Streptomycin and streptothricin

March 27, 1952.

Test (cross-bunch) Sm^R and Sm^S strains vs. old streptothricin 10^4/ml. W1922, W1607, W1177, 58-161, W677 all Sth^S
Lack showed a more ~~gradual~~ abrupt cutoff in streaks. Cf. previous observations of SM-STH cross-resistance != These concerned cross-resistance at low levels only! ($S_{u\,sm} \doteq 10u$ sth).

3/29. Selections for Sth^R:
W1678, W1177 ca. 10^9 ea + 10,000 u STH. No survivors

4/20 ff. Add 10 ml grown culture 58-161 to 100 ml Penassay + 50 u/ml STH. After 4 days streak and test survivors.
all tested were STH^R SM^S. Picks 1 as W1969. When cross-bunched against lawnful of 1000 u/ml, show slight inhibition, whereas 58-161 is completely inhibited. Sth^R may differ from S^R in step-resistance.

Trial run: λ adsorption assay with millipore filter

8/13 ± 152

λ (1439 Iwoffate) diluted to nominal 2×10^6/ml.
W1655 young aer culture in NSB, nominally 2×10^9/ml.

Mix in equal volumes 5+5 $4^{50} - 5^{15}$ 37° Incub. (no aer.)

(A) Assay initial phage ~~(adsorbed to unadsorbed)~~ Dilute 1:100 and filter (uncorrected for 1:1 dilution)
with W1655. (132, 181) $= 1.6 \times 10^6$

(B) Assay filtrate .1 543 .01 42 .53 ~~.543~~ $\times 10^4$

(C) Assay residue λ and bacteria (173 .1) (25 .01) Bacteria: $.143 \times 10^7 = \frac{14}{96}$ %
λ: $.173 \times 10^4$

(D) Assay diluted mixture. .1 >1000

λ	165, 162	1.64×10^4	Expt completed 5:38
Bacteria		$.96 \times 10^7$	

Initial λ ≐ $1.6 \times 10^6 \times \frac{1}{2} \times \frac{1}{100} = .8 \times 10^4$
λ in mixture = 1.6×10^4
Residue λ = $.17 \times 10^4 \times \frac{96}{14} = 1.16 \times 10^4$
Filtrate λ $.54 \times 10^4$

Low e.o.p for λ, measured by uninfected cells?

March 28, 1952.

~~Confirmation~~ Test of Mal- elimination in Hfr crosses.

Streak out cross-mixture on EMS lac. Scoring difficult – ? ca 10% Mal+ 30% Lac+

Pick small lac+, look for lac v

3/31/52. EMB Medium rather poor: characterization doubtful. 5 ? / 24. Restreak

A. Recheck from EMS lac. #1-5

Repeat 40 addtl. test from cross plate 1? #6

		lac	Mal
1		v	—
2		v	—
3		v	—
4		v ?	—
5	X	—	—
6		+	+

~~Save + ↓~~ → T.O. 6/53.

Note 6/52. Unless these are homozygous, which is unlikely, elimination likewise occurs in Hfr x Het. The important point is not readily tested here, namely whether markers such as Xyl or Mtl + in W1177 would be heterozygous. Selection for prototrophy invalidates search for TL, M heterozygosity which would be nearly as useful! Similar cross should be conducted on EMB lac sen.

March 30, 1952

Lac+ S^R had been noted repeatedly in mixtures of W1895 and W1607, etc. These might represent recombinants.
Grow W1895, W1177, W1607 overnight in broth. Mix 1 ml each + 10 ml Pennassay. Incubate 11^{30} AM – 4 PM. Streak out on EMB lac sm.

are sm-inactivated Hfr cells participating?

1895 no colonies
1607 all –
1177

1895 + W1177 ca 1–2% Lac+ S^R some apparently sectored
1895 + W1607 " " " , but Lac+ very weak (Gal – ?)
R → Lac+ Mal Xyl – S^R Aux.

3/31. 1:5 each 12 N.
A. 2 PM EMBlac: clumping not obvious. EMBlac sm 2+ 37–
well cool. streak out.
B 5 PM. EMBMal sm. 1 Mal+ ca 500 – (streak)
EMBlac clumping?} streak → R EMBlac sm
~~some rather small Lac+ or~~ Lac +/– (ca 2–3%) Mal sm
See below

~~#1~~

1 pure +
1 Lac – (+?)

4/3/52 Restreak as 928A

(delight in saline)
4/1, C. As above. 2^{45} PM – 4 PM
D. Grow separately, then plate together

1895 fails to selfagglutinate

D: 4 x 200 EMBLac sm → No Lac+ S^R EMBLac: ca = mixture
numerous overlapping colonies
C.1 EMBLac [redacted] about like D, but ca 1% small sectored colonies.
2 EMBLac+ sm:

Lac+	Lac+/–	Lac –
1	5	78
2	3	92
2	3	63
3	6	116
1	5	82
2 ?	8	113
11	30	546 / 587

ca 2% Lac+
6% Lac+/–

E. Washed cells — to Penassay +

F. Mixed in saline (dense ca 10^9/ml each) 37° —

G " " " 4° —

H as C. 2^{30} PM – 4^{15} ++

I " aerated (to stir!) ++

J 1177 cells + Hfr supernatant. —

streak out EMB lac sm.

K. See 929. 4/4.

Pool data of 929-1 and K:

929-1.	Lac+,+/-	—
#1-11	7	84

K:	+	+/-	—
EMBlac sm	0	1	51
	1	3	63
#12-25.	1	12+1	64
	2	5	48
EMBlac TI	0	0	60
	0	3	45

see over.

3: lac+, lac– RR. ← #26-28.

EMBlac. 67 56

KK. EMBlac see also 931B. 15 Total.

1-12 streaked 4/plate. 13-15 1/plate. a) pick colonies 1-4. b) replica all original plates.

diagnosis is V_1 ; S

	Lac+	Lac–
1	SR	RR
2	RR	RR
3	SR	RR
4	SR	RR
5	SR	RR
6	RR	RR
7	RR	RR
8	RR	RR

	Lac+	Lac–	
9	SS ; RR	RR	no SS seen
10	RR	RR	(SR) — a few
11	SS ; RR	RR	
~~12~~	~~pure lac+ SS.~~		No recomb.
14	SR	RR	
13	RR	RR	
12	SS	RR	Recomb?

See 928 CC

Exp. Time seg; Time by dilution in broth, & time of disappearance of lacV re lac+.

K: Lac+ and − ... all are Mal−, Xyl−.
(3 are V_1^R selectants)

Among Lac+, $10 V_1^S / 17 V_1^R$

Lac−, $1 V_1^S / 19 V_1^R$ (coupled with Lac+ V_1^S).

a) Keep this pair for test (#32)

b) Test all for prototrophy (B, agar).

3/-8 Lac+ } grew on B, agar. both are B_1 −.
2-10 Lac+ }

∴ 2 B_1 − +++ of 47 recombinants (ca ½ Lac+ S^R).

3.2 Lac+ $V_1^S S^R$ Lac− $V_1^S S^R$ sci

both are T− B_1 − L+

In most tests, T− and L− are not distinguished (on replica plates), but only on recheck in tubes. L+ V_1^S may be associated.

4/2/52

C The pure lac+ S^R are especially significant as they exclude the possibility that residual Hfr cells fuselage W1177 microcolonies on the agars (after agglutination and clumping).

C2 - pure lac+ Restreak: 6 ✓ lac+ pure. Pick for Mal, V, test.
C3 - sectored lac+/-

C1. 5: Repl to EMB lac, Incom for suit. lac/S mean.

4/4/52 C2-3. (lac+) all Mal-. C2: $3V,^R$ $3V,^S$

C3: $7V,^R$ 5 S
+5 +5
13^R 13^S

C1 in each are, isolated lac+ S^R identified, fewer no lac+ S^S! ∴ the small sectored colonies presumably are related to Hfr recombination.

928 A) Repicks 8 lac v? colonies:

1	−, v,	+
2	+, −,	v
3	+, −	(v?)
4	−	
5	+ −	v
6	+ −	v
7	+ −	v
8	+ −	v

Repeat lac V. Restreak EMB Lac, Mal, Lac sm; EMS
V − V Xyl− Gal v Auxotrophic

C3 Nutritional tests: agar. all these are Mal Xyl Gal− Tubes:

	V,	BB, TL	B B, M	O		
11	R	+	−			
12	S	+	−			
13	S	+	−			
14	S	−	−	MTL−?		✓ M−T−
15	R	+	−			
16	R	+	−			
17	R	+	−			
18	S	−	−	MTL−?		✓ M−T−L−
19	S	+	+	±[MTL+	I. Probably B− or B_1−	✓ B_1−
20	R	+	−			
21	R	+	−			
22	R	+	−			

Test associated lac− or V_1!

Summary sheets. 928 CC

Re-organize C3 tests lac+ and lac- components — all Mal + | + and -

C3

EMBlac-m

#	Lac+ V_1	Lac- V_1		M Nat.	Reg.	TL
1	S	R		+		–
2	S	R		+		–
3	R	R		+		–
4	R	R		+		–
5	R	R		+		–
6	R	R		+		–
7	S	R		+		–
8	R	R		+		–
9	S	R		+		–
10	S	R		+		–
11	R	S	+	+	–	–
12	S	X	+	X	–	X
13	S	X	+	X	–	X
14	S	R	–	+	–	–
15	R	R	+	+	–	–
16	R	R	+	+	–	–
17	R	R	+	+	–	–
18	S	R	–	–	–	–
19	S	R	+	+	+	–
20	R	S	+	+	~~+~~ –	–
21	R	R	+	+	–	–
22	R	R	+	+	–	–

MTL –

MTL – ; MTL –
Lac+ B_1 –

KK

lac^S
EMBlac

#	Lac +	Lac –	V_1	V_1	S	S	TLB_1 (growth)		BM		B_1 not +
1	+	–	S	R	R	R	+	+	–	–	
2	+	–	R	↓	↓	↓	+	+	–	–	
3	+	–	S				+	+	–	–	
4	+	–	S				–	+	+	–	
5	+	–	S				–	+	+	–	
6	+	–	R				+	+	–	–	
7	+	–	R				+	+	–	–	
8	+	–	R				+	+	–	–	
9	+	–	S		S		–	+	+	–	
10	+	–	R		R		+	+	–	–	
11	+	–	S		S		–	+	+	–	
12	+	–	S		S		–	+	+	–	Cross?
13	+	–	S		R		+	+	–	–	
14	+	–	R		R		+	+	–	–	
9a	+		R		R		+		–		
10a		–	[illegible]	S		R		+		–	
11a	+		R		R		+		–		

Components tend to be all TL- and concordance MTL-. Reminiscent of prototroph tumors. But B_1, not concordant.

Mal, S concordant

See also 931E

#4,5 are exc. BM- Lac+ V_1^S S^R (scoring errors)

#9,11,12 were Hfr type, + usual pair   12 Cross?

#10 had ① usual pair V_1^R ; ② TL- lac- V_1^S S^R ∴ W1177 + 2 Recombinants Lac+ V_1^R lac- V_1^S = 2 zygotes?

∴ 3 TL-^{R} types: Lac- V_1^R Hfr missing

KK: ~~Most sectors are~~ lac+ V_1^R S^R.
lac-

of 14 seg. colonies, 3 showed lac+ S^S / lac- S^R, and may not have been recombinants.

The remaining 11, the lac- component (1 part. exception) was V_1^R S^R. The lac + was also S^R, 5 V_1^S
6 V_1^R.

Sample type colonies from each sector ~~fed~~ for further test.

Hfr combinations.

4/3/52. 1ml each 1/5 Penassay: 2[10] PM - 4[05]

			lac/S recomb.	
1.	W1895 -	W1177 -	+	> 2% No photographs with 100x !
2.	W1895 -	W1607 -	+ or ± (weak lac+)	
3.	W1895 -	W1876 -	±	(≤ 1%)
4.	W1678 -	W1876	—	
~~5.~~	~~W1802 -~~	~~1876 -~~	—	
6.	W1922 -	W677	—	~~22 lac~~ S[R] - Rebuke No.
~~7~~	W1678 -	W1177 -	—	
~~8.~~				

8. W1895+ W1177. Conc. ca 10[10]/ml each fresh Penassay 3[00] PM - 5[25] PM.
0/80 lac -. (Numerous + on EMB lac)

4/4 : W1177 + W1895. overnight.

~~1.5 lac + 4ml~~ 1ml ea + 5ml Penassay 3PM. A
W1177 control. 4:50 B
W1895 " C
Microscopic clumping in C and A.

SM Crosses and F+ gradient.

March 30, 1952.

W1922 = W1895 S^R. Hfr status?
1903 = ~~1895~~ S^R
1678

A)

			D(o)	D(sm)
1	W1895	x W1903	+++	++
2	W1922	x W1678	ca 50	12
3	W1922	x W1876	+++ Hfr? R → ~~[redacted]~~. ~~Lac → Lac+~~ Mal- → Mal+	

closer comparison with lower grades needed. Note that both A1 and A2 are reasonably fertile. S^R segregation here? Does it mean that W1678/sm can act as F+ to W1922 (Hfr?) Or is W1922 no longer high level F+?

B)

			D(sm)	D(o)	Mal	Lac	D(sm)
					D(o) R →		
1	W1895.	1177	~~+++~~	++++	→	·	
2	"	1876	+++	++++	→	→	
3	"	~~1678~~ 1903	+	++			R > S
4	W1922	677	—	++++	·	·	
5		1896	1 col. —	++++	·	·	
6		1678	+	++			· S? > R
7	Hfr ket ✓	1876	/	+++	·	-7+ · -2+	
8	1903	1896	1 col? —	—			
9	"	677	—	++	← ca 2% +	→	
10	"	1802	—	+++			· S > ca 10% R
11	1678	1177	+	+±	·	·	
12		1876	2	13	→	→	
13		1607	~~++++++~~	~~++++~~		X	
14		1875	~~+~~	++, ++		← →	· R>

mor dupl. D(o) D(sm)

all -/+ ratios agree with F gradient. Note Hfr x 1678 less than maximal yield! Does 1678 transduce F+?

Preliminary tests on allelism using 1 diauxotrophs.

3/31/52

EMS Lac

	× W1177 Yield	lac	W1876 Y	L	Cond.
W1765	—		—		background only! λ?
1688	+++	20%+	++	20%−	F < 1876.
1920	++	5%+	±	5−	$F_{1920} \geq 1876$?

Test vs. 1607, etc.

Hfr crosses
W1895 – W1177

April 5, 1952.

A. 1½ directly from gram broth ; no fresh broth 5:10 PM.
B. 1/10 each to " "
C. .01/ml each " " (thermostat is ?)
D Grow together overnight.

B: 20 minute intervals: 0, 20, 40, 60. (ca 2x growth assumed)
Plate at 10^{-6}, 10^{-7} of original cells (~~10^{-5} 10^{-6}~~) (= 10^{-5}, % of susp)

A: EMB lac sm

		−	+−	+
•	(6)	297	7	
	(7)	38	0	
		335	7	

Ca 2%

C: " " 16) 2x 757 ~~3?~~ 4/v ca 1/500. This speaks for non-random contact.

~~B~~ 0 M. 0+ / 30, >1000.
20 M 0, / 34 ; all appear. +/ 12/ca 1000
40 M 1+/139; ~~18/ca 1000~~ mostly +/-. 18/ca 1000
60 M. 7: 0/36 7: 0/32
1v 133
sm. 1v 139 T1 6: 3/322
2/108
6 10 9 13 } − 1000
32.

Use 330 as est'n for other plates at 10^{-7}

∴ ca 3%

Direct mixtures may do nearly as well as moving into fresh broth.

B: pick lac+ colonies on EMB lac. Post with 928 KK.

Hfr crosses 931E

April 6, 1952

E. W1895 + W1177 ca. 50 minutes

18 plates ca 60/plate EMB lac sm 7 lac+ or +/− (1+ others +/−)

16 plates EMB lac. Picks Lac "v". Also

replica to EMB sm; T1 to check on frequency of lac+ S^R, V_1^R. +R normally at junctions of colonies, or at previously picked lacs.

---

13 lacs from EMB lac. On basis of 928K, pick only 1+, 1− from each and check through.

also, plate at 10x, 100x D(0); D(B,).

D(0): 1 100x

D(B,): 25 100x (about half are v. small)

	Lac	Mal		S		V_1		Nutr Require.	
1	+	−	−	R	R	R	R	TLB,	HTL ←
2	+	−	−	R	R	R	R	TLB,	TLB,
3	+	−	+	S	R	S	R	BM	TLB,
4	+	−	−	R	R	R	R	TLB,	TLB,
5	+	−	−	R	R	R	R	TLB,	TLB,
6	+	−	−	R	R	S	R	TLB,	TLB,
7	+	−	−	R	R	R	R	TLB,	TLB,
11	+	−	+	−	S	R	S	R	BM	TLB,
12	+	−	+	−	S	R	S	R	BM	"
13	+	−	+	−	S	R	S	R	BM	"
14	+	−	+	−	R	R	R	R	TLB,	"
15	+	−	−	−	R	R	S	R	TLB,	"
16	+	−	−	−	R	R	R	R	TLB,	"

E

Cross?

} Cross?

Only 2 colonies tested per ~~plate~~ plating

See also 928CC

April 7, 1952

2^15 PM – 4^50

A. Grow W1177 overnight in TZ broth. + W1895 1 hour 1:10 each.

B. Control 1177/1895

C. W677/1922 Also T1 EMB lac.

A) [To test crossability of W1177 TZ). EMB lac sm:

Very high yield!

	lac+/–	–
xc!	5	48
	4	24
	4	32
	13	104

B 3 10.6

prob. underestimate

est. 40 500

C) EMB lac sm. 60. all lac+ 14 339

EMB lac T1 Pick and streak out lac s ~~etc~~! (~~This is mistake~~) (also

20 lac s pairs: lac+ and –: all V1^R ✓ S^s Xyl–.

5 lac+ " " ".

W1922 is clearly still Hfr.

Therefore lac/T1 selection still leaves only the Mal– segregation

? Are Mal+ recombinants present many fam? – Mal vs T1; sm; BM/TLB1 are possible. See D.

D. 4/9/52. = A 5:20 – 8:30 PM. Plate on • EMB Mal, EMB Mal sm ...

E " x ~~[illegible]~~ 1611 EMB lac sm

F " x 1590 EMB lac± sm

4/10: D– ca 40 lac– per plate

	plates	# rec.
EMB Mal sm	3	0
" Mal T1	3	0
EMB M+Il sm	3	0
EMB lac sm	3	7
EMB Mal	2	0 (no Mal ✓)

E ca 600 cols. No lac+ S^R

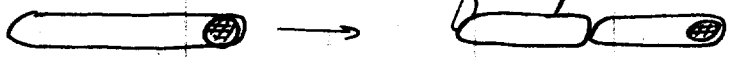
F ca 700 EMB lac sm No ". EMB lac: 8 lac?/ca 1000 lac+, –

Why? Restreak. None lac ✓ Gave a few lac+ S^R in streaks

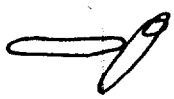
Observations on living cells.

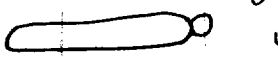
April 6-10, 1952.

A. Use of tetrazolium as tag for parental type. Cells grown in .005% TZ / Penassay broth. W1177. over 95% of cells have 1, usually polar, TZ granule. This does not segregate (observ. on agar - coverglass mounts) PM 4/9. Several cell divisions were of the form:



This does not necessarily mean that the "mitochondrium" itself is genetically discontinuous.

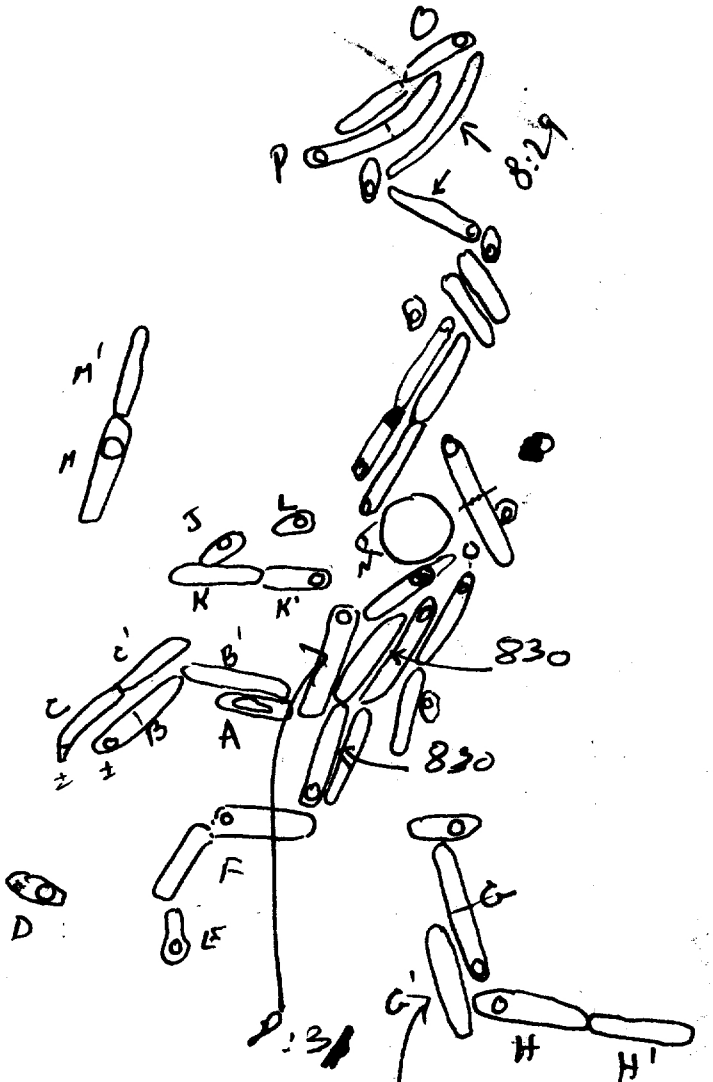
B. In mixtures of W1895 + W1177 numerous pairs have been found  and  These were firmly attached, and in a few cases arose by conjugation, not fission.

C. Interesting cells  with small bud frequently noted.

108.9
51.8

W1177 TT

5:20



P 8:10
F 8:05
B 8:15
G 8:15

April 11, 1952

EML Irradiated W1895 UV 8 sec. EMB Lac. Obtained ~5 lac-
12 plates < 4-8 sec / 8-7 sec. 200-400/plate. Streak out EMB Lac ++

	A 1?	✓	= W1940	most unstable
	B 2?	✓	1941	
(1)	C 1?	✓	1942	
	D No- noted		1943	slow + at R.T.
x1922	E No lac- noted		1944	" " "

Incr A-E + W1922 in Penassay 10 AM. Brief streak-out tests.

(2)	A	- ✓
	B	- ✓
	C	none noted
x W1894.	D	- ✓
	E	?

Use W1941 for further experiments on self-crosses. lac- bacs?

Re 937.

In allelism tests x W677, EML recorded lac+ recombinants in 1945, 6, 7; 1951. 1940-44 not yet tested.

Recheck ~~1948~~ 1941. Use 1948 in further test.

6/25/52. W1941 x W1956 gave few or no lac+ recombinants. W1940, 41, 43, 44 gave lac+. Recheck 1941, 1948. Use others to search for lac+ balanced diploid vs. lac, -., esp. W1940

~~Hoofstaandeling~~ W1941 was used in some subsegment Hfr (X) expts., but its lac, allelism should be further verified.

April 13, 1952.

- A. W1895 + W1177 (overnight no Reassay). Ail + plate on EMB Mal
- B. " " EMB Mal T1 B' = 10 x B" = 100 x [= 10^{-5}].
- C. " " EMS Mal TLB$_1$. 4 plates. all –.
- D. " " EMB Lac sm. (for "control"). and EMB Lac = H.
- E " " together 1 hour. EMB Lac sm (for double cross over test).
- F " " " " D(B$_1$) for mapping: 100 x
- G " " overnight. ... EMS Mal SM (BM).

→ G' 1 G" 3+2. streak out EMB Lac. 4 Lac – 1 Lac +

A. 5 plates ca 100 colonies. Pick all colonies that might conceivably be Mal s or Mal +^{s}.

1 + small +^{s}? all +
2 ◐ prob comic. {EMB(0) Repl. + –
3 tiny ± all +
4 4 like 1 n.g.
5 ● but near +. Mal +; Mal –
6 like 1 +
7 ◐ darker + lighter; not – + dark + light
8 med. size, background mottled? +
9 " " " near –. +
10 ● prob comic. + –
11 like 1 +
12 " +
13 like 8 or 5. +

	Mal		Lac	
1	–	+	–	+
2	–	+	–	+
3	+	+	+	+
4	+	+	+	+
5	–	+	–	+

No evidence of Mal, Lac Recom.

Test on S, V$_1$, Natr. (parental)

B. ca 30 per plate 5 plates. all Mal –.

B' 200-300? 1 Mal + ◐ → all Mal – (uninfected col.?)
B" crowded 1000? None observed.

		Lac –	+	+/–
D.		31	1	0
		42	1	3
	D'	332	6	12

E. 9 plates. Count 1: 109 4 3.

Total 10 plates: 1400 19 ~~52~~ 1~~[illegible]~~. 61

Picks "pure +" 17 total. But only 8 give only + colonies on EMB. Very rare – or others. 1 Lac v? #10 → +, – and mostly v Mal – Xyl – Arex.

See over.

H. Somewhat crowded for separation of Lac s.
(over)

935 E. (lac+ from EMB Lac sm) all are Mal −.
Repl. to EMB Mal T1

"Pure+":

1. S
2. Pure R
3. Pure R
4. "
5. R
6. "
7. "
8. "
9. "
10. R

Note preponderance of Lac+ V_1^R in these colonies.

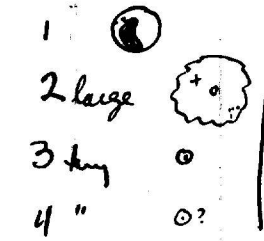
No evidence of $\frac{\text{lac+ } V_1^R}{\text{lac+ } V_1^S}$

Apparent excess of Lac+ V_1^R may be due to selective advantage of certain types.

#11 Pure lac− V_1^R lac+ V_1^S.

F. Picks to EMS Lac B_1:

Lac−	+	+ and −
9	8	1
5	10	2
7	11	2
11	8	1
8	10	1
5	10	2
45	57	9



H	EMB ~~Eos~~ Lac	Lac	Mal	V_1	~~Mal~~	
1	+, −	+	−	+	− S	R −
2 large	+ rare - maccessible	+		+	S	/
3 tiny	+ −	+	−	−	− R	R •
4 "	+ −	+	−	−	− S	R ×
5	+ only (rare, macc −)	+		+	S	/
6 "	+ −	+	−	+	− S	R −
7	+ - rare.	+		+	S	/
8	+ −	+	−	+	− S	R −
9	all +	+		+	S	/
10 "	all + 1 −	+	++	+	S	/
11	+ −	+	−	−	− R	R •
12	+ − (scarce)	+		+	S	−
13	+ −	+	−	+	− S	−
14	+ −	+	−	+	− S	−
15	+ −	+	−	+	− S	−
16	+ −	−		−	R	•
17	+ −	−		−	R	•
18	all +	+		+	S	−
19 tiny	+ −	−		−	R	•
20	+ infr. −	+		−	S	−
21	+ −	+	−	+	− S	R −
22	+	+		+	S	/
23 small	+ −	+	−	−	− R	R •
24	+ rare −	+		+	S	/
25 ?	+ − (infreq)	+	−	+	− S	R −

~~Mal~~ 18 complete pairs (Lac+, −). ∴ only small colonies are likely.

Recom. { 6 are Lac+ Mal− V_1^R / Lac− Mal− V_1^R
1 Lac+ Mal− V_1^S / Lac− Mal− V_1^R

Par { 11 Lac+ V_1^S Mal+ / Lac− ~~Mal~~ V_1^R Mal−.

Incomplete pairs: 7 − parental. Recomb other markers?

April 14, 1952.

Mixed 2:30 – 6:00 PM

J. W1941 × W1922. Plate on EMB lac; EMB lac sm for lac s; lac – s^R.

K. W1922 × W677. Plate on EMB lac sm T1 Plate 10× or (') 100×

L. " " Plate on EMS lac SM TLB1.

M W1895 × W1876. Plate EMB lac; sm; T1 10×

N.

J. ca 150 per plate EMB lac sm: 5 plates

No lac – s^R.

EMB lac ① plates. ca 1/500 l. 4? lac ✓ → lac+ only on EMB sm.

#5: + and –. lac – s^R col. noted. mostly lac+ s^R and lac – s^s 2 lac+ s^s; 1? lac – s^R found. → owing to proximity these might have been secondary recombinants

#5 ⑤ #6? all +. This ⑥ n.g.

K. 5 plates. No recomb.

M. EMB lac 5 × ca 150/plate. No lac s

N. T1. 5 plates → 3? lac s. ✓ → Lac+, –. Repl → EMB lac Mal sm

1 EMB lac sm. → 0.

L. 1 twin colony: 5 plates

all 3 give lac+/lac– & Mal-s^R

~~Hfr × F⁻~~ Mal / T1

~~957~~ 936 /

April 23, 1952

W1957 × W1702 (Overnight growth).
Hfr V_1^R Lac-Mal-S^R

① EMB Lac ssss

② " + T1 100X

3 plates.

Phenotypic delay?

Factors Lac- 7 200

~~+ lac + (Gal)~~

Replica to EMB Lac ~~+~~ T1. after picking lac+, test G.

G tested. – all V_1^S

Replica → no intact V_1^R

3 isolated new colonies, probably mutants

Rechecks W1957 / T1. – as recorded this is $V_{1a}^{\ll r}$, unlike V_1^R!

Hfr ⊗ 937

April 17, 1952

A. ~~W1895~~ × W1955 × W1922 EMB Lac

Lac- V_1^R Lac+ $V_1^S S^R$

B. " " EMB Lac T1

B. 7 plates ca 250/plate No Lac+ V_1^R !

A 5 plates. 6 ?? Lac s. Mostly ⊗ types.

Replica streak plates to ~~[illegible]~~, EMB Lac ± sm.

	Lac-	Lac+
1	S	R
2	S	R
3	S	R
4	S	R
5	S	R
6	S	R

= Only possible Lac/s Recomb. ✓ V_1.

937 A 5.

	Lac	S	V_1	
1	—	R	R	
2	+	R	S	PAR.
3	—	S	S	

Other parent? Should have tested more colonies!

Check these for Hfr.

all 3 were Hfr (× W1956!) Save ① for further use in crosses. W1970.

C 4/28. W1970 × W1895 plate EMB Lac ± sm. (Appropriate Lac- ?)

L sm. 4 × 150 = 600. 3 Lac v.

C' s sm 2 < 300 2 Lac s

Hfr x F+

April 22, 1952

E. W1922 x W1896 (3PM – 8PM). Plate on EMBlac ±sm
lac..+ S^R
and D(8) → no colonies!

low density plates. EMBlac: ca 60/plate

6 EMBlac sm. No lac – S^R
6 EMBlac 1 ?? lacS. As said.

Repeat 4/25. (overnight)
4 x 150 plates. No lac – S^R

These crosses apparently much less fertile than Hfr x F –.
Return to unselected crosses W1895 x ~~W1915~~ W1876 for further study.

April 17, 1952

C W1895 × W1876 EMB lac sm $10^{30} - 6^{00}$ 4 plates ca 250 lac⁻/plate 2 lac±.

D " " EMB lac 7 plates 500/plate but ½ smeared! 6? lac±.

Rate of recombination much less than W1177 experiment.

4/18/52. $1^{35} - 5^{35}$ ~~EMB lac sm.~~

E W1895 × 1177

F. " 1876.

	E: EMB lac sm	+	+/−	−	F:	+	+/−	−
		10	16	443		1	0	1020.
		5	7			0	2	
		4	3	—		0	1	Abandon.

Hfr × F+ is much less fertile than × F−!

F: only 2 likely lac± on EMB lac. ~~Abandon~~

Residuals on EMB lac. add to D.

C. B.[2] and 936N. 3. all Xyl − Mal − (S^R). Abandon.

D		lac	Mal		Xyl		Mtl		S			T1			BMB.	TLB.	
P	+	−	+	−	—	—	+	−	S	R	S	R		P	+	−	+ + every recomb hue is Mal+
P	+	−	+	−			+	−	S	R	S	R		P	+	−	− +
	+	−	+	+			+	−	S	R	S	R	×		+	−	− +
	+	−	+	+			+	−	S	R	S	R	×		+	−	− +
	+	−	+	+			−	−	R	R	S	R	×		−	−	+ +
	+	−	+	+			+	−	S	R	S	R	×		+	−	− +
P	+	−	+	−			+	−	S	R	ts	R		P	+	−	− +
	+	−	+	+			+	−	~~S~~	R	s	R	×		+	−	− +
Par	+	−	+	+			−	−	R	R	s	R	×		−	−	+ +
	+	−	+	−			+	−	S	R	S	R			+	−	− +

control ?

NG 945

Sic! Issues of 1895 × ~~1877~~ 1177? Try W1922 × W1896!

Note crossovers of Mal/S!! Mtl-S-linkage!

5/23/52

2 types:

① Par$_1$ / Par$_2$ Mal+

② Par$_1$ lac+ Mal+ / Par$_2$ − Mal+ !

Further content of these colonies should have been screened!

See 945

all showed a Mal+ S^R comp., but zygotes might be S^S.

E W 1970 x W 1918 EMB Lac ± sm
F " Y10. "

E. 4 x 200. Lac sm. No lac +.
5 x 350 Lac crowded No Lacs sm.

F. 4 x 200 1 Lacs SR!
5 x 350 3? Lacs.

April 21, 1952

A 58-161 10^{10}/ml B W1956. 10^{9}/ml ca 25 minutes in 1mg/ml HN2 in D(m) 37° dilute 1:10 in Penassay.

Streak out. Plate and dilute by spreading serial plates.
A. Sterile B. Ca 200 on plate 2. (10^{7}). pS = ca 5.

Replica to D(0) + W1177 for replica-plate test of F+ or + 58-161.

C 4/22/52. As above. 20 minutes exposure.
ca 50 on plate 2 (i.e. .1ml from 1/10 dil to Penassay)
1 Lac- ~~2 addl. lac-?~~ Pick + restreak. W1165
Gene Hfr occ. prototrophs Replica to D(0) + W1177. (spot W1895 control)

(D) ~~B~~ 4/23. As above Plate 2 only.
only 2-3 coli/plate. B. subtilis likes contaminant.

E 4/25 10^{10}/ml 10ml 5mg HN2 in D(m) 20m. 37°.
at 20m, dilute 1:10 in Penassay ~~express~~ dilutions from this as
10^{0}. 2 > 400.
3 60.
~~1 Lac-?~~
This establishes suitable ~~dose~~ level
No Hfr noted this run.

In preliminary HV runs. 1 colony 58-161/ca 60. plate was noticeably nibbled. This proved to be λ^{s}_{1} similar to W1655. Discard.

Summary.

A) Lac + I −
B) Lac + S^R I −
} Almost all TL− M+ S^R Mal− Xyl− Mtl−. Segregating Lac − V_1 − V_6 in more or less linked pattern. (V_6 − Lac − TL−). Also upheld in C.

C) Prototrophs! → B_1− > B_1+. ca 10:1.

In Hfr x F− there appears to be a constraint favoring TL− ... as well as S^R Mal− M+ .... [Rothfels crossed S^R M− x S^S M+ ... Almost all recombinants in his experiments were also T−L−Lac−V_1^R just as here! This was concluded to be based on the M+ .. Lac− ... linkage. It can be reinterpreted as a Lac ... TL ... Y linkage, with Y a hidden selector. But, the order V_6 Lac V_1 TL Y would (if applicable to Rothfels) would give a different sets of auxotroph single crossovers! The conditions of mating do not preclude a limited degree of F+ − transduction.

It may be conclude that selection for TL+ essentially discounts the effect of Y over the Lac − TL region, but leaves this influence at the left end, so that all of this set of prototrophs are Mal−.

① Further Project: Compare Hfr x F+ crosses.

② Study T−L−V_1 more closely

Map W1895 x W1956 940

April 22, 1952.

A EMB Lac .5 shows. Plate on EMB Lac ± sm and D(B,).
3-4% Lac+ sh 16 Lac s streaked out. Pick +,- to EMB Lac.
B EMB Lac sm. B' (+ recoverable only).
C D(B,) 100x. Ca 50 per plate Pick 40 to EMB Lac B,.

940 A: (Check for possible Lac v true.

B 20 Lac s 3 were pure + (test for V_1 R/S)
20 Lac tor s. Uncertain relationship of Lac -, do not consider these.
isolate Lac + (and -) and test.

B" 1 pure S V_6^S check reaction V_6
1 pure R V_1^S
R/S. both V_6^R Check nutrition of V_1^S. at bottom of test plate

C. Many of the strokes were mixed +/- on Lac EMSB, despite care to obtain single pickings and despersion of the colonies.

a	+ (sup.)	16	Upon restreaking on EMB Lac, almost
b	-	8	all showed a Lac - component.
c.	+,-	18.	Picks + and - wherever observed. (for later confirmation of nutrition).

Again, 9 unselected Lac segregates were all Mal – S^R.

7 Lac$\underline{s}$ also Mal$\underline{s}$ were parental for every marker

and must be assumed to be trivial clumps.

		Lac	Mal		S	Gal		Xyl	Mtl		T1	T6			BMB, agar	TLB, agar	
C	1	−	+	−	− R	R	− ±	−	−	−	− R	R	(S)	S	−	−	+ +
D	2	−	+	−	− R	R	− ±	−	−	−	− R	(S)	R	S	−	+	+ −
	3	×	+		+	S	+		+		+	S		?		+	−
A	4	−	+	−	− R	R	− ±	−	−	−	− R	R	R	S	−	−	+ +
	5	×	+		+	S	+		+		+	S		S		+	−
	6	−	+	−	+ R	S	− +	−	+	−	+ R	S	R	S	−	+	+ −
	7	−	+	−	+ R	S	− +	−	+	−	+ R	S	R	S	−	+	+ −
A	8	−	+	−	− R	R	− ±	−	−	−	− R	R	R	S	−	−	+ +
	9	×	+		+	S	+		+		+	S		S		+	−
D	10	−	+	−	− R	R	− ±	−	−	−	− R	(S)	R	S	−	+	+ −
Par		−	+	−	+ R	S	− +	−	+	−	+ R	S	R	S	−	+	+ −
	11	−	+	−	+ R	S	− ++	−	+		R	S	R	S	−	+	+ −
	12	−	+	−	+ R	S	− ±	−	+		R	S	R	S	−	+	+ −
A	13	−	+	−	− R	R	− ±	−	−		R	R	R	S	−	−	+ +
E	14	−	+	−	− R	R	− ±	−	−		R	S	S	R	−	−	+ +
A	15	−	+	−	− R	R	− ±	−	−		R	R	R	S	−	−	+ +
F	16	−	+	−	− R	R	− ±	−	−		R	R	R	R	−	−	+ +

P. P. P. P. P P

1–10 show 5/10 segregations. 11–16

Mal · Xyl · Mtl · S, linked

Lac .. V$_1$ .. V$_6$ .. TL .. BM

meangel link.

9 total.

4	A	−	+	−	− R	R	−	+	−	−	−	R	R	R	S	−	−	+ +
0	B											R	S	R	S	−	−	+ +
1	C											R	R	S	S	−	−	+ +
2	D											R	S	R	S	−	+	+ −
1	E											R	S	S	R	−	−	+ +
1	F											R	R	R	R	−	−	+ +

clear

Exc. ? 12, None fail to show Lac+ S^R! Further tests needed for Lac− S^R
... recombinants. But note rarity of recomb. in Lac− selections 1:9

Note rarity of crossovers between M–TL despite Lac, V, syn.

Xyl, Gal evidently unlinked but 12

12 maybe recomb (+/Gal)

① Verify Lac-V_6 linkage

② Probably V_1 – TL linkage (all 7 TL+ are V_1^S)

③ Probable Lac-V_1 linkage (?) $\frac{V_1^S}{V_1^R}$ > in Lac+ than –.

These data suggest a constraint favoring TL– as well as Mal– M+. # 11 (unless coincidence) may point to Xyl – M.

?? Are we missing Lac– zygotes? Try looking for rare M+/S recombinants, or S/V_1 [1895 V_1^R × … V_1^S]

B1

	lac		Mal- ↓	S^R ↓	Gal		Xyl		Mtl		T1		T6		BMB, agar		TLB, agar	
1	+ ↓	±			±	−	−	−	−	−	S D	(S)	R✓	(S)	+	(+)	+	+
2	+	−			↓	↓	−	−	−	−	RA	R	S	R	−	−	+	+
3	+	−					−	−	−	−	RA	R	S	R	−	−	+	+
4	+	−					−	−	−	−	RA	R	S	R	−	−	+	+
5	+	−					−	−	−	−	RA	R	S	R	−	−	+	+
6	+	−	—	R			−	−	−	−	S B	R	S	R	−	−	+	+
7	+	−					−	−	−	−	R A	R	S	R	−	−	+	+
8	+	−					+	−	+	−	R F	R	S	R	−	−	+	+
9	+	−					−	−	−	−	S C	R	R✓	R	−	−	+	+
10	+	−					−	−	−	−	RA	R	S	R	−	−	+	+
11	+	−			±	−	++	−	+	−	S G	R	S	R	+	−	−	+
12	+	×			++	↓	−	×	−		S B	↓	S		−	↓	+	↓
13	+	−			±		−	−	−	−	S E		S	R	+		+	
14	+	×			↓		−	×	−		RA		S		−		+	
15	+	−					−	−	−	−	RA		S	(S)	−		+	
16	+	−	—	R			−	−	−	−	R I		R✓	R	−		+	
17	+	−					−	−	−	−	S B		S	R	−		+	
18	+	−					−	−	−	−	S B		S	R	−		+	
19	+	×					−	×	−		R A		S		−		+	
20	+	×			↓	↓	−	×	−		R, S I	↓	R✓		−	↓	+	↓
21- 31	+	+			±	±	−	−			S E	RA	S	S	+	−	+	+
2	+	+			↓	↓	−	−			R A	RA		|	−	−	+	+
3	+	+					−	−			S B	RA		|	|	−	+	+
4	+	+					−	−			R A	RA		|	|	−	+	+
5	+	+	—	R			−	−	—		S B	RA		|	|	−	+	+
6	+	+					−	−			R A	SE		|	|	+	+	+
7	+	+					−	+	see		R A	SH		|	|	+	+	+
8	+	+					−	−			R A	SJ		|	|	+	+	−
9	+	+					−	−			R A	RA		|	|	−	+	+
30 40	+	+			↓	↓	−	−			R A	R I ✓		(R)	↓	−	+	+

Left margin beside rows 11–20: "–L+"; beside rows 21–30: "T-L+ see BB"

Among 16 lac- S^R selections, only 1 recombinant. [complement to lac+ V_6^R?]
40 Lac+ ... no parental (re mutation ... S^R - mut linkage?).
Types of lac+ (Mal- S^R Gal+ or ++).

1-20 are paired +, -
21-40 are unpaired +.

		Xyl	Mtl	T1	T6	BMB,	TLB,	
A. ✓	22	−	−	R	S	−	+	standard
B. ✓	5+1 (Gal++)	−	−	S ↙	S	−	+	V_1
C. ✓	1	−	−	S ↙	R ↙	−	+	$V_6 V_1$
D ✓	1	−	−	S ↙	R ↙	+ ↙	+	$V_1 V_6$ BM
E	(3)	−	−	S ↙	S	+ ↙	+	V_1 BM (TL)
F	1	+ ↙	+ ↙	R	S	−	− ↙	Xyl TL
G	1	+ ↙	+ ↙	S ↙	S	+ ↙	− ↙	Xyl T1 BM TL
H	1	+ ↙	−	S ↙	S	+ ↙	+	
I	3	−	−	R	R ↙	−	+	

J = E

BM TLB, + ← Xyl BM T1 T6

Note preponderance of Mal+!

Prototrophs

940c. Single factor ratios:

	V_6	Lac	V_1
R_1 −	35	32	28
S_1 +	29	32	36

About equal Lac+ : − valid unless single prototrophs are not segregating units.

Linkage:

V_6 \ Lac	+	−
R	7	28
S	25	4

V_1 \ Lac	+	−
R	4	14
S	28	18

V_6 \ V_1	R	S
R	14	21
S	4	25

consistent with

V_6 − Lac —— V_1

B_1 − M 6 B_1+ / 58 B_1− } independent

Xyl − M 2·3 + / −

MH

No Mal+!!

T − L − V_1 linkage

940BB

Compare mutation of (TL) − V_1^R vs. V_1^S. (Lac+generally)
Collect occurrences in following array:

		V_1^R a	V_1^S b
A	1	1	14
B	2	4	3P
	3	2	6
	4	3	9
	5	4	12
	6	5	17
	7	7	18
	8	10	23
	9	14	25
	10	4956	1

D( )

	TLB₁		TL		TB₁		LB₁	
1	+	+	−	−	−	−	−	−
2		−		±				
3		+		−				
7						++		
8						++		
9						−		
10					±	++	± !	++

∴ 16 = B_1 − ✓

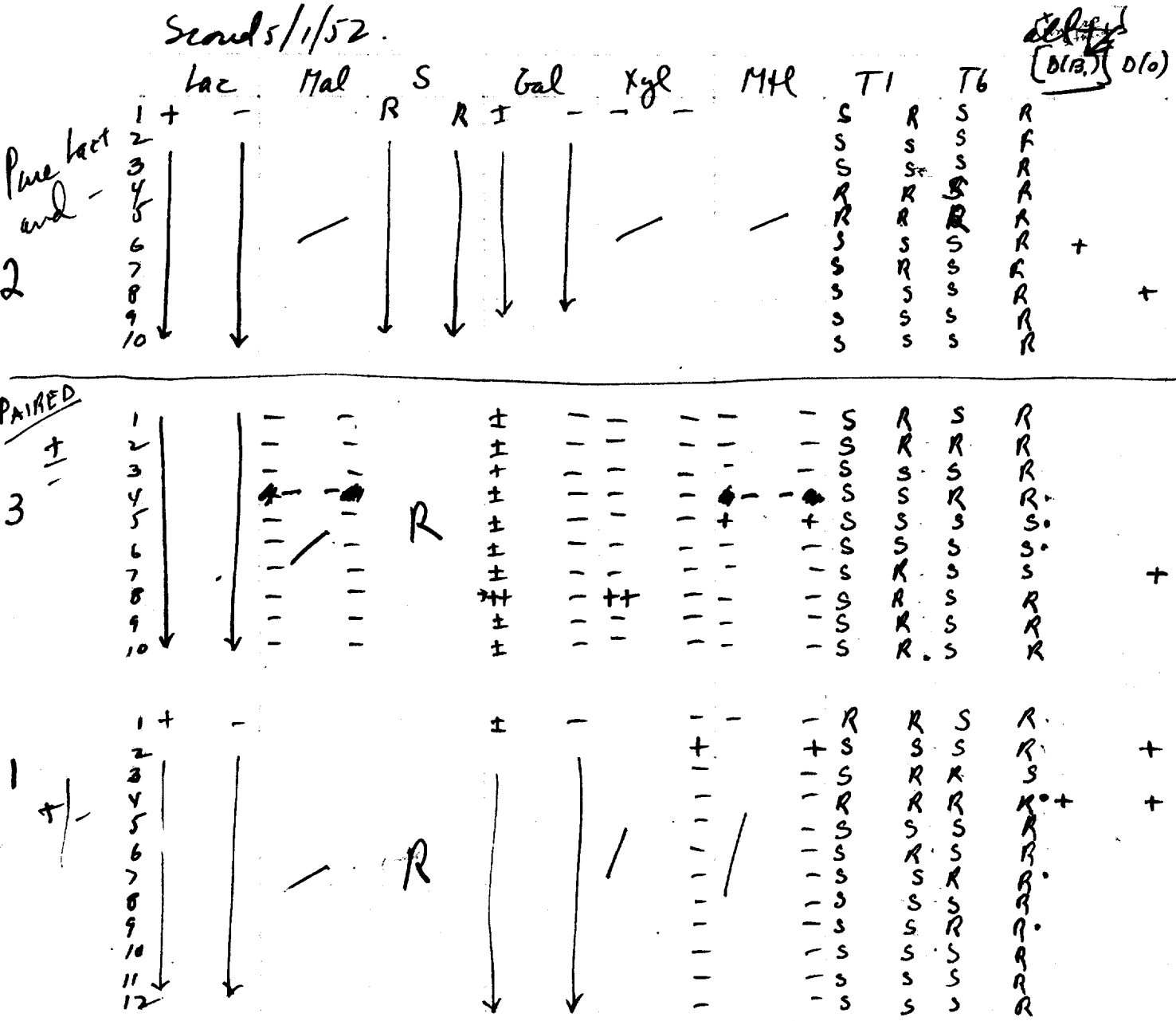
Same ~~T-L~~. Presumably this is correct order: ~~T-L~~ L − T
T-L+

Lac − V_1 − L − T
↑ V_1^S
↑ V_1^S ~~T-L~~ +

D,I+ Prototroph recombinants

9/0C

Seeds 5/1/52.



a). Note high frequency of mixed pairs (22 pairs). Some of these might be lac- → lac+ reversions (especially if concordant for V_6) 7 may well fall in this category, and should be checked further. However, remaining 15 are discordant for V_6 also. Remaining pairs are for V_1: SR 8 RS 0 SS 7 RR 1
~~Dropped lac+ of the 7 concordant pairs~~ But no pair was concordant for Gal!
all pairs: 8 0 12 2

42 colonies streaked out. 20 were substantially pure, 10+, 10-. Remainder were mixed, picked 1 lac+, 1 lac-.

← over

94 @ CC

May 1, 1952

Au 943. W1895 x W1956 on D(B$_1$) and EMS lac B$_1$. Incubate 3 days.

EMS lac B$_1$: Superficial appearance. An unusual proportion of sectored colonies is indicated.

+	−	−(+)	+(−)
34	2	5	7

Owing to size differentials, the figures do not show their proportion adequately. Sectoring for texture is also notable on D(B$_1$). Pick well-separated colonies from D(B$_1$) and streak out on EMB lac.

Yields, as usual experience, about 10^{-4} of inoculum.

5/5/52 Picked 32, "random" streaks on EMB lac

	Proportion lac+ (est.)
1	.5
2	.1
3	.5
4	<.1
5	0
6	<.1
7	0
8	.1
9	0
10	.5
11	1.0
12	.9
13	.4
14	.8
15	0
16	1<
17	<.1
18	.5
19	0
20	.1
21	.9
22	0
23	<.1
24	.1
25	<.1
26	1<
27	0
28	.3
29	0
30	1
31	0
32	.8

110/32

Weighted average: 110/32 ca .3 lac+!

Of 32 pairs, only 3 had Mal+ : 20−
22+
31−

As EMS Mal B$_1$, a much larger proportion appear to be Mal+

11 EMS lac B$_1$, colonies picked to EMS Mal B$_1$: ca 30% +

Effect of EMS medium??

W1895 × W1956
various conditions

April 23, 1952.

B. Unaerated. 5½ hr cultures. Mix 2 hours   ca 5%?
A. Aerate " " (s aeration).

A1 —
A2 heat 60° 5 minutes. Plate 10×.   No kill!
A3 UV 0, 10, 20, 30 sec. on plate
A4 Partially sediment (ca 90%) mixture. Sediment ail. 20+/268
A5 ↓ Supernatant dil. 6+/264.
A5 appears superficially somewhat less. Should be repeated.

A3): 0 Mean plate counts. 22+/350 21/363
10 "
20 "
30 ca 30% surv. 5/184 14/280.

slight effect?

4/24/52.

C. Aerate overnight W1895, W1956TZ. Remove (c air) 10 AM.
Mix and regrow 2 PM.

		EMB lac sm. (Poor est. of counts)	Total counts:
1. 1 ml ea parent / 10 ml Penassay.	Air	3, 6 (+, ±)	40, 34
2. W1956 control	"		
3. .1 ml ea par.	No air	0	40
4. .01 " "	"	6	129

Confirms very high relative rate of recombination in diluted mixtures.

1 C1 (stars) 3 38
2, 3 C2 plate mixture to 5 PM (grow on plate) before fix. 3 heavily overgrown.
" "

4/25/52

D. 1895, 1956Tz from overn. acc. 10AM - 2PM Region.
Mix ca 5ml each + 5ml Tenassay 2PM — 3PM EMB Lac sen.

1	(+ 90 mins room temp.)	< 1/5% lact+
2	supernatant after strong centrif.	"
3	Resuspend in saline. Re-sediment : supn't.	"

cultures may have deteriorated or undiluted added. contact.

E ~~Mix~~ Dilute 1:100 3PM. Mix in 10ml: (assume 10^{10}/ml initial)

1	1ml ea	(ca 10^8/ml)	ca 1/2% lact SR.
2	.1ml each	(ca 10^6/ml)	" "
3.	.01 ml "	(ca 10^5/ml)	0

In view of D these results are meaningful. However, the development of zygotes at extremely low dilutions is confirmed. [Competition expts?]

Flagellar phages: Salmonella

~~944~~ 942

April 24, 1952

Received this date from Boulgakov

1 "Steam" 372 = ~~H901~~ Sutii - Boulgakov Rough
2 377 H901
3 383 = Felix 6.396 V/3

A & VIII - 113 1936 § had been propagated on H901.
B " "
C " Passage 372
D " " "

3/24. Open 1, 2, 3, A. Test by cross-streak on EMBLac

	A
1	S
2	S
3	S (later secondary R)
Stanley	R
O-901	S!
LT-2	R
LT-22	R

3/25.

	A	B	C	D
H901	S++	S ++	S +	S +
O901	S+	S ++	R	R

Apparently C and D fit description of flagellotropic phage. Should be single-plaqued to verify effect of propagation on H901, supposedly the sole destruction of A+B.

Test various Salmonella types on EMBLac vs. C.

1+C cleared after ca 3 hours in Ramsay. Streak out for O/S survivors

Restreak 1+C, 2+C. Pick single colonies: restreak; test motility.

1: 3 MS most promising ← like (#1 →). Retest single colony isolates.

2: 3 motile, 1,2,4 re tested: show limited motility overnight. Retest single colony isolates 1PM – 9AM to 10PM (from #1)

H901 controls! — clump of large bodies seen in soft agar!

Motility of H901, & motility of O901 verified microscopically. (over)

O-forms from S. typhi ste.

942-2-1 4 colonies tested. #1 did not migrate overnight
#4 ++
↓ should be unstable

942-1-4 " " #2 +
#4 +++

Compare motile + non-motile "revts" for sensitivity to φC

---

check phages 942-1 942-2
alike ↓

H901 S (mucoid) [culture old, from liquid]
0901 R
3 motile revts of H901/C } S +++ [from mobility agar]

1 NM-H901 R. save.

			A φ	C φ
1		1	S	P
2		2	S	P
3		3	S	P..
V	(Edw.)	0901	S	R
558	(Kauf.)	0901	S	R
6		stanley	R	S
7		enteric	R P	R
8		para B	S	S
9		gallin.	P	R
10		Ty 2V	S	R
11		Ty T2	R	R
12		LT 2	R	S
13		LT 22	R	R
14	K.	13	R	R
15	K	223	R	R
16	K	248	S	R
17		[] + C]	R	R
18	i	SW 519		
19	i	SW 520		
20		SY 79		

phage C.

	LT2	marked S
SW	519	S ±
SW	520	S +
SY	79	marked S.

Lig growth with C → motile

Inoculate from lytic area to Peressay 10+ AM.

3PM: para B + φA Motile
para B + φC NonMotile → 3/3 bimotile for 8 hours
stanley + φC NonMotile → 1/3 " " " (#1)
LT2 + φC Motile! Recip LT2 + φC also unmarked motile.

phase?

4/28/ Grow 1 plaque of φC on #1. = φ 942-1 } (Peressay 50 ml)
" " #2 = φ 942-2 } behave alike re H, 0901 contra A, C as rec'd!

Responses of SW519, SY79... phase variation? UV variation?

Replicas of A to EMBlac sm; EMB Mtl.

1. of 31 Lac+, 5 were S^R
2. No Mtl+ Lac were seen in 5 plates (ca. 1000 Lac- Mtl-
   31 Lac+ Mtl±)

Owing to disturbed ratio of Lac+ : Lac-
and overall low number (5) of recombinants
this experiment is not conclusive

Bordgalsov flagellotypic phage 942

Summary.

4/29/52.

1. Two phages (A-B) (C-D) received from Bordgalsov.
2. AB is essentially a typhi (also paratyphi B) phage, but independent of somatic (842-1 Rough) or Vi antigen.
3. C-D Accords to description of flagellotypic phage. High (?) titre obtained from single plaques either on H901 (942-2 φ) or Sutic Rough (942-1φ) S. typhi. This acts on H⊕ Typhi, probably mutant on Vi+ (Ty 2V, SV79) It has given non-motile secondary growth and O-forms of various stability with S. typhi (Sutic R unstable; H901 "stable"), para B and Stanley. Although washed lysis is seen with typhi murium LT2 and SW519 (typhi; i —), secondary growth remained motile (Vi, V antigens ??)
4. Motile "mutants" from S. typhi became again sensitive to φC.
5. Saved for further work: NM typhi H901 (para B; Stanley. Should be subcloned. Also lysates from single plaques.
6. L-forms not of motility agar. See 944

10/31 - which para B? Stock book records SW533 (=703) but no explicit notation here

4/28/52.

W1895 + W1956 TZ. Overn. aerate 9:30 to 2:45. 17y to 4y5

				lac sm	lac
	A	1	1	21+, 379-	ca =+,-
	B	1	.01	4-	
*	C	.01	1	3+..;	28 lac+, >-
	D	.01	.01	1+ 22-	68+, 17-, 3s

plate on EMB lac ± sm.
Again note relatively high efficiency of diluted crosses (esp Hfr << F-).

4/29/52.

1895 TZ + W1956. Aerate overnight. No aer 3h. (TZ). 1:10... (no shaking incubation) inocula

				lac sm.	in 10 ml. 2PM...
A	1	+	1	0	!
B	.1		"	0	
C	.01		"	0	
D	.001		"	0	

5/1/52. No aer overnight. 1:10 Pen aerate 10AM - 2PM. Suspend air ca 30 minutes to reduce TZ

Incor 1 or .01 ml/10. No air. 2-4:30

	1895 TZ	1956	EMB Lac	
A	1	1	ca 20-:1+	!! (lac+ fail to grow in central broth?)
B	.1	1	lac+ v. infrequent	only 1 lac+ in several EMB Lac: Lac s.
C	.01	1		
D	.01	.01	only lac- seen.	

rate → 100% of Hfr cells

Replica A to EMB Mfl EMB sm.
← (see over)

~~S. typhi H901~~ large bodies

942C.
944

4/27 Many colonies of ~~large~~ L-type growth noticed in course of motility tests in H901 controls.

H901 was inoculated from EMB plate (?) to semisolid agar incubated ca 24 hours. Room temperature 8 hours.
Inoculate to semi solid agar (5 ml ±) + 500 units penicillin.

4/28. – penicillin showed same interspersal of bacilli and spherules
+ " " no macroscopic growth; spherules were prominent.
These are very transparent, practically invisible except to phase microscopy possibly accounting for infrequency of reports on them.

Similar admixtures of spherules noted to varying extent in semisolid agar smears of S. Stanley, pearB, and 842-1 eswells cytotes of H901!
(Test LT2, K12...).

4/29. — Similar observations without & with penicillin. However the L-colonies are much less prevalent than in H901.
W1895 and W1956 unequivocal in motility agar. Then transfer to nut agar + penicillin 500 u/ml. Occasional L-type, usually not colonial, seen with and without bacteria respectively. Small + very large spheres seen
5/1 Similar structures with B. subtilis and staph. aureus.
Further control examinations showed similar spherules singularly in uninoculated plates, also in freshly poured medium! Doubtful connection with bacteria!

May 3, 1952.

See 938

W1895 x W1876. Grow overnight Penassay. Mix 12N 1ml 1/10 ml each. Incubate to 1 30.

A. EMB Lac 9 plates ca. 100/plate. 2?? lac ±

B. EMB Lac sm ('10 and "100x). B·2. O+; B':2 O+ B" 3: 3+?

C. EMB Mal sm (1 and 10x)

D. D(B,)

C: 78- 7+ / 75- 8+! / 43- 9+ Contrast very low frequency of lac+ S^R. These Mal+ S^R appear to be unselected. Possibility of contamination in parents? 196- 24+ → all lac-.

W1895/1938 "control" - Lac EMB sm. 94-: 6+. Conditions are suitable. Cf 938D which shows similar patterns.

5/4/52. ~~Repeat C and also plate on EMB Mal.~~ Replica A to EMB Mal. As above. Reinoculate ca 5 hours Mix 3 45 - 6 45.

1895 + 1876

E EMB Mal "W1876" streaked out gives 10% Mal+ on EMB Mal sm.

F EMB Mal sm

G EMB Mtl These expts n.g. except rare lac+ S^R

H 1895+1956. Spread on D(B,) 3 45. Incubate to 7 PM. Examine under phase microscope Numerous microcolonies. About 1/100 is partly or fully lysed with many granules of various sizes (such as mentioned by Pogt?) Need controls!