Hfr, F+. miscellany.

June 20, 1952.

Resume Hfr studies. Slant of W1895 showed a yellow contaminant. Restreak on EMB lac. Colony #1 was extreme rough. (continued Hfr) Kept as W2041. Retain a "smooth" culture as W1895. Both cultures test regularly Hfr.

Attempt to resuscitate diploid cultures.

777-semis mostly inviable.

81051. OK. MHv Recorded as lac− Mal− Preserve as H-311
✓MHv lac−.

H245 − U.g. first attempt gave all lac−

6/25. A W1678 × 1607 2 trials No lac+ SR colonies.
B 1956

C W1918 × " 2 trials. No lac+ SR
D. 1956

Pure papillae in thick streak: A, B, C, D
see 954

6/26. W1590 × W1940 EMB lac. No + colonies noted. 1?
E Hold for papillae. 1? See 952.

F H310 × W1895

G. H311 (Het Lac = Mal −) × W1922.
see 955

H267/SM. 7 lac− : all protoclones. 1236 are Mal− 4,5 Mal+ ✓ 7 Mal ✓

see 953. Save # 2: H312

Hfr Lac+ X Hft Lac- 952

June 27, 1952.

W1940 x W1590. Mix in Penassay ca 4 hours, streak out on EMB Lac.
24 hours: opp all Lac-, some colonies have a dense center.
A. ① streaked out, gives a few Lac+, apparently not var, but with var. appearance on EMB Mal — ② Lac+ is pure Mal-.

6/29 48 hours: ca 1% Lac S colonies, and typically Lac+ at colony intersections.
B. Picked and streak EMB Lac. (8)

C x W1956. Similar to B, somewhat fewer Lac+. (4)

6/30: Lac+ appear pure, Hold for further development. ~~3~~ 3 colonies in B restreak EMB Lac.

no change
some begin to papillate

↓

1 Lac v } Mal-
2 Lac v }
3 Lac + }

Restreak 1, 2. Test nutrition. both TL
1 (BM) : - (TLB) + Try B, only.
2 (BM) : + (TLB) +++

Use #1 for further tests. Both are mostly rather weak Lac v+.

↓ H 316

Hfr x diploid (H312)

June 27, 1952.

H312 isolated by sm. selection from H267 as Mal- lac^v prototroph.

W1895 x H312 on EMS Mal for Mal+ prototroph.

7/30 4 hours. streak on EMS Mal. (v H312 parent. ca 10%v)

Mal+ only as papillae.

---

overnight: streak on EMS Mal.

~~Mal+ only as~~ after 3 days, numerous papillae noted.

7/6 8 streaked out directly. 1 clearly Mal^v. (H318)

Restreaks on EMS Mal → Mal+, Mal- colonies.

Restreaks 4 of these to EMS; EMB Mal. also lac^v +/+-

7/9. 40 tests 2 Mal^v. Restreak EMB Mal, EMS Mal.

#1 ✓ (H319) #2? ↓ Mal+ Lac-. Restreak single EMS Mal col.

~~#1~~

H319 on EMB Mal gives almost exclusively Mal- and Mal^v colonies. The latter restreaked do the same. on EMB lac, mostly weak+ lac+ and Lac-, occasional lac+. Triploid? or variety of Mal+, lac+ segregants?

July 16, 1952.

H318 = W1895 x H312 (H267 Mal- s^R).

8 segregants: 4 Mal+ MH+ all lac-

	S	+	D(o)	(Bm)	(TLB$_1$)		S	D(o)	(Bm)	(TLB$_1$)
1	S		−	−	+		R	+	+	+
2	S		−	−	+		R	+	+	+
3	S		−	−	+		R	+	+	+
4	R		÷	−	+		R	+	+	+

∴ Mal+ are TLB$_1$− Mal− are prot. (also segr on EMS/Mal cc 953)

Note crossover Mal+/mutr.

H310 ~~H310~~ x W1895
~~H311~~

955

June 26.. 1952.

H310 = non-disjoined (W1895 x W1177) TLB$_1$ - Mal-S^R Lac^v Xyl-
8 + Prototrophs picked from EMS Lac. (pseud. Lac+ and Mal+).
~~Most appear lac^v. Spot on EMB lac, streak EMS Mal, MtL (or Lyl).~~
4 from EMS Mal.

8 Lac: all pure Lac+ → check on EMB Mal / sm.
↓ 4 Mal- S^R
2 Mal+ S^{R}s
2 Mal+ S^s

4 Mal: 3 pure +, 1 Mal^v ! H313
check on EMB lac.

Pict ↳ S^v Mal^v Lac$^+$ Mtl^v
Restreak on EMS Mal;
Purify Mal+, Mal- for
nutritional and ~~fr~~ test.

24 Mal+ streak on EMB Mal: No likely Mal^v.
↙ vs. EMB Mal / cm. ↙
all Mal+: 4 SR 18 8^s

Pick 8 Mal^v from H313 to purify Mal+, Mal-.

40 Mal+ → no clearcut Mal^v. Restreak 4 on EMB Mal, Lac; check vs sm.
not ^{v} on either
↙ Others come Mal+ S^s (40)
B) 1 seemed to be Mal+ S^s / Mal- S^R Restreak: appears ~~Mal-~~ Lac+ (2)! Retest
vs SM: single colonies and mass.

Replica single colonies No Mal^v left.

July 5, 1952.

Σ 7 pairs:

Mal - Xyl - Mtl - completely linked. All auxotrophs, Lac +

	A	- - -		B + + +		
	S	Nutr: D(4)	D(TLB₁)	S	D(M)	D(TLB₁)
1	R	-	+	s	-	+
2	S	-	+	s	-	-
3	R	-	+	s	-	-
4	S	-	+	s	-	+
5	R	-	+	s	+	-
~~6~~	~~[illegible]~~			s	-	-
7	R	-	+	s	-	-

These types are therefore seen:

	[Lac+]				Repeat	Test EMS Lac, Mal	
						1607	1177
A	MXM-	S^S (TLB₁) -	(A2, 4)	✓	+++ Mal+ >		
2	MXM-	S^R TLB₁ -	(A 1, 3, 5, 7)	✓	++ Mal+ >	x3: ++++	x58·161: ++
3	MXM+ S^S	M-	(B5)			++++ → ?	
4	MXM+ S^S	TLB₁ -	(B1), B4		+++ Lac+ W<		
5	MXM+ S^S	(MTL)? ←	(B2), 3, 6, 7.				1678?

B5 would appear to be ≐ W1895] #3 is certainly Hfr ≐ W1895.
Also test type 1, 2, 4, 5 for Hfr] #1, 2, 4 are either F+ or Hfr.

7/8. Also mix W1607 + 1, B, 4 4 hours. Streak EMB Lac SM. (for recomb + F+ transduct)

B.
1 Scored poorly ?
3 Scored poorly
4 ca 2%+
n repeat, 1, 3, 4 gave 1-5% Lac+ S^R.

C. Repeat 1956
1 3 4 5: 4PM-9PM in broth
all gave 1-5% Lac+ S^R
∴ Hfr.

D) Reisolate Lac- from
B1, 3, 4, 2.
Plate x W1956: all F- ✓
∴ 1, 3, 4 are confirmed
Hfr, not transducible

#2 is only one in doubt.
It reacts with intensity of F+ (non Hfr)
with W1607.

Is #2 Hfr? 8/30, test H310 ✓
Lysed colony noted in plating of B2 on EMB Lac → λ^S (acc EML).
(W1607)

July 13, 1952 ff.

H310 (mass culture) x nDlo)

7/16. 58-161 +++ ∴ H310 is presumably Hfr!
W1607 ++++
58-161 x 1956 ++±
1607 x 1956 −
1802 x 1956 −

and W1895 x H310 is not especially significant.

(Cf. 955D).

7/16. Test Lac+, Lac− from H310. compare with from hybrid W1895 x W1956.

1 E.R.L. 7/10/52 Single cell-zygote-syn. Lac+ SR #7 TL ← W1607
2 " " " " Lac− SR #7 BM
3 " " " " Lac− SR #11 TL
4

5 H310 Lac+ (TL+)
6 " Lac− (TL−)

+
what does this mean?

All gave 0 prototrophs x W1607!, #2 x 1956
Repeat H310, Lac+ and Lac− x W1607; #2 x W1956.
(F+) F− probably F− but cant.

Repeat H310 and Lac−

H310 x 1607	+++
H310 Lac− x 1607	−
" x 58-161	+

∴ at least two segments of H310 are not Hfr.
H310 itself appears to be.
Try crossing H310 x F−! Streak out H310 x W1607 on EMB, EMS Mal
= 964

7/21/52

a. 8 Lac+ S^R from W1895 x W1956. 5 were also associated with Lac- S^R. Check nutrition: 2 Lac+ were B_1 -; remainder were TL B_1 -. Test these 6 Lac+ and 5 Lac- for F states.

1-8 = Lac + 11-18 Lac -. x W1607.

	x 1607	
1	-	++ x 58-161.
3	-	
4	-	
6	-	
7	-	
8	+++ → (3) 2 types of colonies on Lac	→ A Lac ++ → ~~MH+ and -~~
		→ B, C Lac+ weaker sl. mucoid

12	-
13	-
14	-
15	-
16	-

all appear to be compatible ♀ F-.

Restreak #8; The others appear to be F -.

b. Isolate Lac -, + segregants from individual H3/0.
Use early after checking purity.

Lac+		Lac-	
21	-	31	-
22	/		
23	-	33	
24	-	34	
25	-		
26	-		
27	-		
28	-		
29	-	39	
30	-	♀ 40	

all were incompatible x W1607.

---

955 F 8A = MH - but MH+ papillae and one + colony noted!
Restreak. Test MH+, and MH - W2068
MH- papillae after several days. ♂. W1956 stuff?

~~H311~~ x ~~W1895~~
H311 1922

956 ~~956~~ 956

June 26,.. 1952

H-311 = Het diploid: lac- Mal- MtlV. prot. EMS lac. Picks 8 lac+→lacV.
A) Prot. mostly lac-, occasional lac+. (Maybe revrsents of H-311?)
8 picked: all are Mal- S^{s} MtlV (exc. #1, #8 Mtl-). Restreak these on EMB lac. (prob. not diploid) (Should select for Mal+ prototrophs)
: these two are lac+ Mal- Mtl- not diploid.

? Are the lacV reversions of H311 or recombinants with W1895?

Restreak from EMS lac (ctg. lacV) to EMS lac., Mal. B)
This streak is ca 30% lacV but very few if any Mal+ on EMB.

C) Rest EMS Mal.
~~Almost all Mal-. 2?? Mal+ prototrophs. Restreak to EMB Mal.~~
4/5 show a +V rx on EMB Mal. Possibly Mal+ lacV? Restreak on Lac, Mal: all 4 are lacV Mal+V, as surmised.
These must be results of H311 x W1922, and presumably hemizygous for Mal. Het diploid may not bypass elimination!

A test additional
Test #2A for Mal hemizygosity: 8 Mal EMS papilla → Mal+ on EMB. Restreak on EMB lac: all were lac- → lac-
~~4 lac+, 4 lac- regressants. All were Mtl-! See 956A.~~ 7/10: 3 addl.

D. 4/0 lact ~~tested~~: 23 were clearly lacV.
Spot on EMS lac, EMB Mal: all 23 are Mal-. Do not save

7/15/52 Segregate (H320) A2. 4 Lac+, 4 Lac− (all Mal− unfixed test) (Hfr x Mal dyploid Mal v)

	Lac	D(o)	D(BM)	D(TLB)
1	+	—	—	+
2	+	—	—	+
3	+	—	—	+
4	+	—	—	+
5	−	—	+	—
6	−	—	+	—
7	−	—	±	+
8	−	—	—	+

where are Mal+ ?
Picks on EMB/Mal.

Btypes: #1, #5, #8. All should be S^S Mal−.
TL− M− TL−

[Test for Hfr by Lac S cross. (exc. #5 and 8 are Lac−)]

H320: Selct for reversions on EMS/Mal. Picks ~~88~~ 48 papillae to EMB Lac.

1 was still Lac v → lac v Mal+ ∴ H320 is hemizygous Mal−.

~~June~~ July 1, 1952.

lysis = +

Seed plates of:

Spot	W1827B	W1485
λ	+	+
K12	+	+
1485	−	−
1827 ABC	−	−
B	−	−
518	−	−

no background plaquing

Cross-streaks on EMB Sec.

	B	λ	1485
1827A	−	−	−
" B	−	+	−
" C	−	+	−
1827 B + λ	+? plaquey	(B: + !!*)	+ pl.
K12	− ?	−	+
1827B + K12	−	+ , +	+

* prob. used 1827B. (= 1827B)

No evidence that 1827 is lysogenic vs. 1485 or B.

Include B / λ ✓ B is λ^R.

7/2. B seed plate:

1 ✓ (λ + 1827B plaques)

3 (λ) + (1827)

4 1827

Plate ca 100 particles of λ on

A) 1827 → 100 plaques, clear,

B) 1827 + B → no 100 turbid plaques

Pick plaques A, B to 1485, 518, B. None grew on B

all streaks equally + on 1485, 518.

No gross evidence of phage modification.

Use 1027?

EML now reports that W1055 was responsible for the modification.

H245 x W1922

958

July 2, 1952

7/3. 40 Mal+ colonies streaked EMB Mal. Mostly Mal+ or +v Sare ability Malv. Restreak EMB Lac, Mal; EMS Mal.

#2 3 6 78 are Mal+/- v?

1 2 6 78 Lac+/- prob. Lacv. #3 are -+

1,6,7 certainly Lacv 6 certainly Malv. #4 pure -

Retest from EMS, single colonies: 1, 2, 3, (4), (5), 6.

1-5 are Malv Lacv #6 Malv Lac+

At first glance #2 appears Lac+/- , 1,3,4,5 Lac +-/-+

Repurify Lac+/-. H321

H295 secondary. ....
H324-325

June 26 ff. 1952.

Plate H295 from D(lac) on EMB Gal. Replica to Lac. Pick apparent Gal-lac$\underline{v}$ and test for λ...

1 Gal-lac$\underline{v}$ secondary isolated. It is apparently still Lp$^+$/Lp^s.
= H317

Later proved to be Gal$\underline{v}$ still, although with slower expression of Gal+.

Difference between H295 - H317 obscure. May be primarily a shift from segregation ratio + > - to - > +. Lysid colonies (Gal-) are very prominent.

June 9, 1953. H324-325. Plate out from D(lac) to EMBlac, Repl. to Lac, Mal, Gal. 3 plates each. >95% lacv(+). ca 150/plate

possible lacv Mal-

1. H325
2. H325

Lacv Gal-

3. H324

See 1056

4. Galv lac- H324

5. Malv Gal-lac- H324

H316 x V1895

July 4, 1952.

EMS Mal.

v. high yields. ca 20% Mal+. Picks smallest colonies, nestwalk EMB Mal

16 tests: 3 likely Mal v. hold to pool with further tests.

+ 40 tests } . No Mal v noted !

Some possibly Mal+ v but no Mal- segregants seen in estwalks.

F- via sonic disruption

7/7/52. A) B)

1 plateful W1895; W1678 in ca 15 ml. H_2O.
Subjected to Raytheon machine at full power, 15 min.
Then filter (14 lb Mandler).

4:30 brot 1 ml filtrate (+ 1 ml W1607) in 5 ml Penassay.

9A8: Controls clear. Plate Exp. with W1177 as D(0) or EMS tac.

	× W1956	on EMS Gal.
A = 1607 + 1895 fil.	· 0	· 15
B = 1607 + 1678 fil.	· 00	· 7
C = 1607	· 0	· 13
D = 1607 + 10^9 λ (K-12 UV)		· 315 (confirm ML/Rose).
E 58-161 × 1956	$+10^2$	

48h.

∴ Ultrasonates lacks both F+ and Gal+ transduction activity

Streak out papillae from C, D (8) (12) to confirm Gal segregation; test possibility of F transfer coincidentally.
"D1" was a very dark papillae. C papillae do not show such clear fermentation generally.
E (V) are areas between papillae for control use re F+ transduction?

In addition to streaking-out, cross-bucsh pushby picked papillae from EMS Gal; all uniform spot on D(0) - all auxotr.
Brot pooled samples to Penassay to prepare pulsum. F+ test:

C 1-8 D 11-20
D 9-10

8C: all appear to give pure +

12 P: at least 9 show Gal v, possibly all.

Repurify from single (selected) clones.

Pooled Gals (C, D) and Gal- (E) remained F- (× 1956)

7/14/52

Restr single colonies.

C (spont rev.) 8: all pure Gal+

D. (should be 19/20 Galduced). 12 tests:

1 + −
2 + −
3 + −
4 + −
5 + −
6 + −

7 + −
8 + only
9 + only
10 + only
11 + −
12 + −

4.5 8	3.5 0	8
6.6 3	5.49	12
11	9	20

is comparison. ~~No serious doubt of effect.~~

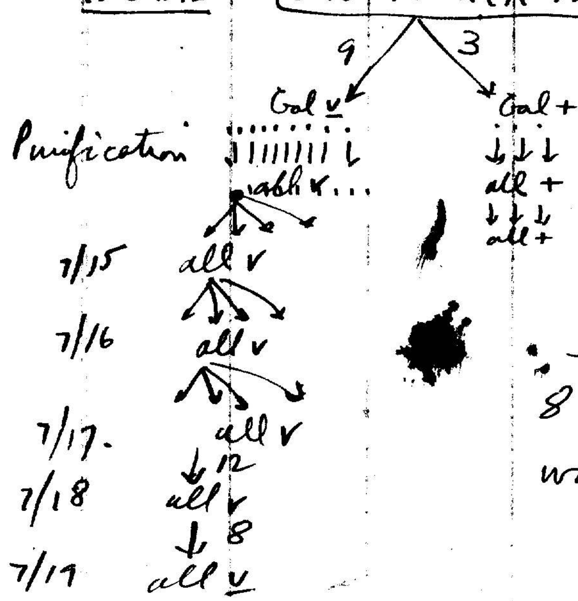
For further study, Restreak single colonies of # "1" for acquired stability, and 8, 9, 10 for present status.

7/15. On restreaks, #8 showed 1 Gal− ? (Residuals). 9, 10 were entirely +. but restreak single colonies → all Gal+ A. (but background has poss − ?)

7/15 #1 showed 3 +, − 1 "pure +". Restreak

7/16. All colonies were +, −. Restreak + and v → 7/17 all +, −, v colonies.

RESUME: (W1607+ λ(K-12))



Instability of Gal v seems to be persistent. No truly stable Gal+ noted!, if retested by further studying.

Save as slants: 1v, 8, 9, 10+.

Restreak to look for + from v

8 spont. reversions from Gal− from #1 were all stable + for 2 transfers

July 18, 1952.

5 plates D N Z Lac harvested to ca 20 ml. Ultrasonic (10,080 cps) 2 minutes. K12, W1895 (H)

A) Survival ratio (measured 7/21/52). Plate .1 ml 10^{-8}. } Initial counts
B) untreated — estimate density at ca 5% (early est.) : } ca. 10^{10}/ml
treated cells ca 10^8/ml

Filter through 14# Mandlers: Highly opalescent filtrates.

1: K12 2: 1895 . test sterility 1 ml samples.
add 1956.

Sterility affirmed in 4 day incubation.

Reinoculate samples to fresh broth for F tests.

(# 1956 - ~~of K3~~) ~~3~~ Test × W1607: Both F− !

∴ strains inactive re F

A 1895 / 5 ml Pimassay + 0.1, .2, .4, .6, 1.0 ml of 0.1% BT
B 1956 " 0.1 5% Tetr. (blue tetrazolium)
blue coloration by BT, test inhibition of small molds

ca 7/15-16. ERL pulled a single long cell from 1895 x 1956. This divided to 3 cells → microcolonies.
Only parental combinations were recorded, however. (no detectable crossovers or plasmogamy). Save
-A1 (1895 type) -B1 (1956 type).

F+ transduction via λ

Lysate # EML 269-9

A	1 ml in 5 ml Penassay		① clear	② turbid!
B	.5 " "	+ W1956		
C	.2	"		
D	.1			
E	B 1/5	"		
F	D 1/5			
G	0	"		

1st testing at ca 6 hours.
2'd " at ~~30~~ 24 ".

Lysate may have a "dormant" contamination. Streak A on Gal, NA.
① → Mal+ Gal+ (very likely the W750 Gal+ used to prepare lysate)
∴ Experiment above is confounded. Do not pursue.
~~Ep~~ This crossing suspensions of treated W1956 showed same Mal+ on EMB Mal: #9 (750+) and #10 (1939)

Tentative conclusion: F transduction is due to "dormant contamination".

4P20: Inoc 1 ml lysate #10 in 5 ml broth
P 10A21: clear
~~10A22~~ "

Repeat c̄ # 10.

~~1937 with the cap~~ [illegible]

1. 1 ml /5 sterility test
2. 1 ml + W1956 F-transduction test
3. Inoc test of 4P20 c̄ W1956

48h. CLEAR. Add 1956

2 and 3 were both F− (x 1607).

✓ #1 : F−

July 21/1952.

See 955E. EMB, EMS/Mal.

Repeat cross EMS Lac 7/29.

8/1 24 lac+ → EMBlac.

8/3 Repurify 6 possible lacv.

	Lac	Mal	EMSlac	
1	V	+ V		Mal+
2	+?	+		
3	−	−	N.G.	
4	V	−		Mal− H322
5	V	?		Mal+.
6	+?	−		

Save for hemizygosity tests on Mal−lacv.

H310 Revertants! EMBMal.

#1, 2 Mal+ Lac− no test.

6 addnl Mal+ lac− " "

see 1057

Phage tests on W[illegible]...

965

Reoves

7/15/52. Chuke phages: (small vial stocks)

	T1	T2r	T3K	T4	T5	T6	T7	λ-2	Bacty. X
B	✓	+	±	±	✓	✓	✓	—	
K12	✓	+ v.sm.pl.	—	+	✓	✓	✓	✓	

T2 T3 T4 should be reviewed.

T2	1.	T2:	6/8/48	Norris?	✓	✓	K12 R	B S	K12 S	B S
	2	T2r	12.3.46	Lu?	(contam.)		R	S	S±	S
	3	T2h	6/8/48	Nou?			R$_s$	S	S	S
	4	T2r	6/8/48	K12			R	S	S±	S
	5	T2r	7/17/48				R	1 plaque	S±	S
	~~6~~	~~T2r~~					R	S		

T2 should be further grown on B. EOP probably reduced.

T3	1	CSH 10/23/51	✓	✓	K12 R	B S	K-12 is
	2	7/30/51 (13)	✓		R	S	
	3	9/24/51			R$_s$ (hyp. pl.)	S	clearly resistant
	4	T3C 11/2/51			R	S	
	5	T3K 7-17/48			R	1 plaque	to T3 proper.
	6	T3K 7-18/48.			R	S	
	7	T3 6/8/48	A.N.		R	S	What is T3K? Test
	8	T3K	A.N.		S	S	various stocks for history.

Possible host-modifications?

T4	1	6/8/48 A.N.			S	S
	2	" (11.12.)	✓		S	S
	3	7/18/48.			S±	S
	4	7/17	✓	✓	S	S

HT5" A.N. 6/8/48. what phage? : not T1, 3?4? or mod?

	T5 AN	T5 ✓
~~B~~	S	S
K12	S	S
B/1,t	S	S
B/4	S	S
B/347	S	R
W1955	S	R
W1956	S	R
W1957	S	S
W1979	S	R

Our present T5 stock seems ok. Identity of T5 from Norris?!

---

cf. T3 - T3K. B^S, B/3,4,7; K-12^R, 58^R-161, W1678^R, W1590, W1485^R, W1918, 1956, 1802

	T3 S	T3K	plaque.
B	S	S	
B/347	R	R	
K12	R	S	
58-161, 1678, ~~1590~~ 7	S	S	
1590, 1485	Plaques	S	
1918 1956 1802	S	S	

Note: T3K ≠ T3H.

Plaques on 1485!

T3 strain is evidently mature on K-12 ....

It shows a limited e.op. on W1485 full action on W1918 (Y10.... acc'g Novick).

T3K was selected by growth (on K12?)

9/16... T3, T3K spotted on E coli B, 1918.

Repeated and tested:

	T3/B	T3/1918	T3K/B	T3K/1918
B	+++	+++	~~B~~ +++	+++
K	−	±	—	± (1pl.)
1918	+++	+++	++	+++

Question of possible host modification should be studied more explicitly. T3K originally gave +++ on K as well as B.

For testing WG series, use T3K initially; then T3 for T3K ˢ types.

9/22/52.

(A) Stock "PLT22/LT2" 10° 3/52 found quite moldy.

(B) 10AM. to 100 ml Penassay incubate c aeration

1 1ml LT2 + 1ml stock PLT22
2 " .1 "
3 " .01 "
4 " 1ml SW435 (LA22)

2PM. Heat shock #2, #4 60° 1 hour. Sediment. Treat supernatant c $CHCl_3$.

Test .1ml "4" / SW435 EMB Gal.

(C) Gal+ transduction

9/21 ca .1ml SW435 ± .1ml stock FA LA22/LT2.

9/23 SW435 1 papilla → Streak out 16

SW435 + FA ca 30 Gal+ papillae 1st plating: apparently clear Gal+, –

FA sterile 0 2d "(+): all apparently pure +. Repick a few lightest (incl ? –): 3d plating: unstable cones)

(D). Assay FA #2 .1ml on SW435/Gal : papillae ~~—~~ 5

D (0) : colonies. 6

Thrown out ~~—~~ FA: sterile

(E). 1ml each SW603 (para B O ca 10^{10}/ml); 966-4

→ trails and swarms small foci ful

(F) SW603 5ml/petri dish UV
No trails / no swarms

UV	9/26 (48+ hours)
0	~~—~~: isolate
20	–
40	–
60	–
120	–

(G) Assay 4: <u>800×10^8</u> phage / LT2;

∴ phage/FA = ca 10^8!

Gal+ papillae/ml / SW435
61/.1ml = <u>610</u>

Note — SW552 "Group D Rough" – not transmitted by PLT22/2.

966 C 16 tests of SW435 transinduced Gal+
all stable

9/29/52. Second run: SW 435 + .1 ml 966 B 4. (see C)
Picks 8 papillae (smallest or appearantly mottled)

↓

↓

all stable cf. 9/23.

---

24 tests total

UV-induced O → H 966F

9/27/52

In previous run, 20 seconds gave a swarm. Repeat on a larger scale.

b̲ − = F1

9/27 2 plates SW603 / 10 ml.
Irradiate 5 ml aliquot 25 seconds (F2). 5 ml − 0 (F1)
Concentrate to ca .5 ml Plate .02 ml / plate

9/29 UV-0. 5 plates: 3 show swarms (late develop; no trails)
Moz to Pen. s purifying = F2, 3, 4. = all b̲
UV-25 secs 5 plates 0 swarms 0 trails

Recap.

① WG 5,6,7,8 found to grow poorly on D(0)

② Indole-negative citrate positive (presumably slow)

③ Crossability tests (= 776-34 = W1395)

a). C: 0 (certainly not detectable!) × >300/plate but all pave.

(aerogenes or intermed. (cellobiose-not gummy))

b)

④ Auxotroph hunt. Grew poorly on D(0), apparently resistant to penicillin. Should be repeated with D(succ)

A. Repeat SRP (interim growth)

			Mal	Lac
9/24	K-12 x 2058	+++	+, −	
1	wg6 x 2058	5	all+	
2	wg6 −	5	all+	
3	wg6 x 1956	9	"	all +
4	wg6 x 1970	1	+	
5	wg6 x 1817	9	all+	

no suggestion of fertility!

B. Penicillinicum. ca. 3×10^8 /ml. 3:15 – 930

	succ	penic.	Turbidity
1	+	−	+++
9/26 2	−	−	+++
3	+	100	++
4	+	300	+
5	+	500	± } plate: ca 300 / 10^{-2} ml
6	+	1000	±
7	−	500	±

→ But poor growth when replicated to D(0) + spread succinate
1? mutant

(over)

~~SRR~~ + SRP test do not support patristy of wg 6

Similarly wg 7 never gave a clear response.

ca 9/26 Try crosses:

W1987 x 1817
1978 x 1987
1978
1987
1978 x 1956
1978 x 2058
1978 x 1817
1987 x 2058
1987 x 1956

} on D(0)
all barren

~~Lab~~ Observations on recombinants Hfr X

TCN expts.

9/23/52.

TCN IV. 23 lac$^{\pm}$s on EMB Lac. All tested also had lac+ S^R, ... recomb. Confirst streaking, all appeared to give pure +, − (no nondisjunctions).

9/26 Restreak ± to look for possible persistent lac ± (like H3 10) all pure lac+

9/28 VIII TCN. Pick Lac ± colonies from EMB Lac various times to EMB Mal.

		Mal −	Mal +
1895 × 1956 Penassay	45 min	3	0
	90"	19	0
	120"	19	1 *
	180	14	0
	240	7	1 *

Replicate to EMB Lac sm to verify. Restreak * to verify whether they are actual zygotes or trivial conjunctions.

→ on streaking and replicating, very few lac+ S^R.

~~Lac~~ These two are probably trivial sectored colonies.

FA from other Salmonella.

sensitivity tests and productions

~~903~~ 970

9/26/52.

1. Test sensitivity of var. stocks by cross-brush of broth cultures on EMBlac

FA

		PLT22/2	PLT7/7
✓	LT2	S	S
✓	SW435	R	S
	SW603	2 plaques	R
✓	stanley	sens SR	R
dublin	SW553	S	S
	eastbourne	R	R
	Boyd 4	S	S
	" 11	S	S
	typhi H901	S !	R?

second run

	PLT22/2	PLT7/7
LT7	S	S
coli 2	R	R
coli 3	R	R
Boyd 4	S	S
Boyd 11	S	S
altendorf	S	R
typhi Bo	R	R
typhi H901	S lytic	S lytic!

		phage (contyuum)	FA (SW603)	
~~A1~~	1A	—	—	} Thrown out
~~B2~~	1B	—	—	
~~B~~	2A	+++	+++	
B	2B	+++	++0	
	7B	+++		
	8B	+++		

	PLT22		
LT 1	S±	12	S
3	S	15	S
4	S	typhi V,F,	R
5	S		
6a	S		
8	S		
9	S		
10	S		
~~13~~			

Note host specificity of PLT22/2 on para B as previously unnoticed.
PLT22 remains the phage of choice. Grow in broth and on plates on

agar = A
broth = B

(1) stanley B d 1,2 (2) SW553 (dublin) D gp (3) altendorf B c 1,7 and (4) H901 D d

(6) enteritidis D gm (7) abony B b enx (8) sandiego B eh enz,15

		22	7
✓ 6	enteritidis	S	1 pl.?
✓ 8	Sandiego	S	S
✓ 7	abony	●	few pl R
		R	R
	derby	R	R
	montevideo	R	R
	coli 5	R	R
	coli 1	R	R
	539	R	R
	~~typhi~~		

	PLT22	7	
SW 609	R	R	spont b
618	S tiny pl.	R	transind b
623	2 plaques	1 plaque	transind i (PLT22/2)
H 901	S	faint S	
SW 664	R	R	
SW 623	1 plaque	R	

Although H901 is readily lysed by PLT22, no satisf. preps. have been obtained in liquid culture. Adapted plaques should be used. Ditto for stanley

→ Prepare PLT22/603 609 618 623 starting with single plaques from PLT22/2. SW603 and derivatives are evidently poorly sens. to original PLT22/2

B g - derby essen calif. reed.
D b muenchen
D e eastbourne

phage - lysogenicity tests on

stanley LT4 / plaque. PLT22++

H901

eastbourne LT10 several, (LT1, LT3) probably severe lysog.
PLT22 very slight

typhi 60

Boyd 1104 eastbourne+++) but filtrate acted on neither

A B C D Layer plating test for lysogenicity: did not work too well owing to overgrowth.

Plaques:

	1	2	3	4	5
A	stanley	London	derby	eastbourne	
B	LT1				5
C	LT6				10
D	PLT22+	+	+	+	

FH / Salm. misc. 970

B
d 1,2 ① STANLEY, typhi ...
Repeated efforts to grow PLT22 on stanley, typhi H901 or 60 have failed, despite plaguing on agar.

~~④ S. typhi H901~~

⑤ heidelberg 5a LT-7 5b LT-22 n.g. Repeat: o.k. ... see 971D5 ...
PLT22/heidelberg

? eastbourne
coli 1-5
london derby montevideo

SW694 — resistant to PLT22, PLT7

not FA against SW666.

9/29/52.

A SW603 / motility agar

B SW435 / " + typhimurium serum 1:500 (~~inadequate, try 1:200~~) (OK) .

C styphi H901 / d-antiserum 1:500 ~~[illegible]~~ (inadequate) ; 1:200

A! see SW666

1 + "FA 970-1A,B". no trails no swarms (FA: no phage)

2 FA 970-2A,B numerous trails and swarms. Streak out and

Keep non-b as 971A-1,2 ~~= SW663,662~~ ✓✓

b 971A-3 test single colonies : Seach 2A,2A' < 2A: 1 b+++ [illegible] / 2A': 1,2,3,5 b:+++ later shown

Check non-b on available sera: b, i, d, e, 1,5; 1,7, 1,2 Inagglutinable GP but motility OK under scope

9/39. 7.

Swarms + trails. 2 plates Test c̄ b, e.

a } 16 isolates all b. = 971A4

b } = 971A5

b e

9/29 8. swarms + trails. 2 plates.

a 7 isolates all b ~~[illegible]~~ SW664 ebony + +

b 8 have b 2 are ? (#5,8.) A7, A8, [illegible] sandiego [illegible] +

possibly IX XII enx - ?

→ react c̄ enx 1:100 para B +++

abony is mostly b, very faint enx

1:10 reacted c̄ #1, etc. (non spec?) e serum?

Repeat 7 for non-b

3. few trails; swarm

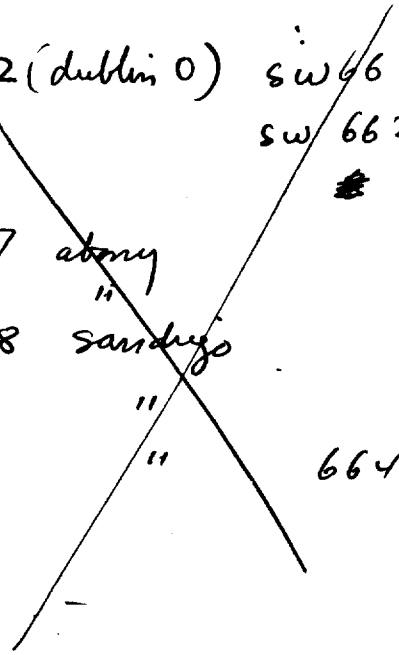
6. " "

Notes: SW603, A1, A2 are strong Xyl+ SW553 is v. weak ±.

recover to stock tab.

97A := sw603 +

	FA			antigen
1	97a-2 (dublin 0)		sw663	gp ?
2	"		sw662	gp ?
3	"			b
4	970-7	albany		b
5	"	"		b
6	970-8	sandiego		b
7	"	"		b
8	"	"	664	env ?



Recap.
10/7/52

A SW603 [= para B/o]
B SW935 + tymus serum
C S. typhi H901 / d serum 1:200
D SW666 = SW603 Gal−

A. 1. FA 970-1 fractive

(dublin) 2 970-2 2 plates, 5 colonies each. 1/5 : b 1/5 : b
D gp ✓ others presumably gp to be sent to Chamblee to verify. Save 2 nas-b as SW-663,662 = 971A1, A2 resp. Save 1 b as 971A3.

altendorf 3 970-3 1 plate: several fractive and swarms. Streak out and
B c; 1,9 test single colonies. 10/10 b

4 970-4 H901. No satisf FA to date

B a; 1,2,3 5 S. heidelberg

entucky 6 970-6 1 plate: as 3. 6/11 b, save 5 nas b
D gm ✓ [illegible]. See D6

10/2/52

971A

abony B enx b

7. 1st test: 2 plates many T and S (tracks + swarms). 16 isolates all b.
parent culture is largely in b phase [anti b serum would be invaluable].
2d plating 10/2/52 4 plates. 8/8 single colonies: b. save 2 b as 971A-4 and 5
Inoc #1 (strong b) #7 (weak b) and mass into b agar. Also abony to isolate enx phase.

10/4. Both 1 and 7 were blocked on b agar, gave 2-3 swarms = 971A7-1B, -7B.
[abony swarmed out directly (single cols. should be compared). also Cf. spont rev.]

B: orig. 1 b, 7 b
3 swarms ea. not b, 12, enx ... ??

T.O. all of reverse cultures (probably 233)

8 2 plates many T+S. 7/7 all b 6/8 b 2/8 reacted ± enx at 1:100
This serum aggl. para B at 1:10. Rx not very strong but confirmed microscopically. ~~Save~~ 2 b as 971A-6, 7. 1 presumptive "c" as 971A8 = SW664 (✓ ch: —)

San Diego B ch enx 15

Antigen transductions to typhimurium 971B
SW 435

Sept 29 - 1952

(dublin) D gp — B 2 9/30 2 plates no buds 10/3 2 buds replant to i, 1, 2
#1 did not grow out well on i. → not Salm on EMB
#2 inaggl. i, b, 1,2 (trace, or i each) . Presumably gp. Phasicity? → (Gal -) SW674

abony B b enx — B 7 9/30 4 plates no buds PM 1 swarm. Grew poorly. probably contaminant
10/3 2 buds. Pick to broth #1: b → isolate as SW672. Plant on b
#2: inagg: b, 1,2, enx, i → contam (not Salm).

san diego B eh en2,15 — B8 9/30 4 plates no buds
1 plate: filled with growth contam? 10/3 no buds or swarms

B-0 Immobilization OK on 1:200, 1:400 1:800 H-typhimurium antiserum
In 1:500, para B grew well. However, buds are growing out very poorly!

Antigenic transductants to S. typhi (H901) 971 C

Sept 29, 1952

C - 0 9/30 H901 not completely suppressed: dense spreading growth may obscure some swarms.

C - 2 9/30 1 plate no buds PM 1 bud-swarm → agglut b, d, i
D gf Isolate as SW ~~668~~ 667

C - 7 9/30 2 plates 5 buds 1-5
B beur all 5 are b (abnormal pmt prod. this phase)
Isolate as SW 670

C - 8 9/30 2 plates no buds PM. 2 buds? ✓ → swarmers
B ch en315 both react with ent (a?) Purify and isolate as SW 668-9

C - 15 (about 2) cf. C7. 10/25 - v. faint turbidity away from inoc. Pick + test

C - PLT22 1 plate 2 (or 3?) buds. → 8 swarmers later
B i 123 3 isolates: all 3 are i-, not ~~b~~, d; 1,2 = same as 971 C 22

no tails associated with the buds. Inoc buds to fresh plates, broths

C5 (anti D inadequate) but no kidsor swam

transductions to paratyphi B
SW-666

971D

~~#~~ 10/1/52

D7: many swarms pooled: all Gal− n streak. 5/5 b

Reactness to b agar. → several buds. Pick for test (D7B:1−)
save for test

10/4 B. Plant individual colonies from original pool to b agar. (Two kinds of dysbiosis??)
→ 3 swarms: inagglutinable b, 12, cmx

1 ~~so~~ does not swarm on b agar 2 swarms slowly.

D7A2 gives weak reaction after swarming!! cmx ?

streak and save D7B1 Rebuds.

of A7

Rev D7A1 A2: 4/6 b strong = D7A1 = b; 2/6 b weak! D7A2 b
and B0 give weak b SW671
B1 maggl (2d phase?) 678

D3 10/3 From swarm from transplant to b agar. → non b, i. 1,7 ?
streak out and save as SW675.
c 1,7 z 33

10/5. Second run, plant swarms to b agar and save selection: slow out growths
(probably second phase again) < 1 − still b
2 − non-b

D3: ~~a : c ~~ ~~equify as SW 903~~ z 33) T.O.
a, b : not c. (probably "j") 3/5/53: D3b = b

D5 10/3 − FA mg. 10/?.. 3 buds → streak and test single colonies. see SW683
v; 1, 2, 3

D15. 10/24. Numerous single swarms. Pick + test : see 979-4

D18 (LT-2II) 1/8/53. FA18 × 666. 5i 52b Note concordance
with 22 × 666 see 986. 4/5 were Lp3.

D6 10/7 3 swarms. 2 non-b 1 b. Save non-b as SW 669
FA39 (Sendai) 12/4/52. 30b : 2a (22? : 4/ mixed) swarms may have been mixed. SW
& some rough

3/4/53 "weak" and "strong" b have not been carefully studied. May represent features of b? Throw out

#3

533 → 534 ⟶ 588

parB 0 sp+ (1,2)

note: 588 still sens. like 533

543b+ are resist

S W. cultures.

Rack 1

SW			
351	538	548	566
414	539	549	567
435	540	550	568
530	541	550R	569
531	542	552	573
534	543	553	574
534R	544	556	576
534	545	558	577
536	546	563	578
537	547	565	579

Rack 2

SW		
580	600	628
584	609	629
586	618	630
587	619	
588	620	
595	621	
596	622	
597	623	
598	625	
599	626	

Rack 3

SW			
655	669	680	696
654	670	681	697
655	671	682	698
656	672	683	699
662	673	684	700
664	674	685	
665	676	687	
666	677	688	
667	678	694	
668	679	695	

Rack 4

701	770R	829	LT-2 I
703A	771	834	Staphi VI FI
703B	774	837	LT-2 II
704	775	839	2nd PRE TO
704B	776	840 A	LT-15
706	787	840 B	SY 79
715	791	842	LT-12
721	803A	862	
760	803B		
764	825		

Rack 5

901	913	923	933
902	914	925	933
904	915	926	934
905	916	926 mix	935
906	917	927	936
908	918	928	937
909	919	929	938
910	920	930	939
911	921	932	940
912	922	932 b1,2	941

Rack 6

942	954
943	954+P
944	956
945	979
946	
947	
948	
949	
950	
952	

FA 56 (SW 960) appears inactive re Fla. (SW 666; SW 967)
FA 55 and FA 57 –x SW 616 give b only
~~f#~~ –x SW 967 " swarms:
(57–x 967 seems unusually slow)

3 trials each FA 54 –x maize (d, a, 123 runs) to secure z_6 phase gave only 1, 5; –x TM 1, 2 resp. FA 54/A is designated as $\underline{z_6}$ but should be reraised.

missing:

A	B	C	D	D
1394	112	1827	1728	618
1177	125	1969		
1895	1368			
1922	1606			
1918				
#310				

pulled for remedial

8848
1979
1895
1896
1570
1635
518
1325
1452
1618
884
96108
760
1015
1022
1023
1024
1033
1327
2069 H+
1674
1666
1649
1688
1517
1529
1541
1585
2069 pt
1832
1852
1846
1903
1920
1939X
1970
2020
2019
1957
Y 28
Y 679
1801
383
1742
1813
1730
1734
1729
543
513
404

X-phage

9/29/52

Survey host range by cross-streak on EMB Lac:

		R
1	ty[redacted]	↓
2	S[redacted]	
3	[redacted]	
4	Boyd 1411	
5	coli 3	
6	LT2	
7	altendorf	
8	eastbourne R	
9	san diego	
10	albany	↓

11	SW541	R
12	enteritidis	R
13	Boyd 1404	R
14	coli 5	R
15	stanley	S
16	derby	R
17	london	R
18	SW553	R
19	montevideo	R
20	eastbourne	R

(2)

1	coli 2	R
2	coli 1	R
3	H901	RS
4	SW435	R
5	"coli 4"	R
6	SW539	R
7	typhi F1	R
8		
9		
10		

11	LT15 #292	R
12	12 114	R
13	1 ✓in 1,2	S
14	3	R
15	4	R
16	5	R
17	6	R
18	8	R
19	9	R
20	10	R

check motility of S, RS. 9/30

A

		R
1	971 "A2"	↓
2		
3		
4	=A1	
5	A3	
6	"A2"	
7	=A2	
8		
9		
10		↓

F		R
1		↓
2		
3		
4		
5	SW 583 / 653	S
6	582	
7	581	
8	5~~7~~	
9	588 (circled)	SR ← SR
10	547	↓

reported sensitive to adapted X (→ 588)

C

	R
634	↓
635	
636	
637	
638	
639	
641	
2	
3	
4	↓

	R
645	↓
6	
7	
8	
9	
650	
51	
52	↓

B

		R
1	592	
2	594	
3	546	S
4	609	
5	610	
6	611	
7	12	
8	13	
9	14	
10	15	↓

	R
616	↓
617	
618	
623	
25	
26	
28	
631	
32	
33	↓

Records on SW543 B+?

Notes: Stocker reported that SW 588 was more susceptible to X grown on SW592.

H901, H901 i both S
S479; S479 i both R

History of 588: ~~[illegible]~~ 544 (tyrosine (O form)) spont + → 588 :. vej mal sham iscus. ~~[illegible]~~

10/7/52

Compare X 942-1 and regrown on SW592

	V_{or}	X_{942}
609	R	R
610		
~~673~~		
617		
618		
623		
625		
633		
LT1	S	S
LT2	R	R
SW435 ~~LT22~~	I	I

No improvement.

SW543. Line seems resistant.

Typhi derivatives:

Later, recheck of X/SW592

		942-1	X/592
	703	+	±
	588	++	±
	633	±	—
(typhi)	537	+	+
	976-5H.	-	—

A SW541 5EMB Gal 3 Xyl 8sec UV (°K).

1 each?? — Gal n.g. Xyl - lookalook. SW665

excellent mutant: no transductions, papillae are clear in 48 hours.

Apparent rather high yield compared to SW435. (should be compared closely)

Plate c PLT22/2. Pick papillae + streak to test stability

12 papillae. Streaks to give large but poorly fermenting colonies!

Compare 541, 665 and papillae! (possible a Xyl+ weak allele, in this background).

In various tests, inconsistent scoring. Best result: incubate at 37, then room temp. No sign of segregation - not many tests

B SW603 5 Gal 1 Xyl → 1 sectored col. → good Gal - SW666

considerable dimorphism on gal, especially after UV, not remedied by repicking large colony.

excellent mutant colour. Pick papillae & streak to test stability. +PLT22 → plaque ridden (e.o.p. ca 10^{-4})!

8 papillae. 1 appears Gal v = 973 B1 Restreak several colonies.

All others appear stable.

10/9 Pick added. 16, (all large papillae only). All + appear stable on 1st streaks. Restreaks: no - segs, but + # 2c shows some mottling. Review this as 973B2: upright + and + mottled (phage?). Repeat once again:

→ appears to throw stable, mosaic types. Repick B1A to look for new stable, "stable" ✓ 1 colony is stable.

Streaks of B1A mosaic Gal v → -, v, +. Check +, restreak v. → pure + → -+v

∴ This unstable transduction segregates pure + and -.

~~Can~~ (over) Isolate pure + and -

973C PLT22 + SW665

973D ϕ SW541 + SW665 Xyl.

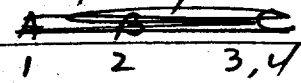
973E PLT22 SW435 Gal. – 20 : all pure + !

D) SW541, transductions of SW665 behave very peculiarly on xylose. Sharp, Xyl+ colonies give – restreakings; SW541 itself gives inconsistent responses on EMS xylose, but not to cause a false negative reading. Only one restreaking gave a typical + reaction. 24 tests 22 – 1++ 1 weak + !

These colonies gave typical + reactions on original plating!

Possibility: interaction with SW655? Try mixture.

Save 1 plate which carries 1++, 1+, 2–.



Efficiency of transduction and effect of serum.

973 B

FA is PLT22 966-4.

Mix .5ml ca 10^{10}+ cells/ml with .5ml FA (or dil. as ind). 966-4
Let stand 5-10 minutes · plate .1ml.

1 = ~~SW665~~ as EMBXyl Note self plaquing!

2 = SW666 EMB Gal

3 SW435 EMB Gal

---

papillae / ml FA →	.05	.025	.01	.025 + .12 serum .02 ml	0	
SW665/Xyl	477	218	40	31	1	self plaq.
SW666/Gal	15	6	5	~~4~~	0	
				.5 + .02 ml b serum.		

Nearly plaqued all plates. Obvious reason for non-linearity less heavy plaquing 16

SW435/Gal. 53 50 31

why non-linear?

8000 / 8000 x 10^7

JAN 2 6 1955 This calculate at only 10^{-6} papillae per phage of BADS claim of

Spara ϕ/o serial transduction

10/4/52

= FA22

Streak out PLT22/2 on SW666.

Pick single plaque to broth = FA9

streak out FA9 on SW609 = FA10 (masked plaques)
618 = FA11
623 = FA12

10/2 974-1 SW666 + PLT7/7 → 1 trace-swarm: i

Purify again and keep as SW671. No swarm on i, 1-2 agar. (i alone ??)

11/5/52. SW666 + FA as indicated

				grows on glucose	plant on agar	
				b i	anti b	i
9	NoTorS	(o)	→	no swarms		
10	Many T+ S	(b sp.)		+	−	++
11	"	(b transd.)		+	−	++
12	"	(i transd.)		+	no swarm on i agar	−
22	"	(i)		+	" " "	++ ++

why PLT22 × 666 different on i agar than FA12 × ? (test further for full sample)

(over)

Pick swarms to broth. Streak out on EMB Gal to get joint transductants. all Gal−.

Plant on i and b agar to screen for diverse types

After 48 hours, second phases appear i 10 and 11.

22: 5/7 i
2/7 b

Do not bother

B.

1	FA12 + SW609	grow together on agar; plant on b motility agar.		many buds
2	+ SW618	b	6 buds/plate → 4 nmb, nai	5 tested → all nai b, i
3	+ SW673	b	many buds → i. Streak out on SW682	
4	FA9 + SW623	i-12	ca 10/7: no buds; 10/10: 2?	not b or i
5	FA9 + SW435	i-12	no buds ... solid	
6	FA10 + SW623	i-12	no buds; 0	
7	FA10 + SW435	i-12	no buds ... 0	
8	FA11 + SW623.	i-12	no buds; 0	
9	FA11 + SW435	i-12	no buds ... 10/10: solid growth	

Repeat series using larger amts. of FA.

i-12 always negative

Repeat 12.

10/5 4+ swarms from 2 addnl. plates
to i, 12. no swarm.

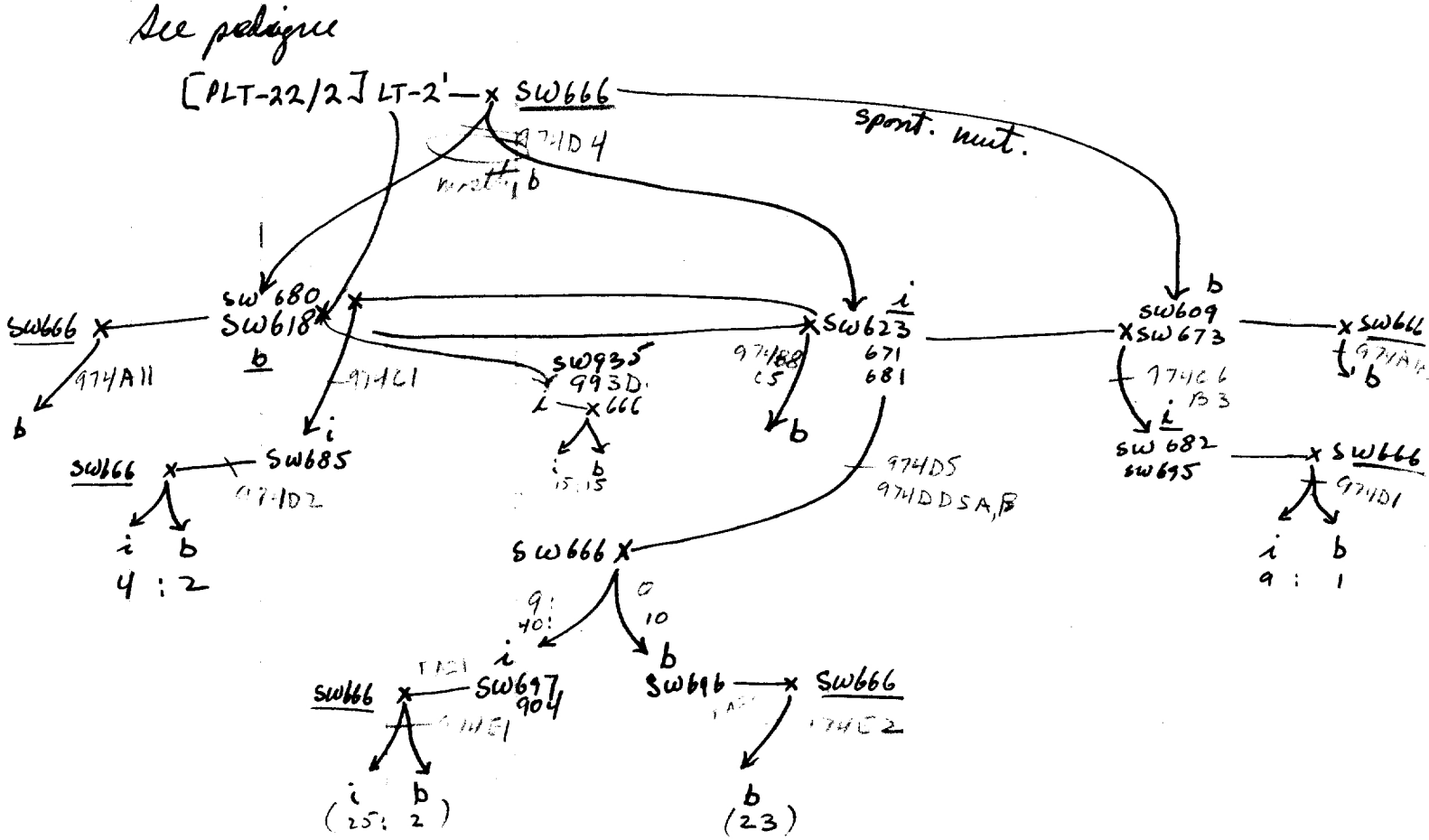
10/7 4 addnl. plates: resol buds. 6 to i, 12
2 showed slight movement (moisture mvmt?):
both are b
others remained stationary.

10/8 18 addnl swarms from 2 large plates (including pooled swarms)
all stationary as i' ago.

Total 30 swarms: all i.
?

Progeny tests on SW666 x — i

974



Note: 974DD5B

① seems to show purity of individual screams, but controls may be indecisive.

② In three cases tested, failed to isolate O-forms from initial flare. Treated themselves not tested. Established growth seems to be inhibitory to remoculation. Mayard both methods: see 680

974/B C

	FA	SW		Plate in	
1	12	609	$A^i \rightarrow H + A^b$?	b	no buds. of B3
2	12	618	$A^i \rightarrow H + A^b$	b → c	✓ pur. SW 685
3	22	618	$A^i \rightarrow H + A^b$	b → i	SW 686.
4	11	435	$A^b \rightarrow H + A^i$	i	no buds! → 1,2 swarmed → i ✓ isolated colony i
5	11	623	$A^b \rightarrow H - A^i$	i	no buds! → 1,2 swarmed → i ✓ isolated colony i
Repeat 6	12	609		b → i	SW 695
7	22	609		b	no buds i.e. "swarm" is still i
8					
9					

c5. 10 buds 9/10 b 1/10 still i or mixed.
natural.

974/C 3. Repeat 11/21/52. Use relatively light bacterial inoculum: this seems to work much better. Numerous swarms (not well separated when finally seen). Includes 2-3 flares. Pick these as possibly representing segregation of b.

c3A Flares (fundamm contam. by blocked b not certain.) Grow in Pendassay, test probably and select on i agar. : all i

c3B Distinct swarms for any Lp^s i: 16 - all i., Lp^s Prepare FA. (#10, #15 may be Lp+) See 993.

4/5 blocked i 1/5 → b. (possibility of contamination). In further experiments use added R cells as a contamination marker. (e.g. 666R).

after pul. test save #1, #4 as SW9

FA from two-step transduction 974D

10/13/52

1. FA16 (SW682) + SW666. only 2 swarms → both i
2. PA (SW685) " plant on i, b agar
3. " (C8C) no "Tor S!" "
4. " PLT22 " many swarms: pool and streak out
5. FA 12 (623 = trans di) " individual swarms pulled out

1 Repeat: mixed swarms - no motility on i-agar.
test pool growth: (2) a no agglut! → b, i± as results.
b: reisted b, (i ± ?)

2. " as above (2): do.

i and b!

although no swarm on i.
Perhaps medium n.g.
Would acct. for failure of
C4-5.

✓ Immotile as before on old (i, 12)

5 i
i
b
b
i
b
i
i
i
i
i
i
i
b
i
i
i
i
b

12 i
5 b

4

This experiment was recalled to be confused, and these results bear it out! From previous experience, 4 should give a mixture of b and i, 5 i only.

REPEAT 10/17.

This is actually mixed b, i

4 b+i?) pool was streaked out. Test colonies: 14/14 b
7/7 b pool rx b, not i
7/7 b test rx b agar

migrated on b agar

D1 separate T+S 7i 1b

D2 1 plate T+S pool - to both: reacts i ++ b + . Colony tests from strain: 4i : 2b

D3 No T or S med new PA

D5 ~~both~~ individual swarms: i agar b agar

	b and i	Total
b swarmed.	8	19
i swarmed.		

pervasive difficulty not reproduced.

D2 affirms F-2 progeny test of limited transduction

974DD

10/21: Resolate SW623 single colony (b) and prepare FA (FA9 + ...)
FA(SW623) + SW666 → many T+S. (very well defined on large agar plates)

5A Pick reasonably individ. swarms + retest b or i:
10: i 1: b (weak, maybe b + i) → purify and retest

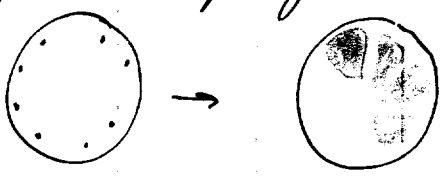
5B. FA12B → SW666 on motility agar, well separated swarms and tracks.
Count: 14 lateral swarms (mostly i tracklets) 46 tracks. In many cases, the tracks flare out into swarms. Pick well isolated swarms (may overgrow) for later selection test. Pick flecks + streak out to reisolate presumed O component.

at least 2-3 mm. from streak "A" from SW686 (2 batches) – no T or S. (check sensitivity to PLT-22) see over.
SW66 controls No T or S.

→ Spot on i (or b) agar to test purity 5/5 i immobilized.
6 add tests spoiled by bad agar, but spirals. Also single b stopped around and retest → all are immobilized. ∴ each swarm was pure.
To check, spot i on b plate and v.v. 11/16 swarms. 5/i grow spont. (plate dried out?)
Repeat test by serial inoculum.

6. FA18 (PLT22 / LT2 phase II 1, 2) + SW666.
2/2 b.
16/16 b.
Total 18/18 all b
no 1/2 ?!

Spot 16 on b agar: no swarms. typical slow spr. growth, some mutual inhibition.



Conclude: these are monophasic b identical with SW618.

(over)

see 979

5B: 3 flares streaked out.
15 colonies tested from each: all i no O forms!
(45 tests!)

Perhaps the flares are not actually O microcolonies!

---

12 isolated buds tested: (made from rear source)
11 → i
1 → b (#12)

(as over) Spot on NSA., transfer to homologous serum agar.
all uniciliged. ∴ each swarm is pure

This agrees with 15/15 individual tests on each of 3 swarms.

Note 11/8. SW688 (Boyd 1/rough) gives very coarse flares on motility agar, prior to later "smooth" swarm. Reisolate and compare morphology, swarms & original unselected.
This may be the basics of flares. see 978

progeny tests on SW666x — i, b

974E

FA21 → SW666 1st test: agar too sloppy 2d test activity?
Repeat preps: try FA9, LT7.
new FA9 prep. apparently lysed but activity still poor.

---

new PLT7 prep. inactive — v. few Trailers. 2? swarmers.
but might be carryover?

Test other hybrid i's for assoc. to PLT22. cf. 697 which is app. resistant
e.g. 974DD5b: 1–11 and SW697. All show no lysis on plate (but hybrid?)
with FA9 except #6. = SW904. However in both 697 does seem to be inhibited by FA9!

FA21A (904) → SW666

27 swarms. 25 : i 2 : b → 1^R / 1^S

Note: in 974DD5b of 11 i 10 are LP22^R 1^S / 4^S | 31 R : 5 S
were of 25 28

2 FA20 → activity? / a number of T+ swarms developed later. Maybe contaminants: quasi-poxlysin NSA
as above. 1 swarm → b.
new prep (FA9) → T+ swarms fairly separable.
22 → all 6 (not i) ∴ 23 total
Test on FA9: 1^S 21^R of 986

Serum effect on transmotilityation.

~~974~~ 975

October 4, 1952.

Many fewer swarms occur in antigen substitution experiments (e.g. typhi x typhimurium) than in "transmotilizations" (eg. SW543 x typhimur), and it seems unlikely that this is due to a lower frequency of occurrence. The SW543 transductions allow a comparison of the techniques.

10/3. Plant D22 (SW-666 + FA 14722/2) on motility agar ± b, i 1,2.

No serum: many tracks and swarms (ca. 60 tracks, diff. swarm)

i: 1 bud } A4: transplant — to ~~E~~ i

b no buds, no tracks. }

Could this effect be due to O-antibodies? Absorbed sera should be used.

Also of. 971D.

D3: 19 ~~buds~~ tracks. many swarms D3/b: no buds, swarms or tracks.

Repeat P4, D3/-, /b, /i (to test specificity of i effect)

A5: D3/- swarm; ca 15 tracks

D3/i 1 sw ca 12 tracks.

D3/b No tracks or swarms.

D3/d T+S as above.

In this case, b is specific of 974

$D3_0$/b 1 swarm. See D3. Nonagglutinable.

NOTE. Nonagglutinable phases might come either from FA or alternate phases in SW603. Occasional buds appear in each plate of SW(666)(H+)/b, e.g. SW673. → nonagglutinable by b, i, 1.2, enx ... Furthermore colony/b (occurs very readily) = enx shows very weak reaction with our enx serum!