12/17. Estimated bacteria = 2×10^{10} FA12 ≐ 10^9

① Mix .1ml E3 + .1ml undiluted bacteria. Plate .1ml on EMB lac + ~~[illegible]~~

② " " bacteria 1:10 + ~~.1ml undil bac~~

③ " " " 1:100 + ~~.1ml undil bac~~

no extra light.

After 10 mins RT. add .1ml saline to ①, .1ml undil bac to ②, ③ and plate .1ml samples on EMB Lac., afterthought also Gal

	EMB lac		Gal: plaques. Gal +
1	6,7	(.075ml)	2
2	2,8	(.05ml)	4
3	1,5	(.06ml)	1

Multiplicity reactivation probably does not account for the residual plaques.

12/22

See 1003: Streak out plaques 1-4 to isolate pure lines. On initial streaking #1 was mix. of clear and turbid plaques, both small; 2 + 3 also mixed, mostly larger clear plaques, 4 small and larger clear plaques. For fast purps. pick well isolated clear plaques, grown on LT-2

In spot tests, 927 and 928 was not lysed by any plaque. LT-2 was grossly lysed. 666 showed only few weak plaques on #4.

Grow plaques on LT-2/2 in broth. All but #3 gave v. clear lysates.

a) test vs LT-2, 927 etc.

b. Add ca. 2×10^5 cells LT-2 to eq. vol ~~[illegible]~~ LT-22. Plate $\bar{c}$ .1 ml 22V4 (1/2 dil). at various intervals, incl. 0. Then there is a final plating $\bar{c}$ 1/4 x .1 ml of diluted cells. All platings equiv volumes

		ca. log surv.
1	No FA22, No 22V	10^3
2	No FA22, 22V	(1 or 2)
3	60M FA22	10^3
4	" + 22V	10^3
5	1M FA22 + 22V	10^3
6	~~25~~M FA22 + 22V	10^3
7	25 " , no 22V	10^3
8	45M FA22	10^3
9	45M " + 22V	10^3

~~10 (-2)~~

of 5 $\bar{c}$ 2

Immunity exp. to 22 protects against 22V! In view of very high multiplicity of 22, this may be adsorption - interference!

Titer of V4: 12×10^9, +++ $\times 10^7$ Confl. 10^5. All plaques clear! (For more accurate titration, use 10^8.)

Qualitative tests:

		V: 1	2	3	4
LT2		S	S	S	S
927	λ^+	R	R	R	R
666	λ^+	odd plaques			
928	λ^+	R	R	R	R
912		S	S	S	S
688	R	R	R	R	R

∴ can also be used to select rough.

(over)

Some "smooth" revisions of ~~II~~ 22V + LT2.

Follow 4 through serial streaking: gave self-nibbled ~~p~~ streaks until isolated as PLT-22, 22V-sensitive, nonlysogenic.

On SW666 22V gave scattered plaques at 10^{-3} (e.o.p. ca 10^{-5}). Plaques not very sharp, not lytic.

Restreak on SW666 to get 22BV — retains lytic action on LT2, gives turbid plaques on SW666.

However, attempt to induce lysogenicity in SW666 gave 4/4 still sensitive

10^{-15} m. .2 ml cty $10^3, 10^6, 10^8$ PLT22 + 10^9 LT2/ml (2ml)
RT.

	PLT22				
A	10^8	count: 111×10^7	+V 3×10^7	288×10^5	
B	10^6	count: 120×10^7	+V: 0×10^7	22×10^5	(589×10^3) sic!
C	10^3	plaques: 23×10^1 (+ [illegible])	with V: +++ $\times 10^1$		
D	0	count: 129×10^7,	with 22V: 6×10^5	1073×10^3	+++ $\times 10^1$

Plate c 22V at various dilutions. also φ titrations c C.
Stock PLT22 assumed 3×10^{10} of plaque titre and "protective titre".

Results. Phage added was 2.3×10^8 initial rather than 10^9.
Bacterial count was 1.2×10^9 containing 1.07×10^6 background resistant.

	Phage added	V22^R recovered.
A.	2.3×10^7	+ 2.78×10^5
B	2.3×10^5	10 + 12 $\times 10^5$
C	230	—
D	0	6×10^5 10×10^5

∴ ~~2.~~ $\frac{28}{23}$ = 1.2 resistant bacteria per plaque-former!

7 contaminated colonies appear at A ($\times 10^{-7}$) acctg. for all added virus!

E Test transduction of FA43 (22V4). .1 ml
SW435 D(0) 8   Gal+ 10   SW928: no swarms

all Gal+ stable. Test lysogenicity after purification: all lysogenic possibly exc. #1a (grows poorly!)
This is neither lysog. nor sensitive

Type of lysogenicity:

Kill susp. c̄ $CHCl_3$, streak on LT-2

22 type is turbid plaque

	type		
1	22	7	—
2	"	8	22
3	"	9	"
4	"	10	"
5	22	~~11~~	
6	—	~~12~~	

6, 7 need retest MOI in Renaassay c̄ LT-2

✓ 6, 7 → turbid plaques.

FA43 → LA22.

10 Gal+ transductions. Restreaked and replicated to LT2.
All strains are lysogenic. #1 and 2 had possibly non-lysogenic components : E2 lysogenic

E1 appears to be non-lysogenic; resistant to 22, 22V.
It still reacts with anti IX - XII. May be an "immune - 1" type. Test for transducibility.

By growing E1 c LT2, turbid plaques were secured.
1006E1 may thus be another "weak lysogenic". cf.
1009B4, 1009C1.

1006E1 plated alone gave numerous small colonies
D(a) + FA43 gave 7 large + " " .
+ FA22, .1ml ca 300± large colonies
1/6/53 435 + FA43 : 1,3,5
435 alone gave 6 !
Do SW435 now spont. revertible ?
May be "intermediate" allele

Reincubate. Small colonies may be living on added broth carryover.
Check these colonies (5 each)

			Gal -
1	SW435 sp.	++ ++ ++ ++ ++	—
2	1006E1 sp.	++ + + ++ ++	+
3	1006E1 FA43	+++ +++ ++ ++ ++	+
4	" FA22	+++ +++ +++ +++ +++	+
5	SW435 FA43	++ ++ + ++ ++	—

+++ strong growth in 24h.
++ " " 48h., mid low temp. (temp. time?) (over)
+ moderate " " .

Apparent revertants may be weaker (correspond to intermediate?)
SW435 now may be unreliable. Check requirements; reisolate if nec. Compare 240, 279

Cf. supernates of

1006E1, SW435

= 1006E1A 1006E1C

X-ray of 22B

12/23/52.

X-ray courtesy A Novick / U of Chicago

SW686ko (pur. titr. 3×10⁹) A no R B 100,000 r C 200,000 r O: original stock

	Q ×10⁷	Repeat		Gal+ /666 .1ml	Q assay on large plates ×10⁷
A	92	53,61	57	36	61,70
B	26	24,31	28	48	~~48~~
C	117	3,7	4	12	(7.3; 6.2) 6.8 (73×10⁻⁶....)
					original stock: 64

N/9 [bracketing A, B, C] sic!

Repeat! anyhow shows v. little effect of x-ray!

~~approximately 1 [illegible] per 10⁶ r.~~

∴ Q is attenuated ca 1 decade/200,000 r
FA is diminished (?) from 36-12

In view of tremendous doses required, this avenue does not appear to be promising

C streaked out: all stable Gal+.
Incidence of heterogeneity: 10/12

ca 12/10/53

SW948 - 949.

+ FA 9, 10, 12 gave no T or S except
1 extensive trail 12 → x 948
a few swarms? 10 → x 948

FA10 → x 948 2b, 2a FA22 → x 948 2a.

SW948 (-9) grows smoothly in broth but gives very rough colonies on NSA. Nevertheless, these react strongly with anti - I - II; IX XII

Check adsorption of PLT-22. of 925

see 1045

Where are results of 22 → x para A-O?

Dec. 24, 1952

11:35 PM. LT-2 Total ($\times 10^7$) PLT22 dil to "10^9". Contaminated.

A B: count 117, 134

Drain along:
B B 1 ml + φ 1 ml 78, 78 (sic)
C B .1 ml + φ 1 ml 85, 88

D plaque assay (Gratia plates: sal susp LT2: $\times 10^7$: 67, 73. c fresh cell "10" phage 130
dil in terms of original B or φ.

condition of cells may affect the quantitative recovery of φ (as there may be dead cells or other inhibitors

No effect of multiplicity on survival. The actual ratio φ/B was
~~130/78~~ in B and 130/
130/125 in B and 130/82 in C but amount actually adsorbed was not established.

Detail on B, C:
r ≑ 10 C₁ 85 plating 4 obviously contaminated. Many additional are vaguely mottled. Restreak these. Replicate to 22V.
central plaque general.
C₂ 88 Replicate to ~~[illegible]~~ LT-2 4 contam.

B (r ≑ 1) Many colonies have a central clearing, difficult to tell whether definite plaque or not. Obvious plaques in 17/78; almost all others have a central plaque.

recover

Small central plaque are multiply infected?
Larger plaque & lysed are singly??

1009151
Total 78   Test lysogenicity by replication

B ~~#~~ lysogenic   all non lys. were more or less lysed by 22V.

21 nonlysogenic
57 lys.   of these, 17 showed obvious plaguing and are therefore contaminated.

On replication, 8 additional were ~~soft plaq~~ sector-plagued

streak out remaining lysogenic for purity (15 can be picked)

25 contam. or colony   27 not
21 non-lysogenic

15 picked   12 not adequately tested on replica or not pickable.

$\frac{21}{78}$ non lysogenic $= .269 = e^{-1.3}$

actual ratio $B:\varphi = \frac{130}{125} = 1.04$

C: ~~B:φ~~ φ:B ca 10.

c2: 7/88 nonlysogenic $= .0795 = e^{-2.5}$

Are these non-lysogenized? Test for sensitivity (all were 22V^s)

also test "non-contaminated" lysogenics.

of remaining 81, only 4 were obviously contaminated

A: non-lysogenic on PLT22
B streak out "non-contaminated" lysogenics

In preliminary test of C1, 8 colonies were picked (7 resist 22V)
All showed a few lysed colonies on streak. Conclude that all are actually contaminated.

12/25-26 A dilute stock FA22 ca 1:30
B UV A 20 minutes (10 ml) at 50 cm.

Add .1 ml to 1 ml LT2 (10 hour broth)
Plate out c̄ 22V. at 10^{-5},

O: LT2 s̄ FA22 +22V, 10^{-5}: 5 survivors.
(A) A. LT2 c̄ FA22 ~ 10^3 plaques on LT2; ~10^3 survivors c̄ 22V at 10^{-5}
(B) B. plaques: 0×10^5, 0×10^3, 0×10^1
survivors c̄ 22V: 0×10^7 8×10^5. (check these for lysogenicity).
Note extreme killing (does dilution of the broth increase virulence?)
UV'd PLT22 seems to have lost its protective function, but the dose may have been excessive. Check transducing ability.

C (J) as above A, FA22 UV 10 min. (11 nas rough) No plaques at 1, 3, 5, 7.
K D. FA22 UV 0 1 10^{-6} (nas rough > rough) 58 × 10^6 recovery ok!
E noFA22 16 nas rough surv.

(F) 22V UV 10 min. assay. (in terms of 1/30 diluted)
at 10^{-5}, C, D, E ~10^2-10^3 survivors with V. Expt n.g. owing to high survivors in the control. Y.D. Indication that this dose also removed protective power.
Diluted phage is killed by UV much faster than broth, as one should expect.

K: 10 plaqued colonies. See L-M for more thorough expt.
Any non-plaqued lysogenic? 2 lysogenic were not obviously self-plaqued in original Restreak these.

B-B 8 "non-contam." colonies streaked out. 6/8 showed 1-5% plagued colonies and are therefore contaminated. 2/8 showed no overt plaguing. Replicate for further test.
1 - colonies evidently not lysogenic. Restreak, pick whole brush, also an isol colony
2. " " " "

✓ sensitive! all sensitive. Initial scoring as lysogenic - non contam was incorrect

Conclude that most or all colonies are initially "contaminated".

C-B 8 non-cont. colonies streaked out. 3 overtly clear.
Replicate
crude test, apparently pure lysogenic (might harbour up to 2% nonlys.)
Of the 3 "clear" pick possible sens. for retest.
2 single colonies not sens. No sig. lysis noted in restreaks.

Thus, multiple infection may give some uncontaminated lysogenic clones

B-A 15 tests 10/15 simple LP22^{S} 5 are not 22^{S} (try 22V?)
1 self-plagued. → all lysogenic (reduced?)

C-A 8 tests: 7 22^{S} 1 22^{R} → turns out lysogenic (reduced).

∴ There is too high an incidence of non-lysogenized. Some infected cells → pure sensitive clones? (one!)

Save B4 and C1 as 1009BA and 1009CA

1009 BA, 2A · seem pure on streaks.
Cf. lysogenicity i a typical "lysogenized colony

Transduction – lysogenization –
protection vs. 22V

1009 L7

also UV'd FA22

1/1/53

L. Add 10^8 FA22 to 10^9 LT-2. Absorb 10 min. Plate out to count incidence of lysogenization. (assume stock = 3×10^{10}/LT22)
Also plate ~~[illegible]~~ ̄c 22V for purity of these selections.

M. No FA.

N. as L, UV 500 seconds.

L. 1ml SW414 + .2 ml FA22 (1/30) 9:45 –

M " + 1 ml "

N " + 1 ml FA22 (1/30) UV 500 sec.

A: Plate .2 ml on D(0) + M or H.

B. Plate 10^{-5} ml with 22V, EMB...

C. Plate .2 ml + 22V on D(0) + M or H

B): L ca ~~400~~ 878
M ca ~~800~~ (note 1:1 dilution)
N ca 7. Plate .1 ml directly: ca 100 plaques.
O ca 15

stocks all right; UVd phage (PLT22) does not protect.

The design of this experiment is faulty for using 22V/LT2 rather than 22V/LT-14 in the last step.

(over)

medium useful?

O: (no FA)		D(Hist)	D(Meth)	D(0)
	0	0	2	—
A	L	ca 50 v. small	ca 300	
	M	2	ca 300	
C	L	ca 10 v. small	ca 100	
	M			
O + 22V		0 (pinpoints background)	4	

Important comparison:

LA – MA – LC

Note MA ≯ LA although 5x as much FA was used.
absolute counts seem rather high also, for amount of FA used!
Transduction by 22V may be ignored.

Survival of transductions $= \frac{LA}{LC} = \frac{248}{113}$

Survival of population: $= \frac{400 \times 10^5}{10^9} = \frac{4}{100} = .04$ (crossed out) $\frac{878 \times 10^5}{\text{"}10^9\text{"}}$

$\frac{LB}{{}^{0}B}$

Expected surv $= {}^{0}\varphi/B = \frac{10^8}{4 \times 10^7} =$ ca 2.5

New LT-2 mutants
UV FA22

ca 12/31

SW414 9 sec UV (ca 4000 survivors/plate).
Gal 13 plates
Xyl 5 "
Mal 10 "

1 clear Gal− (SW950) and Mal− (SW951)

SW950 also had some "fuzzy" colonies as well as clear ~~plates~~ −.
these → pure +.

∴ SW950 = SW414 (LT-2) Gal−.

SW951 is slow +, not suitable as selective marker although unequivocally scoreable

Response of SW950:
A Spont Rev: 1 + colony in no spread plate

B + FA22 .1 ml >10^2

C + FA22 (1:30) see 1009 LM 23 papillae

D + FA22 UV (1009 N) 9 papillae + 59 plaques.

Thus FA22 behaves like FA10,12 in response to UV.
UV'd phage transduces, (though lysogenicity). though it does not infect or protect

All Gal + stable.

A: Lp^s
~~B~~ C: 20/20 Lp^+
D: 9/9 Lp^s

It can be inferred that FA22 can also be UVd so as to separate phage from transduction.

SW950 /EMB Gal. ca 15 plates, 1 sectoral → SW952.
This is slow +, like 951, and may be suitable only as an unselected marker.

1/14/53 A SW950
B 694
C 948.
O –

add (3× 10⁷) PLT22 (in 0.1ml) to 1ml heat-killed bact. (10^9)
9:00 – 9:20. Plate 10^{-1}, 10^{-3}, 10^{-5} c̄ SW950.

A: ca 20 $\times 10^5$ discrete at 10^3, barely confluent at 10^1!
B: ca 100 $\times 10^5$ at 10^1, ca 100 small sharp plaques.
C: ca 60-80 $\times 10^5$ (smeared) not discrete at 10^3
O: ca 100 $\times 10^5$

694 lysogenic for LT-2?

Unfortunately, plates smeared.

Consistent with adsorpt: 950 > 948 > 694 > 0. Rates seem unaccountably low.

Has antigen been destroyed? Perhaps should use live cells for adsorption, then kill. Each killed resp. aggl. (slide) c̄ XII IX serum.

Adsorption of PLT-22

2/2/53

overnight broth cult. Boil 10 mins.

Add ca ~~10~~ 10^4 FA22 in .1ml to 1 ml 10^8 killed bacteria.
Room temp 10 minutes. Add 1ml 10^9 ~~LT22~~ SW414. ~~Assay (.1ml, .01ml)~~
Assay .1ml

A	LT2	156
B	694	344
C	948	81
0	—	270

Expt. n.g. Use higher densities.

1/4/53.

37° 4:40 – 5:10 Nominal 10^{10} B + 10^9 PLT22 (SW950) /ml.

A. Assay initial Bact. 10^{10}

B. Assay infected Bact. 10^{10}

C. Assay infective centers B. 10^9

D. Assay FA44 surv. A $10^7 - 10^8$

E Assay FA 44 surv. B 10^9

✓ F Assay Gal + A .1 ml

✓ G Assay Gal+B .1 ml .05 ml .01 ml

✓ H Assay Gal + B + FA44. .1 ml.... (used 46 by mistake on Gal plates)

✓ FF - HH same on D (meth)

A 43, 38 × 10^8, – 40 × 10^8

B 46, 38 × 10^8 = 42 × 10^8

C 34 × 10^7 8 × 10^7 poor plate (dimorphism of plaques: spreaders coated?)

D 114 × 10^5 4 × 10^7 >10^3 × 10^3

E 14 × 10^7 (FA44), 13 (+45) × 10^7

Actual initial conditions. Bacteria = 4 × 10^9 PLT22 = 3 × 10^8

But 22V survivors : s phage = 1 × 10^7

c phage = 1.4 × 10^7

i.e., protection was not achieved. Note conditions: absorption in dense washed suspension! (Adsorption?) Platings of B showed 1? contaminated colony in two plates (Expect ca 10% = 8). Test by replica plating.

Also note: plaques in C are of two types, 25 clear and 13 turbid.

(over)

check plaque type in LT2

22V1-4 : all lytic; similar action

1012B suspension : all turbid

Stock PLT-22 : turbid

2-clear plaques : lytic

2 turbid plaques : turbid

Especially in view of 1012B, clear plaques must have been contaminations (improperly flamed spreader?)

Replica tests of B: 1 lysogenic colony on two plates.

F	4	+(FA 43)	2		
G	9, 11 ~~[illegible]~~ (.1)	.02	1	.05	1
H	6, 6 (.1)	5 (.05)		5	.02

Overall result is almost negligible transduction or infection c̄ PLT-22.

(D-meth)

FF	.1	4	+ FA 43	6
GG	~~[illegible]~~ " 156,	.05 56		.02 ml: 34
HH	the same. 160			.02 ml : 40

v. effective transduction. This titer seems very high!

Apparently phage was insufficient. All transductions were presumed, but survivorship is obscured. Also note Gal+ transductions deficient monolysins may have been too many.

SUMMARY. ~~Also~~

Bacteria 4×10^9 PLT22 3×10^8 $22V^R$ 10×10^7

$22V^R$ (s̄ phage) $= 4 \times 10^7 / 4 \times 10^9 = 1\%$ (cf.

	Gal+	Fla+
SW666 + FA46	− 1	—
928 + "	− 1	—

Host adaptation

1/8/53 of ·, on SW666 (X); [illegible]950 (Y):
FA21, 22, 41A, B, C. — FA41 = FA21 plaque resolution on LT-2.
~~22 44.~~ 43, 46

.1 ml.

		X	Gal X	Y	Gal Y
FA22	A	50 × 10^7	7	~~→~~ 800^{++} × 10^7	→ > 10^2
FA21	B	100 × 10^7	0	~~50 × 10^5~~	1
41A	C	100 × 10^3	0	> 100 × 10^7	60
41B	D	~~[illegible]~~ 50 × 10^3		100 × 10^7	50
41C	E	20 × 10^3	0	100 × 10^7	
43	F	10 × 10^7 (turbid)	(12)	500 × 10^7	lysed. 10^{-7}: 3
46	G	100 × 10^7 (turbid)	0	300 × 10^5 (clear)	0 lysed

log ratio, assay on Y ~~X~~.

	adapted to	
A	Y	7
B	X	− 1+
C	Y (X)	4
D	Y (X)	4+
E	Y (X)	4+
F	Y	7
G	X	− 1+

The adaptation of PLT22 to SW666 is ~~therefore~~ reversible, and is presumably a host-induced adaptation.
It also affects 22V, which gives ~~[illegible]~~ turbid plaques on SW666 when adapted but does not induce lysogenicity.

Note that FA22 has a differential of 7 while the readapted phages FA41 have a differential of 4. This suggests a dual effect, part reversible, part not (host-adaptation; mutation). This may also account for previous impression that host adaptation was not reversible.

→ Purify, test lysogenicity → pure Gal+: all show ~~same~~ lytic interaction with SW950! (suggesting that 950 is lytic for 666!).

Transduction by lytic variant

1/3/53

FA46 (FA45/SW618) ⟶ × 666 Probable lysis. No Tor S. (Long)

A. 928: 4 trailers in 3 plates; no swarms.

Isolate 3 of the trailers. Also pick empty nearby agar. Test on LT-2
#2 and 3 OK. show typical PLT22 plaques. #1 did not.

1/9 Retest isolated colonies: all lysogenic (PLT22type)

see 1017,

FA43 = 22V/LT2 FA44 = 43/SW950 FA45 = 43/666 FA46 = 45/618.

1017: FA43 ⟶ × 953 → H+

And ~~But~~ see FA44 increase of H+ from 22 → × 950.

1006 FA43 → × 435 → Tr+ Gal+ but low yields.

~~#~~

- see also 1017

Persistence of H_1^{i*}

1/8/53 FA 18 → × SW666   see 971D18.

4 are i, Lp^s.   a, b, c, d.

Prepare FA48 = FA22/a – d.   save cultures under these numbers.

1/19/53. Plating ca 300 PLT2 + SW954 ± FA53
+FA53 ca 300 plaques, all clear.
1009N .1ml → confluent lysis on part of plate; clear plaques on remainder
+ FA53 2×10⁻⁷ ⇒ 0. ∴ same primative particles are working.

Prepare 53A = FA53/LT2. Plaques are very small, scarcely discernible. Titer 100 or 104? × 10⁶.

D) 1-2 resistants ⇒ SW954/FA53 + PLT22

954 + FA53 seems to show some spotty, general lysis. Restreak to separate possible components.

E) SW951, 956 ✓ not lysogenic /950.
Some very vague interactions of 950 → 948, 956?

F) 954 Lp⁺ (PLT22) showed no lytic response to FA53A or 53A + PLT22. (v. sl. inhibition of growth or spreading noted).
G Grow FA53 A (53/LT2'). Titre (SW950) 64×10⁸

January 15, 1953.

Ampoules received (duplicate) 1/14/53 as #1, 2, 3 respectively. (described by letter and Spicer's conversation as "cosmopolitan" Salmonella phages. label FA 51-3 resp.

a) Test on misc. serotypes. All behaved alike except as indicated:

SW950	S	all lysed clear (exc. one rough ?)
666		SW948 had background regrowth to all 3.
948		SW688 and S. coli 3 showed no response to any.
927		SW900 had considerable background for #2.
927R1		927Rough1 is morphologically very rough from aged broth.
H901	S	
SW688	R	
550	S	
S. florida	S	
coli 3	R	
London	S	

b) Plate .1 ml #3 as rec'd c̄ SW666, 950. no papillae.

SW666, 550; c̄ #3 on motility no swarms

c. Select resistants in streaks of broth mixtures. Clones noted:

	Retests:		
1. LT-2/51	53^R	FA22, 44^S	!
2. 948/52	53^R	" ^{R}	
3 950/52.	53^S	" ^{S}	

C1 was noted to show full lysis where streaks from /53 adjoined /221 restest. In region of mixture of FA22, 53. Note also resistance to 53, sensitivity to FA22!

Repeat ✓ C1 is lysed by FA22 + FA53, not separately!

a) obtain C1 lysogenic! C1=SW954

C1 and C2 remain serologically smooth (IV, XII); (I XII resp.)

C2 is not sensi. to PLT22 + FA 53.

1/13/52

A. Titrate FA22 (stock) on SW950, SW953 on EMB Gal, D(H), D(M)

Titrations are needed for further studies.

1009LB showed FA22 to have activity of inv. 1/6 × 1/30 × 1/10 × 500 ±

~~250,100/ml.~~ ca 100,000/ml.

(also 22V, and WV 22 and check transductions for Gal, M–.

1012GG showed 1/1/30 × 1/10 × 150 = 50,000/ml.

of ca. 3000 previously assayed c SW 435.

	FA22	D(meth)	cells have to be washed for D(Hist)
SW 950	.001 ml	30	(count may be cons. higher – plate somewhat smeared). ≟ 30,000
	.005, .01, .002	>>100	
	0	6, 8	
	FA47 .1 ml	>30 smeared	sic!

SW953	.005	3		seems real transduce! for lytic variant. 8 tested, app. Lp+ normal.
	FA43.1	~~ca 100~~	226.	
	FA44	2		
	FA47	9		
	~~[illegible]~~ EMB Galactose			

SW950	FA22 .1, .05	>>100	
	.02	54	ca 7500/ml.
	0	5, 2 (small, delayed,	
	1009N .1 ml	8 + 3 delayed	25 plaques
		10 + 3 "	21 plaques

Thus H+/Gal+ = ca. 10:1 for SW950.

SW953 has relatively poor response

(over)

D'meth) plates:

SW950 —	7
" + .1 mlbroth	8
+ FA47	ca 20 (sm.)
+ FA47B	10
+ FA52.	8

(presumably residual FA22)

Protection - transduction

January 16, 1953

A. B = SW950, 4 hour culture.
B. B 2ml + FA22 ("10^{10}") .2 ml
C B 3ml + " .1ml ~~[illegible]~~ } ~~[illegible]~~ 8^{35}PM - 8^{55} - 9^{10} R.T.

As assays involve a dilution of FA22 1:300 and 1:~~7~~000, Gal+ is only practicable for A, B.

---

1. ✓A, B, C at 10^{-7} for survival
2.– A, B, C at 10^{-5}, 10^{-6}, 10^{-7} with FA44, 10^{-1}.
3. B, C at 10^{-6}, 10^{-7} for plaques on SW950.
4 ✓A BC .1ml D(meth)
5 – ✓A BC .1ml D(meth) + FA44
6 ✓A B .1ml EMBGal
7– ✓A B .1ml EMBGal+ FA44.
~~(8) A 1.1ml [illegible]~~

A)
1 106, 130 x 10^7
2 ca 1000 x 10^3, 2~~7~~ x 10^5
3
4 8, 7
5 2
6 0, 0, 1
7 1, (lysed)

B) 93, 100 x 10^7
78, 87 x 10^7 10^3 x 10^6
; 14, 25 x 10^6, 1000± x 10^7
>10^2 – not ca 3X difference
>7 10^2
19, 24
56, 85

c) 107, 88 x 10^7
3, 6 x 10^6 ; 0 x 10^7
same ; 1000 x 10^6
phage ridden ! 4, 4
2, 2
10^{-1} plaque ridden. 2, 1
0, 1 lysed

8) 1009N + 1A : ~~[illegible]~~, ca 10 plaques. ~~seen in background.~~ 694 H+ colonies : test 8 for Lp+ ; all are Lp^s
verifies transduction by inactive phage.

Note excessive phage in 3B, 3C. Inadequate in 6C !

Summary : initial bacteria : 1.2 x 10^9 + PLT22 B) .97 x 10^9 c) 1.03 x 10^9 (possibly same but uncertain)
FA44 SURV. 2.1 x 10^5 = ca 10^{-4} = .83 x 10^9 5 x 10^6
= 85% = ½% !

Add PLT22, calculated 10^9 ⟶ .3 x 10^9
Why discrepancy between B and C ?
C was allowed to progress only slightly longer, but FA44^R is 50X too low.
(see over)

accessory incomp. phage

A = .02 ml FA22 EMBCal ; .002 ml D(meth)
B = .1 ml FA 47D
C = .1 ml FA 43
D = SW950 .1
E = SW955 .1

		Cal		D(meth)
1 - 11	D	.	1	.
2 - 12	E	.	4	.
3 - 13	D + A	..	26, 20	..
4 - 14	E + A	..	0, 6	..
5 - 15	D + B	•	4,	•
6 - 16	D + B + A	..	13, 27	..
7 - 17	E + B	.	3	.
8 - 18	E + B + A	..	10, 7	..
9 - 19	E + C	..		..

cf. 3/6 no accessory.
4/8 ? lower yield.

Cetrimidine

1/21/53 Cetyl TriMethyl Ammonium Bromide (Cetrimidine). rec'd from D. Gorin (Baker Lot # 1.1446 — opened.) Acc'g Bradley + Boyd inhibits phage adsorption.

Mix 10^4 PLT22 + 10^9 ~~[illegible]~~ SW950.

Add .1ml successive dilutions of CTMA, plate .1ml.

0.
1. .1ml 5% = 5/1000 — ca 10^3 bacterial survivors
[illegible] × 1/5 = 1/1000 — " 10^4; no. plaques
[illegible] 1/5 = 1/5000 — 1/25 — perhaps 50% reduct. count;
4 × 1/5 = 1/25,000 — 1/125 } full count
5 × 1/2 = 1/50,000 } full count
6 × 1/2 = 1/100,000 } full count
7 × 1/10 = $1/10^6$ } full count

Not usable for limiting cross-infection.

~~Diploid~~ Hfr crosses
further commentary 6/10/53

952. (Hfr Lac$_x^-$ x Het F$^+$) Abandon → H316 Mal-Lac v TL-
W1940 W1590
see 960
F status? (Prob. Elimin.)

953 H312 x Hfr → Mal v Lac v Sv TLv M+ No pure Mal+
F$^+$ H267 Mal$^-$ H318, 319. (self eliminating?)
(2n F$^+$ x Hfr) Held

F status? Elimin 1/3 strands?

955 H310 x 1895 1/12 Mal v. H313.
0/24 (Malt)
But appears to have segregated already in previous expts.

H310 then noted to give very high yields x F-
H310 segregants: 2 each Lac-, Lac+ are F$^-$. Test
more extensive pools! → Then 10+, 5- all F-.

956 H311 (Het 2n) x Hfr. No Mal v. ~~Reversion not tested not~~
~~perfected~~ 1 Reversion test.
23 Lac v tested: all Mal-! (Very few Mal+, but
some found).

Abandon all these.

Continue H310 x
Hfr Lac$_1$ x Lac$_4$ .... (1940 x 1956 / EMS Lac ...)

958. H245 x W1922. Some Mal v lac v
Het2n F^+ Hfr.
Abandon

Hfr F^-
964 H310 x W1607 nEMS lac. ~~6~~/24 lac+
$Mal^- S^R$ Mal^+

→ 3 lac v. 2 Mal^+ 1 Mal^- (H322)

need hemizygosity test! (note H310 is Mal =).

superseded by 1057

July 4. 1952. Summary of Hfr x diploid crosses etc.

952. Hfr Lac_x - x Het Lac_1 - $\xrightarrow{EMB\ Lac}$ occasional Lac v (balanced)
but mostly Lac+ recombinants. H 316 = Mal- Lacv TL-
should be crossed
x 1545
x 1617

953 (H267 s^R Mal-) = H 312 prot. x W1895 on EMS Mal.
not ready yet ⟶ H318, 319 7/1. ~~should be segregated.~~
✓ Mal+ are TLB_1-. Cross - Malv

(mostly Lac+, Mal+).
955 H310 x W1895 (1895 x 1177 nondisj. TL- Mal- Lacv) EMS Mal
In first trial gave 1/4 Malv H313 Lac+ MHv S^v
second: 0/24. But H310 itself is Hfr! Mal+ Lacv?
8/1. Recheck Hfr x F- ⟶ F- haploids only.

prot
956. H 311 x W1922 (Het diploid Lac- Mal- MHv prot). EMS Lac.
gave numerous Lac+, 6/6 dip were Lacv MHv Mal- S^s
Rare Mal+ 5/5 Mal+ Lacv
Further tests needed, but presumably may mean that elimination is
not bypassed in n x 2n (still n for Mal).

H320
A: Mal - Check for hemizygosity
B Cross s. g. zygotes

958 H245 × W1922

TL LacV Mal- EMS Mal. under way

MalV isolated. Test segregants for mutation, [illegible].

H321

960 TL H316 × BM W1895 EMS Mal. Check LacV Mal- for homozygosity.

Tested only on Mal!

959 H295 sec. Continue

To be done

a) Further tests on ~~[illegible]~~ 955: for Lacv Mal-pure (is Mal ever eliminated?). H310 itself may be hemizygous?

b) Segregation of H313 → recovery of Hfr?

c) Transmission of Hfr to Mal+ progeny?

d) Transmission of Hfr in H316 × Hfr? etc.

959: H295 secondaries: H317 Lp +/s. Lp2 R segregating? Obtain Gal+ recessives for coupling; repulsion.

954: Lac+ S^R recomb from F+ × F−. Check mesic markers to compare pattern with 1895 + 1956

957. What peculiarities of λ/1827 can be demonstrated? In gross test, λ^4 did not recur.

phase variation of Kunzendorf   1020

2/22/53

Kunzendorf. 6145-52 = A. single colony picked and motilized = A1. = SW961

list of serums:

k1 Kunzendorf
2 minnesota 240
3 berlin
4 new mexico 20
5 para A 228 new
6 morehead
7 para A 228 old

2/22. Inoculate A into serum tubes:
k1, k6.
After 48 hours, k1 showed rough blebs which spread very slowly; 2/28: spread through.
k6 grew out and filled the tube = A/k6. Repick + test antigens.
On slides, unpurified A/k6 =B1 reacts c c, 1,5(k1) but not 1,5(k6) or 1, 2 (over)
Reactions are quite weak.

2/22 Inoculate A1 on plates of k1, k6 agar.
A23: bulbs on k6, not on k1 — slow overspread. (surface spreading makes plates less satisfactory than tubes for serological mutations).
P23 pick → D.   =E spread may obscure blebs.
A24: numerous B+S small k6 plates
1 small rough bulb on k1. Reincubate to try to pick later.
C = cholerae suis c: — #153

D = A1/k6 plates (4 swarms / 3 small plates).
4 mass cultures all react c: + k1: ± k6 — in slide tests. use D3 as strongest reaction and test single colonies.
Each of 4 colonies react c+, k1±, k6 - in slide (= D)
Prepare broth culture for filtration. Inoc D1 in k1. k6; E:
grows promptly through k6 is slightly inhibited in k1, strongly inhibited in c but definite diffuse spread. 2/28 still not spread.
E : one plate showed two rough blebs after 4 days. Pick to Pen assay. (May have undergone two steps of selection)

SW
D1 = 958

	c	k1	a6
B1 (unpur.) at 1:500	+	—	—
A1	—	+f	+?
B1. 1:100	++	+	—

(overnight : same)
supernates fairly clear.

1:500 overnight	++	+	—

D: inoc in k1, k6 55. harvested k6 overnight; k1 in 48 hours = D2

2/26 D3 inoc D2 in k1

Titrate D1, living of. heated 58° 1 hour & formalized.

living	c: 1000 +++	k1:	50 +++	100 ++	200 +	500 −
heated	c: 1000 ++	k1:	50 −	100 −		

Repeat

living	k1 : 100	++	k6 : 50	−		
form	: 100	÷	: 50	−		
heated	: 100	−	: 50	−		
D2 living	k1 : 50	÷	k6 : 50	−	c : 1000	+ (poor susp.)
E1 "	k1 : 100	+	k6 : 100	−		++
E2 "	ditto					

~~Retest kauffmann for c: use D2....~~

confusing notation of kauffmann = 1,5, x... : c, x.., with x-component absent in other 1,5 sera. (cf. berlin serum). Heat lability makes somatic antigen unlikely, but reactivity of living cells is much better than formalized. Same type is secured by selection in kauffmann sera.

Titrate berlin serum : D1 living

1:50	100	200	500	1000
+++	++	++	+	÷

∴ ~~berlin~~ berlin is even more satisfactory serum (titer of 1:500)

2/23-4 B1 } (not pur.) into tubes of k1, k6 SS:
A1 }

overnight:

	k1	k6
A1	—	2 v. small bulbs.
B1	slow diffuse spread!	+++ near end of tube

2/25. Titrate D1, A, C: (1 hour 37°).

A.	c:	100 —	500 —					< 100
	k1	: 500 +++	1.000 +++	2. +++	5. ++	10. +	20. ±	1: 10,000
	k6	: 500 +++	1. +++	2. +++	5. ++	10. ++	20. ++	> 20,000
C:	c	1.000 +++	10. ++	100.000 —				< 100,000
	k1	100 —		k6	100 —			< 100
D	c	1,000 ++	2. ++	10. +				10,000
	k1	50 —	100 —	200 —	500 —	1000 —	2. —	< 50
	k6	50 —	100 —	200 —	500 —	1. —	2. — !	< 50

cf B1 at 1:100, 1:500! 4 hours: ib. (± k1 1:50, 1:100)

Ax to c++ k1±. still shows in slide aggl. from colonies from streak of D1. Cf. heat killed cells (somatic O component?)

Kunzendorf - 958
further titrations

~~2/27 G- live cells and heated in boiling water 10 mins.~~

	c 1:1000	berlin 1:500	berlin 1:100
SW958 liv.	++	+	++
heat.	—	—	—
cholerae suis 153 liv.	++	—	++
liv. D2	++	—	÷
liv. E1 purified	++	+	++
liv. E2	++	+	++

∴ factor is also present in 153, presumably selected by a raw kunzendorf serum. It has been substantially eliminated in D2. The factor is presumably absent from diphasic cholerae suis which migrates readily in kunzendorf serum. It is heat-labile (presumably H∴), but also formalin-labile!

Test serums at 1:50 with D1 living

berlin 1,5 (k3) and ⑤ (berlin absorbed c 157) ++++

all others negative: k2, k4, k6, k7, 1,2; lw, enx, d., 1,2,3 (colindale)

To select ≤ ~~ph~~ only phase from C:1,5, presumably should use 1,2 serum. "phase stability" of javiana may be due to similar cross reactions of ~~bo~~ l-1,5.

Effect of formalin. (Add .5% formalin to D1 cells)

	c 1:1000	berlin 1:100
liv.	+++	+++
form.	+++	—

	c 1:100	berlin 1:100	TVi 1:50	c 1:50
SW 958 l/v.	+	+++	—	
SW 961 l/v.	—	++++	—	—
D3 (not pur.)	+++	—	—	

(cf. slide agg. series 153)

c' factor is evidently absent from Colindale c.

Further questions:
① Does this explain all anomalies (cf. k5-k7).
② Reversion of c
a) 958 appears to be stable in c (Colindale) SS. cf. D3.
b) does Colindale c inhibit migration of 961: tested 2/18 no.
③ Is c' present in Edwards' c serum? (not previously detected with 1,5,c'... owing to formalin-lability.) If so, c' reagent possibly best prepared by absorbing cc' with D3 ~~or with tested cc' cells~~. Absorption of berlin serum with, e.g., para A 1,5 would be less safe.

Misc. tests:
3/1 E.153 is somewhat restrained in berlin serum. (verify c')
SW961 is not " " c (Colindale).

Titrate in c, berlin:

		c 1:1000	1,5,c' berlin 1:100
(mono. c-) 902		++	—
D3 pura. 958C		+++	—
(b.) SW 958C		~~+++~~	—
958	2c (slide aggl.) —	++	+++
961	++		

3/4/53. Stability of c phases:

F) Moc c: SS 3/4/53.
1. SW958 3/7: no 3/10: idem 3/13 idem 3/15 no motility
2. 958C motility whatever.
3. #153
(really perfect stability throughout.)

Other serums: SW961 in SS:

G.
1. 1,2,3 binns — 24-48 hours swarmed through
2. k5, k7 (para A 278) - 2-3 days " " (k5 slightly slower)
3. k3 — substantially immotile in 4 days: rough buds.
"1,2,3" serum probably perfunct (try Edwards 1, 2. if low!)

However, k5 and k7 are "effective serums", may still have some residual components (agg. titre < 1:50)

4. 961 / 1,2E (157 serum): swarms about equally prompt in k6, 12 Edwards, 1, 2, 3.

Stability of O-901

2/27/53.

Fresh stocks of 0901 received from A. Felix. Also test SW 542, on slant, and SW 556 resuscitated from lyophil. (0901 from Kauffmann and Edwards respectively).
In tubes, 0901 Felix 1-2 remained stable 4 days. On plates, (1 each) #1 stable; #2 gave 1 swarm 48h., 2d 72h.
Repurify for further test. → both (d).

A: 0901 I — 0 sw. 1st. 2d plate: 1 swarm : (d)
C — SW542 — 0 swarms 1st plate; 2d plate: 1 swarm : (d)
D — SW546 — 1 swarm : (d)

∴ All cultures are about equally stable (ca 1 swarm/2 plates)

E — 0901 #1 + FA22: 8 swarms after 24 hours (control 0) } all d
v. numerous trails — 48h. — 1

F — + FA9 (SW663) — 3 swarms, several trails
+ FA12 (SW628) — 4 swarms several trails

0901 + F19.

10/23 ~~Pour 5 tubes each SS W6, W1678.~~ (A above W6, B above W1678)
~~overnight: motility not diffuse but from coalescent swarms~~
~~flack.~~

C – D single colonies as above.
W6 W1678
C1-3 "smooth" 4-6 "rough".

10/24 overnight : C 1 spreading is initially rough and patchy; later diffuse from localized origins
2
3
4 "
5
6

D 1 "
2
3 "
4

10/27 Reincubate All reached bottom except D4

Second passage: all reached bottom. ca 24h.

A. 3/2/53 Streak out on N.A. Purified isolates to (H.L.B.) for compatibility test

	× 1876 ①	1956	② 1876	1956
C1	+	+	+	+.
2	↓	—	+	—
3	↓	+	+++	++
4	↓	—	—	—
5	↓	—	+	—
6	↓	—	+	—
D 1	↓	+	—	±
2	↓	+	+	—
3	↓	—	—	—
4		+	+	—
C6-0	↓	+	+	—
D-0	—	+	+	+

① Grown in broth together 24h plated on EMB Lac.
② Grown separately D (0)
③ by SL on D(o) A × 1177 B × 1817

see over.

	xA W1177	xB W1876		
c2	—	+++		W 2207
c3	++++	++	D1-0 +	W 2206
c5	—	+++		W 2208
D2	+	1		
D3	—	4	1607 —	c60 1, c3 +, W 2209
c6-0 (58-161)	+	+ (+)	D1-0 +	
D1-0 (W1678)	++	1	1[illegible] ++	
1607	—	++		
D4	++	±		still F+

~~c3 is ~~ ~~High fq.~~ sl.

D3 seems to be F^- but also sterile.

D2 is poorly fertile, not F^-

c2, c5 seem F^-

try D4 now.

1177 x 2209	—
1876 x 2209	20
1177 x 2209 (1876)	40
1817 x 2209 (1876)	~~10~~ ~~20~~ 3
1177 (2209) x 1607	—

Refertilization and F test: (2209: after grown c 1876 becomes fertile c 1177 and 1607 (but also fertilizes W1177 ! ?) Recheck this test after purification.)

needs to be checked.

2205 fertilizes 1986

2207-2208 are fertilized by W1876.

F/ mot. Recap.

4/6/53. 6 cultures 58-161, 4 of W1678 sent through motility tubes (2 pass.) 3 and 1, resp. were altered.

58-161: W2207, 2208 : F⁻, ~~noninfective~~, infectable
W2206 F⁺ nearly Hfr. Infective. Not studied for mixture. (see TCN) (found no SR+) see also 1113

W1678 W2209 F⁻, non-infective

3/ Prepare fresh F A (54A) from ~~[illegible]~~ purified z_6 phase of Z6g4.

3/13/53 P: 54A —X LT2[2] <24h → d:1,2

~~(R)(S)(T) 54A —x SW[illegible]~~

(R) 891 x— | 54A. swarms overnight. → d
(S) 959 x— | → d
(T) 960 x— |

Inoc these broths, unpurified, into d+1,2 for possible z_6 phases.

3/18. No alt. phases.

Repeat 3/18/53 using motilized 891, 959., in d-1,2 serum.

3/19/53. Swarm in R, S. $\underline{S}$ (in a 1,2 tube). Other R, S (2 d:1,2 each): no swarm overnight ~~~~ ↓d. (T.O.) T.O. 3/28.

3/20/53. 1235 swarmed. 3/21. z_6: — purified, no swarm in z_6 in 24h. Send to Edwards as $\underline{SW999}$.

SW999 / z_6 = 999B: reacts z_6 ++ 1,2 ++ 1,5 ++
2 — 5 ++.

∴ new phase is z_6, 1,5... or mixture. Restudy

cf.	z_6	1,2	1,5	2	5
SW999	+++	—	— +.	—	—
999B (not pur).	++	++	+++	—	+++
SW959	—	+++	÷.	+++	—

↓ 5/5 single colonies behave same way. SW999 apparently reacts fairly specifically $\bar{c}$ z_6

Still, motilize in 1,5 SS for further selection

SW999 and 999/1,5 serum still react $\bar{c}$ Bakers 1,5 serum at 1:100 but are not inhibited by it.

A. SW938 (1,2 : enx) x— FA40 (sendai a:1,5) [2; enx serum SS]

B. " x— FA3 (altendorf c + 1,7) ["]

C. SW676 (z_{33}) x— FA22 (i :1,2). [z_{33} SS]

D. SW 933 (i:enx) x— FA40 [i; enx]
dense bulb but no swarm. 2/28 swarmed through.

E. SW676 FA49 (z_{33}) x. L4-2 I (i:1,2) [i; 1,2]. 246: Moderate spread of inoculum in control (inadequate i ?) Reincubate: 3/1 no buds. Continued slow spread: 3/3 all are i; exp. not adequate

2/26 ...
F. abony 1+2 x— FA55
G. " x— "56
H. " x— "57
(—1,2) } No buds in exp. or control. 3/3 later slow spread of v. dense bulbs. all still b see over
[b, enx serum ss]

24-48/. A: controls and test show bulb-acties but no swarms.
B. " " "
} 2/28 Throw out see note *

C. control: no spread 3 tests: all swarmed + through.
c1 : i test s.c. in i ss: no buds.
c2 : i " " "
c3 (incomplete swarm) : still z_{33}. + slow spread (i probably weak)

D. after motilization, a:

J FA54 76 x sw891 (—1,2). Swarm through in 24 hours. d
K " 959 " d
L " 960 " Controls nonmotilized. (959 shows v. slow diffusion) d See 1023 J,K,L
suggests all of these are H, 1,2 like #153! 1,5 – (slide)

2/28. M. FA50 (sw546) x manai (6500-51, a:1,5) [a/5]
3/2. Slow spread in exptl., not control. → all react 1,2 + (see over) and ② + ⑤ –

3/2 N 15 (abony b: enx) x pinang (732-49) (leo: 1,5) [leo: 1,2] :
~~3/4 no ...~~
see 1028

* Note (2)-serum seems to restrain altendorf (1,7) (+ c serum) but not sendai (1,5) (+ a serum)

A - failed ? Needs multiple replications

B - " . Probably ⑤ has anti 1,7

C - i : 1,2 —x z_{33} ⟶ i : — best evidence so far that z_{33} is a phase 1 homologue.

save one as 1023C1

D (parallel to A).

"i : enx" x— a : 1,5 ⟶ B : a : enx. SW 975 (cf 925 = D, a, enx)

FA 40 (nominally phase 2) may be ~~?~~ mixed?

~~check phase 2:~~

FGH : (— : 1,2) —x abony b : enx 3/7 : no consistent spread in any exptl. or control. Activity of PA? 3/10 Isolate spreading dense bulbs.

23F Rep. - 3/31 still b.
G Inapp. →
H "

SW 973 (3 cultures)

M. 1,2 : — —x a : 1,5 ⟶ 1,2 : 1,5 S.c.i all passed through [3]

② serum ⟶ ⑤ ++ ② - (slide) M1 in 1,2,3 serum gave subbacteria from restricted growth. 3/7: no serum.

546 in ② : immobilized

M (control) eventually swarmed through: ⟶
② —
⑤ —
1,5 ++
1,2 ++
maybe 1,10 or 1,11? single colonies again reacted c ⑤ as did older broth.

~~SW974~~

∴ control gave no change

$z_6 - x$: — : 1,2

SW959 eventually gave spread through tube; 891 and 960 remained immobile. → inagg. b, i, 1,2. pv ± Aubuta ✓ inagg. Put through SS.

3/1 J K, L, [struck]. reacted (weakly) in d. Plant single colonies in d [struck] serum:

3/2. J inhibition bulb, ī subsq. spread = J' — through d: 1,2 → ② + ⑤ −
K " " s? " "
L. " " ī " " = L' — 1,2 ↓ ② +++ ⑤ ++ !

zega/z_6 uninhibited spread.

Single colonies of both J and L / [struck]d react both in ② and ⑤ — note L, originally behaves similarly.

This would suggest

d: $\underline{z_6}$ (phase not necessarily pure) → x: 1,2 → d: 1,2 ✓!

Test J', L' in 1,2 SS. → J'', L'' [struck] d. ∴ J, L are d: 1,2

→ single colonies:

	②		⑤	
J:	② [struck] +++		⑤ −	} z_6; lw —
L:	②	+++	⑤ +++	
SW 960:		+++	+ ?	

In tubes

	② 1:2000	⑤ 1:1000
J'	++	—
L'	++	—
960	++	—
891	++	—

∴ ⑤ cross-reactions appear on slides (at higher conc.)

K finally grew out in d serum: inagglutinable.
Select in SS: → inaggl. (j?) K itself lost. save K' (inaggl.).

J'' = SW 974
K = SW 977 v. wk. reactions even after passage twice in SS d?
L'' = SW 978

B phases [struck] 891 959 960 →) see 1031. Hold off further work until → x — ; exp. ch. is understood.

1023 FGH repeats

3/29: F —

GH small buds (not progressive)

(56,57 → abnorm)

G: z_{33}:

maybe 22s. Test single clones +

verify z33 : lux

Miscellaneous φ and stability tests ([illegible])

2/26/53 et seq.

Test unpurified stocks.

		12	22
	SW676	++	—
	SW546	—	++

A.

		12	22
	938	++	+
	altendorf	—	++
	0901-1	±	+
SW959	Hines 1,2	(++)	+
SW960	5394-51 1,2	(+)	—
dunbar	SW843	—	—
kedenny	SW730	—	—
bispebjerg (type II)	SW725	—	±
	SW714	++	++
J. Taylor	ebony 133-52	—	±

i + 12 ++
b ++ enx ++

mutated readily in 1,2 serum to give i-reacting culture. not macroscopic but might be somewhat stable

B

		12	22
	ebony 177-53	—	—
	ball 11-50	—	—
argyra SDH -1,2	l.l. 783-52	+	++
	SW891	++	(++)
	java 732-49	++	++
	denv 135-52	—	—
	schutio V726 52	—	—
argyra SDH	l.l. 4102-52	—	+
	zega 317	—	++
	shoreditch 208	—	—

a ++ enx ++

lw: — 1,5: +++ z_6: +++ of stocks.

a — enx — after neutralizing ± ±

d ± z_6 ++ — isolate 2 phases; d agglutinates poorly.

C

		12	22
→	stanley 5099/50	—	—
	miami 1570/51	++	—
	ball 268	—	—
→	l.l. 874	++	++
	848 salin.	—	—
	i: - 890A	+	++
	miami 1885-52	++	—
	" 3840-52	—	—

d +± 12 +±
a ++ 15 ++
a +' enx +++ — enx is poorly [illegible]
a + 15 ++

of ebony: (try zega!)

SW874 stocks: slide 4a : 5 mayagl! aggl. on colonies + ca 2-3 enx!

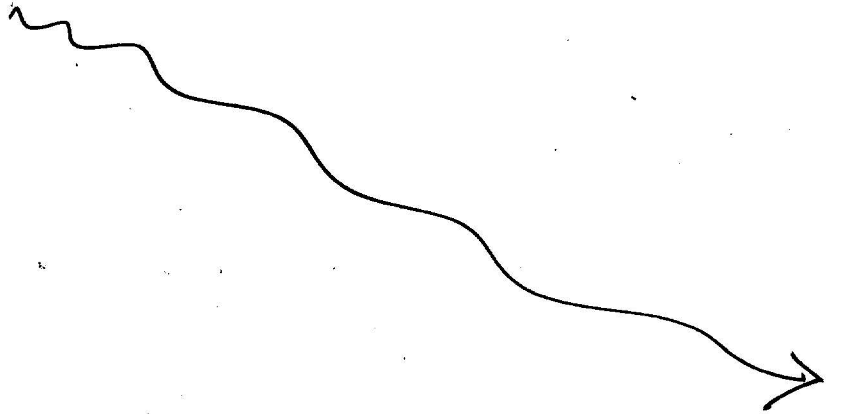
874: passage thru single a, enx colonies through 3 SS agar gave cultures each aggl. heavily both c a, enx. too unstable for present purpose

(over for page B)

Test φ type para B stocks for phase purity.
Bottles direct from stocks.

a) slide aggl.

	b	12
~~Jersey~~		
Dundee	++	+
1	++	++
2	++	++
Taunton	++	++
Beules	++	−
Jersey	++	−
3B	++	+++
BAOR	++	+++
3aI	++	++
3a	++	+++



Repeat

			12	22	
	brandenburg	24	—	—	
—:enx	ab. equi	26	+	++	
	ab. ovis	28	—	—	
	" "	29	—	—	
	dublin	65	+	+	
D -	d.e.sal.	72	—	—	✓
	para A	2	—	—	
-	"	229	? -	? -	✓ numerous faint, tiny plaques!
	wien	281	—	—	
	wagenia	290	—	—	

S. budapest strains from Edwards (non-~~XXVII~~)

E

		PLT 22
1	3807-52	R shaded
2	4102-52	S num. plaques.
3	4641 52	shaded
4	5435 52	"
5	5437 52	"
6	6524 52	"
7	6612 52	"
8	303 53	"
9	517 53	"

lv: 1,7 ~~Sw 986~~

4102 proved to react D:a ! Cf previous page: this culture is recorded as Loma Linda.

F

Tube agglutination:

		b	1,2
1	Beccle	+++	—
2	Jersey	+++	—

b : —

~~Mac~~ b serum to isolate 1, 2 phase for subsequent transduction to b:enx : no aggregation — these are monophasic:

Notes on cultures from Chamblee

3/1/53

(Brought back 2/15+/53)

SW961 = S. cholerae suis Kunzendorf recently isolated, 6145-52, purified and motilized. = —:1,5,(c'?),...

SW958 = SW961 selected at 4W / Minnesota serum. = c,c':—

958C = 958/Kunzendorf serum = c:—

Non-motiles: 1520- to 1526-51 (see SW552-553) presumably all nonmotile dublin from outbreak in Guatemala. See letter to Munoz ca 2/53. 1523, 1526 should not be found. Other NM not searched in accessions. Relationships of 3010-49 to 3012-49 not questioned.

? Kauffmann's D nm: is recorded as highly aberrant = Stocks 234?

Monophasics: —1,2 Stocks 191: history? listed as typhimurium,

SW960 = 5594-51 (Kauffmann...) Isolated in Berlin. Phage types as para B but is —1,2. (Belebsen ortsbezr?)

SW 959 = Hormes VA H -? no other label. listed as —1,2

3550-51: 2 cultures found, marked monophasic 1,2 and b resp. Worksheets name 3550-51 as b:—, 3551-51 as b:1,2. [3550-51 is probably a fairly stable b that later did give a 1,2 phase. This should be verified.]

SW 891, acc. to PRE letter, is I:1,2,3 (Thiel-Cornell) ∴ TM.

z33 homologues

3/4/53 et seq — occurrence: (see also SW.in)

991H1A = SW937/b → z33 ✓ ++.
K1A
K3

See 979-J and K. J14, K15-16 kept as inagglutinable.
Now tested as possible z33 variants. — not z33

After motilization: +		(through z33) ↓	through enx
J14	enx, i	i enx	
K15	enx	enx: z33? v. weak	enx ! z33?
K16	enx	enx	z33 ↓ enx

∴ K16 = z33:enx i typical variability = SW981
K15 ~~ny~~ also reacts z33; 1,2 at 1:500/-1:1000!
but strong spont aggl: maybe rough. Recheck! T.O.

3/13 Note 1023. SW676 —x.. failed. Repeat

3/15. FA49 —x SW666 — swarm. 1b slow buds only. eventually grew out. discard, as basic inprogress? — but
—x LT-2 " " still i.
—x abony no mot. at all. T.O 10/21

Cross-rx i b & 12 presumably prevent outgrowth.

Test 676 in ~~[struck out]~~ b, i:12 serum. markedly inhibited in b. Not especially in i:1,2,3.

ca 4/15. SW1036 selected from SW703 /b+1,2 in tube (1/3, after ~~[struck out]~~ motilization): z33:1,2. The ph2 appears by motility selection of ph1.

SW1005 abony (mot.) | b, enx plates — indistinct swarm.
z33: enx (z33 verified / enx. Motilization → enx.