October 7, 1951.

A. For S^D/S^S Cross on EMS lac, EMS Malsm.

B. 10/8/51. H292 x W1709

No yield!!!

Check fertility of W1709. Grow on Pennassay + 1000 u sac/ml (Hi) 50 u (Lo)

10/11/51

A	1709 Lo	x W1490	High yield	
B	"	x H292	n g. - overgrown	H292 ? Probably used 295 by error
C	1709 Hi	x W1490	V. High yield	
D	Hi	x H292	n g.	
E	1709 Hi	x 58-161	3-4	
F	1709 Lo	x 58-161	3	

Note reduced ~~yield~~ yields of $S^D \times S^S$ cross. (Residual sm in S^D cells?). Should compare $S^S \times S^D$; S^R grown on comparable sm medium, and $S^S \times S^R$ on plain nap-sm broth.

sm 876A	A	W1734 x H290	low yield on EMS lac (Ca 5/); Ca 200/EMS Malsm.
sm 877	B	" x H291	Very low yields Ca 4/ EMS lac ± sm.

876 A

W1734 × H290
Mal+ S^D Mal- S^S ||

A. EMS Lac.
B EMS Mal sm.

Streaked out EMS lac, EMB Mal, EMB Mal sm
High yield, bit n.g. 24h.

A.

	Mal	Mal sm.
1	v?	
2	v	
3	v	
4	?	
5	v	
6	v?	
7	v	
8	v	

growth much sparser. colonies indeterminate. May be ~~SS~~ S^D/S^S with intermixed S^S segregants.

Presumably S^S dom S^D.

Repeat 10/19/51. see 877.

10/22: Ca 5-10 purplate Picks 1-4.

10/22 Picks 5-8. Incl. 1 or 2 v. small Lac?

	Lac	Mal	EMS lac sm	
1	+	nov	+ nov +	S
2	+	"	+ " +	S
3	+	"	+ " +	S
4	+	"	— " +	S

not S v

No colonies on replica to EMB sm.

see 878.

Picks from EMB Mal.

	Lac	Mal	lac sm	Mal sm
5	++		- few sly	+ (few) only
6	++		"	"
7	++		"	"
8	++		"	"

Pick single colonies from EMS. Brush /sm EMB lac, Mal.

No Mal v.

11/2/51

	S^D		S^R Mal	
C	W1734	x	H 301 v	No yield.
D	"		H 302 -	Yields very few, improved mac sm.

Plate on EMS lac Stroke sm on some plates to establish gradient of sm concentrations.

D. Experiments continued in desultory fashion.

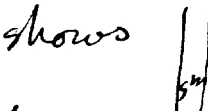
Many prototrophs fail to grow further. In numerous (20-40?) tests, lac- were mostly Mal-, some Mal+, 2-3 Mal v but no S^D were noticed.

Investigate further S^D prototrophs. 10 restreaked

	EMB Mal	
1	+D +R	-
2	+D	-
3	+D	-
4	-	
5	-	maybe S^D or S^D/S^R
6	-	
7	-	
8	-	
9	+D	-
10	+D	-

3, 4, 7, 9, 10 did not produce good colonies on EMS lac sm (1004/ml)

4 (single colony?) shows most definite signs of being a heterozygote. Picks single "Mal v" colonies



o Mal- 4D 1-3 s.c.
4D4 grows from EMS lac sm

5 shows strong Mal+ S^D reactions and a single Mal- S^R.
Purity of initial inocula in doubt

		EMB Mal //sm	EMB lac //sm	EMB lac sm	EMB Mal sm
D4:	1	+D	-D	-	+
	3	+D, -R v?	V, -	(D?) V -	- v? +
	4	"	-	(D?) -	+, -, v?
?	4-mix	- R	V; -	V -	-

Pick clear single colonies, Mal v or lac v" and Streak on EMB, EMS ± sm

W1734 × H291
Mal+ S^D Mal- S^R

877

A EMSLac
B " Malsm

A.

	EMBMal	do. + sm.
1	V	V
2	V	V
3	V	V
4	V	V
5	V	V
6	V	V
7	V	?
8	V	V

On EMBMal, a more or less "normal" segregation is observed, Mal+ predominant (not necessarily not V.) With sm, Mal- is much more prominent, suggesting that S^D/S^R in presence of sm does not compete well with ~~Mal-~~ S^R.

If S^D is present here, it is presumably recessive to S^R.

B. Mal V very doubtful. Same appearance ± sm.

Lost by overheated incubator.

Repeat 10/19/51. 10/21/51. Ca 5-10 colonies per plate EMS Lac. Picks (1-4.)
10/22 Picks (5-8) including numerous small lac?

	Lac	Mal. EMS Lac	sm
1	nov.	+	S
2		+	S
3		+	S
4		+	S

nov? + " ? + " + " + S S S S

Test colonies on EMS, EMB ± sm. from EMB by replica plate.

#39, orc. colonies from thick streaks gave Mal- colonies on EMB mal sm. Mostly Mal+ S^S.

Picks doubtful colony from 1, 2, 3. 1a, 2a are Mal+
3a is Mal V. Mal- on EMB Mal sm. ~~Probably S on EMB~~
~~Maybe either~~ S^R/S^S or S^D/S^S, ~~probably the former~~
Not photographic: probably H291 parent!

	Lac sm	Mal sm
5	- only	- only
6	"	"
7	"	"
8	"	"

all are S^R/S^S

Note H291 found to be Mal V.

Preserve H298

878

4PM 10/16/51

A. Dilute 1:100 in

1. Water abc = 1-3
2. Saline abc = 4-6
3. Peptone 2% abc = 7-9

Initial assay: lost
Probably ca 2×10^9

B. Throw .05 ml in
a Old Silica
b New "
c Sand "

C. New Silica, Peptone dil. ca. 1.5g silica per tube.

.01	10	0
.02	11	0
.05	12	0
.1	13	0
.2	14	5
.5	15	4
1.0 ml.	16	Remained wet 5×10^4

11/18/51. Initiate assays. Predicted initial would be ca. $2 \times 10^7 \times$ [illegible] $\times .05$ per 10 ml tube, $= 10^5$/ml. Plate .1 ml and .001 ml samples.

#1,2,3,5,8 showed no survival in .1 ml samples. However, every tube grew out after 48 hours (Cells bound to silica??)

∴ Water dil., ~~on peptone dil~~ on any, or saline or pep on "new" silica not very good.

#15. 4 colonies at 10^{-1}

#16 5 colonies at 10^{-5} ! (but this not dried)

Many apparently "Lac+" colonies.

11/20/51 Plates of Remaining tubes: 4,6,7,9,10,11,12,13 Sterile #14 shows 5 colonies at 10^{-1} But all tubes except 4,6,11 are turbid.

(over)

These results are very discouraging. However, they may be the result of early destruction or a poor medium for the initial assay was lost in an overheated incubator. Y 872 B1.

Glu- × Gal- = Lac-?

10/20/51.

W990 = Y10 Glu-Lac+
×
W618 = 58-161 Gal-Lac+

Cross on EMS/Gal. Replica to Gal, Lac, Mtl.
Picks the 4 combinations: all are Lac+!

	Gal	Glu	W
	+	+	1741
	−	−	1742
par.	+	−	1743
par.	−	+	1744

In the cross, Gal+ >> Gal−
Mtl− >> Mtl+

suggesting that the Gal and Glu− are both linked to Brl.

[W619 was also tested, but this is Lac ÷. The genetics of the Lac line is not known. Should try W618 × W251, 252 I]

On fermentation tubes, rather slow ambiguous reactions were seen.

W1742 was grown on Y+Lac plates, harvested to water and suspensions tested for glycolysis in 1/100 buffer, 1% sugar BCP.

(15 minute test).

Lactose	++
Glucose	+++
Galactose	±

This suggests differential adaptation of glucozymase to lactose! Compare glucose, lactose grown cells.

W251 (Lac+ Glu- Gal±) × W618. Good yield
Isolate several Glu−Lac+ Gal−
W1752 1753.

W251 in fermentation tubes is Gal++ Glu+ Lac±! in contrast to appearance on EMB plates.

BB 545

Streptomyces griseus.

11/8/51.

Culture WAc 1 Received from E.S. McCoy. Transfer to Nutrient Agar slants, streak out mother culture. Limited ~~superficial~~ growth on EMS bac. Gly, bal. No growth on EMS. Good growth, limited sporulation, on D(o) agar. Culture inhibited by streaked loopful of ~~sus~~ 10^5/ml.

11/14 (1) Harvest spores from n.a. slant in 10 ml H_2O with vibrator, ca 10 minutes. Count ca 2×10^8 in counting chamber. Adjust to ca 10^8/ml in H_2O. Many clumps; ca ~~80~~ 80% single spores. Dilute + plate out on nutrient agar.

A) control B) uv 10 sec. C) uv + 120 sec.

Plate 10^{-6}, 10^{-5}, 10^{-4} A., B, C.

For irradiation, dilute ~~to~~ to 10^5/ml predicted count.

A 6 111 → 4 poss. tested, all +

B 6 125 ← Replica to minimal agar transfer to tryptone

8 poss. retested
2 auxotrophs: WAc-2, -3

No appr. kill at 60 sec.
Try 90 sec. 120 sec. 150

11/16 Dilute to nominal 10^4/ml

A 1 .05 ml
A 2 .02 ml
B 90 sec. 1 ml
C 120 " " 0
D 150 " " 0
E 180 " " 0

survival.

204
233
48

No auxotrophs

6, 14, 4, 8, = 32 ~~aux~~ no auxt

p. 8 F 90 sec, Dilute [redacted] to nominal 10^5/ml .05 ml/plate
5 plates, ca 80 scorable/plate (+ bacterial contaminants!)

2? aux. No.

(See over)

G 60 sec ca 500 colonies 1?? WAc 4

Grows slowly, compactly on minimal agar.

Probably more slowly than WAc 2.

1 colony noted as producing pale yellow pigment on minimal agar.

I	60 sec	8 plates	ca 90/	720
J	90 sec.	12 "	ca 45/	540
				1260 colonies.

10 possible mutants:

2 from J

8 " I (5 on 1 plate!)

Eventually 13 possible mutants.

4 show v. low residual growth on minimal agar (1, 2, 6, 10) = WAc 5-8

Others are mixtures or slow type. Rechecks replicas from streak plates

S. griseus mutants and crosses.

WAc 3 x 4

11/20/51.

WAc 2	A1	Slow growth on minimal, vits?	Arginineless
3	A2		Leucineless.
4	A4		

WAc-2 grows more slowly than + but eventually (3-5 days) gives considerable growth. Admixtures with WAc 1 show no improvement, either by cross-streaking on minimal agar (D(0)) or by restreaking from X on nutrient agar.

879

WAc 5	6	A1	
6	10	A2 ? Syph. WAc 3	
7	1	A4	
8	2	A4?	
9		Slow growth.	

? When 1st streaked on minimal agar scattered colonies with poor growth in periphery of streak

---

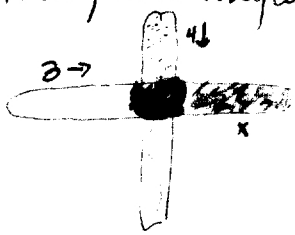
12/5 Cross brush suspensions of WAc 2, 3, 4 on minimal agar.

12/2/51. Controls: WAc 3 has a barely visible residual growth; 2, 4 show definite residue, (2 > 4).

2 x 3 Definite improvement (aerial mycelium) at cross-brush.

2 x 4 Marked " " " " "

3 x 4 Heavy aerial mycelium at cross brush and at carryover regions:



Proper demonstration of interaction may depend on using suitably "negative" mutants.

12/6. Restreak from X, cf. parent

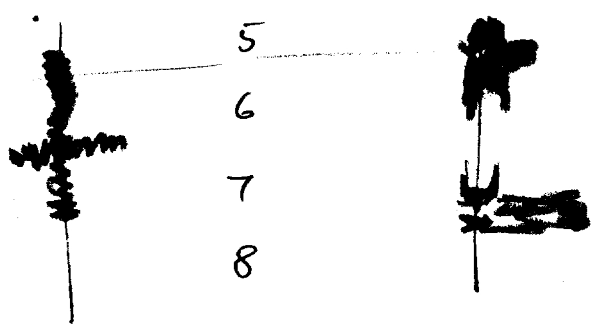
---

4 A4 ✓ A1 ?
5 A1 ✓
6 V.+, A4, A1, A3, A5 esp. A2. — on others, reaction is graded with ++ toward old spot of WAc 3
7 A4 A1 ?
8 A4 (weak ?)

[illegible]

WAc8 shows strong syntrophism with 880 - 9 (WAc 9)

Cross brush var. cont WAc: Drops spread, but apparent interactions are
5 x 3 (maybe 5 x 6, actually)
6 x 8 " ?
3 x 7 very clear.



WAc 8 WAc 3

Repeat on separate plates 12/8/51.

	12/10	12/12	
3 x 5	-	++	(no spont reversions in controls)
6 x 8	++		streaks are indefinite owing to syntrophism. All +++
5 x 6	++		

11/20/51.

See 765

Original strains W1327–1330 were restreaked for purposes of recovery and storage. All are predominantly stable +, –, or slow, no unstable forms seen at first sight. Mal– forms are apparently stable now! This is contrary to former result of high instability. Attempt repeated replatings!

W1729 was noticed as a similar Mal v type. Lyophil culture predominantly Mal v. Following experiments are designed to ① establish patterns of instability and ② (experimentally reproduce the peculiarities of W1327 which is probably now lost.

Possible of W1729 Mal– → Mal v+, thus resembling W1327 original behavior.

11/21/51. Restreak ① and strain W1729 ca = Pure Mal+ / Mal v, Mal–

1. Plate Permassay susp. EMB Mal. ① Hold in incubator at 37.
② store 1:100 dilution in ref. tube.

A Streak plate from slant 28 +v 36 –P No stable.
B Plate from ①. all scorable either +v or –P

C Plate 11/21 : 1, 2. All +v or –P 1 pure + v
D 11/22 all +v or –P
E 11/24 " "
F " "

see 884.

3. Incr. both with a –, + colony.

W1327 x W1394. 882

November 23, 1951.

EMB Lac, Mal

Mal++ > -

No Mal V noted in platings of pooled probings

November 22, 1951.

See correspondence ca. 11/51 with Umbreit & Ogrisley

Stanier recalled the report (J. Bact 1949) that S^R mutants of the Hinman + Bratia strains of E. coli were not improved in growth by aeration. RYS suggested that this might be a directive effect of sm on spontaneous S^R survivors (similar to Ephrussi's petite forms). Several S^R mutants of E. coli have now been tested, and did not differ from S^s in improved growth with aeration on Penassay, Minimal or Tryptone broth. (mid. W1177, & other K-12 S^R).

Ogrisley sent two cultures labelled S and R respectively (Hinman strain). These did not show NAI. effect, but Ogrisley later wrote that the early experiments were not readily reproducible.

The cultures when streaked out are highly heterogeneous, and show a majority of minute colonies that do not grow for ± 48 hours.

11/20 Test S1, R1 (sm. colo.). Both show AI on Penassay.

P21 Restreak S1 and R1 (as received). on EMB Lac, Nutr. Ag.

P22: large colony forms apparent on EMB lac Practically no growth visible TSA. Remainder

12/16/51.

WAc 3 grown on nutrient agar bottle.

Susp. ca 2.5×10^8/ml. Dilute to 2×10^3/ml. 4V 90 sec
plate on nutrient agar. n.g.: survival ca 500/plate!

12/28/51. Same suspension. Dilute from nominal 2.5×10^8
to 10^3/ml. 90 sec uv. Plate .2 .1 .05 ml
ca 10×35 colonies. 4?? None mutants. Rechecks.

Plate WAc 3 on TSA + sm 100. (ca 10^8 plated; 10^2 colonies appeared)
Restreak on TSA sm. Pick clean decay to WAc slant as presumed sR mutant

W1729 : Selection in Series on non-Maltose medium.

November 24, 1951.

Streakout from EMB Mal to nutrient agar A: Mal+v
B: Mal−

Note: A forms somewhat larger, rougher colonies

11/24. Picks 4 colonies from A, B. Restreak on EMB Mal and NA

11/25 A: Mal v (ca 94%) and Mal− in all 4 streaks
B: Mal − P " " " "

∴ Maltose is not immediately necessary for instability.
Inoculate from nutrient agar to Pomassay, EMB Mal and NA
Difference between Mal v and Mal− noted again in these streakings.
(Look for mutational equilibrium)

	A	B	
1	20+ 8−	some+?	ca 1%+
2	84+ 34−	some+ slow	100+: 17−
3	37+: 40−		
	70+ 71−		
		ca =	

A3 shows no + stable
B3 + stable ?

stable ??

A state approaching pseudoequilibrium is reached from either side.
Stable +, (− ?) also occur in B series.

Repeat with colony streaks. Streak Mal+v colony (A3) on EMB Mal; = C1
Nutrient agar

6/24/52. Restreak slant of A1, B1. A1 → mostly Mal ±, and − P
B1 → all Mal − P

∴ no change in stability.

Stable + seems to accumulate in successive transfers, but so gradually that selective maintenance pressure can't be excluded.

December 7, 1951 et seq

see 880

12/2 Cross bench 3 x 4 on minimal agar. Prototrophic interactions seen
12/6 Restreak from X, also parents D(o) agar.
12/9. WAc 1 heavy spor. growth
WAc 3 no "
WAc 4 faint colonial background.
WAc 3x4 Mostly like WAc 4. About 20 heavily spor. colonies in
spores thick streak.

mycelium Ditto., especially around mycelial fragments.
12/9. Restreak (spores) from spores A, mycelium B to minimal agar.

12/11 +++ growth, apparently pure in A, a few residual - colonies in B. Restreak spores on minimal medium.
12/14. A. 12 colonies in thick part of streak
B. Blank.

Restreak from 12/11 plate, A, B and from 12/14 A. to minimal and complete. Also replica 12/9 to " " . to all prototrophs.

12/16. All streaks +++

Compare 886.

WAc 3 x WAc 4. 8859.

1/2/52.

Repeat cross brush. Heavy residual growth of WAc 4, but sporulation only of intersects. Restreak 4 sporulating ~~sg~~ sectors 1/5/52.

1/10/52 Occasional sporulated colonies finally developed. (5 days!) Restreak

1/16/52 Residual growth resembling WAc 4. WAc 4 shows too much residuum to be a satisfactory mutant. New growth probably at coincidence of WAc 3 - WAc 4

WAc 3 x 5

December 8, 1951.

A 12/7/51. Crossed on minimal, but WAc5 ap contam. → shows spots of +.

B See 880 Reverse. Cross-Brush WAc3 x WAc5 on minimal agar. 12/8/51

A+B after 3-4 days show prototrophic interaction, no signs of syntrophism.

A. 12/11 Restreak spores on minimal. 12/14. ~~Very few colonies A, none B. (good mountain?)~~

~~12/14 Restreak from 12/9 plate to minimal, complete, also replicate~~

12/12/51 No stable prototrophs found in this series!

((12/12-12/14. at room temp.))

B. 12/12 Restreak spores on minimal agar (separate areas). ~~Isolated~~

12/16. prototroph areas; background mostly auxotrophic. Repicks to minimal medium.

12/20/51. 2: Scant large colonies, background smaller; (new heterokaryons?) residual background heavy — (auxotroph parent) Restreak from 12/16 plating

2: no prototrophs.

12/25/51. One or two prototrophs, mainly satellited. By late growth diffuse prototrophy (reformation of heterocaryons?) Restreak 1, 2 (centers of prototroph growth).

→ complete. Replica to minimal +, - leucine (a) 20γ/each.

↓ gave sporadic prototrophs on minimal. (over)

Heterokaryosis only?

see 883

1/1/51. plate 12/16/51 [illegible]

streak large + prototrophs in minimal.

1/6/51 1 sector largely prototrophic, about 50%

"prototrophic" colonies, may be partly sectored? Restreak

some of these —

886A. 1-4. 1/10 flat growth not sporulated.

WAc 11 : Mutation mm.

WAc 19 x x.

12/29/51.

Harvest from 4 day bottle nutrient agar.
Yield: 3×10^8 (ca 15 ml.) cell count

Dilute to 1.5×10^3/ml. UV 90 sec. Plate on D(Ac)

1/1/52 Considerable variation in pigmentation. Moderate # sectored colonies (sp. texture; pigment ?) on our plates. 1 yellowish colony, isolated on one plate: streaked out + compared with col. from control plating (might be S. griseus). Methylene blue slight but measureable difference permitted.
ca 14 x 10² colonies replicated. 11 poss. mutants recovered by touching spores on D(0); D(Ac). 3 are clearcut mutants with little or no residuum. Pick from D(Ac) to eg. spore suspensions:

WAc 18, 19, 20. Slant. Cross brush these with each other & with WAc 3 on minimal agar.

	WAc 18	WAc 19	WAc 20.
WAc 3	0	~~++~~ past intersection	0
WAc 18			
WAc 19	++ past intersection		
WAc 20	± " "	0	
	no residue	? reversal ?, sl. residual	no residue

1/10/51. Restreak 1/16/52

A WAc 19 x 18 dark gum growth at intersections of smaller colonies.
B WAc 19 x 3 Numerous, moderate sized white colonies
C ~~WAc 18 x 20~~ occasional large dark colonies against micro. background.

1/16/52. (room temperature:) WAc 3 x WAc 19 very heavy dark gum growth at intersections. Background very light.
WAc 18 x 20 similar, no background.
WAc 19 on this plate shows considerable background (from incubation?)

12/31/51.

Cultures received

a WAc 13 S. venezuelae
b 14 S. lavendulae
c 16 S. coelicolor

16 and 17 sporulate poorly on D(0), DAc yellow color

Scrape spores directly from slants to ½ ml H_2O. Estimate density by cytometer. (ca 10^8). Dilute to calculated 10^3/ml; Treat as 883, 887.

1/3/52 c OK.

b, a. n.g. in uv set (too much killing?)
b sporulated poorly on D(Ac), OK on D(0). sign uv killing
a. count on D(Ac) low, on D(0) o. low uv surv.

Retransfer single colonies to DAc bottles for spores
WAc 16 showed two (more?) types of colonies: dark red (on DAc) and v light orange red. Repick & test inheritance of difference. Red colony was used for stock. Lighter colonies are also relatively asporogenous. No mutants in preliminary uv.

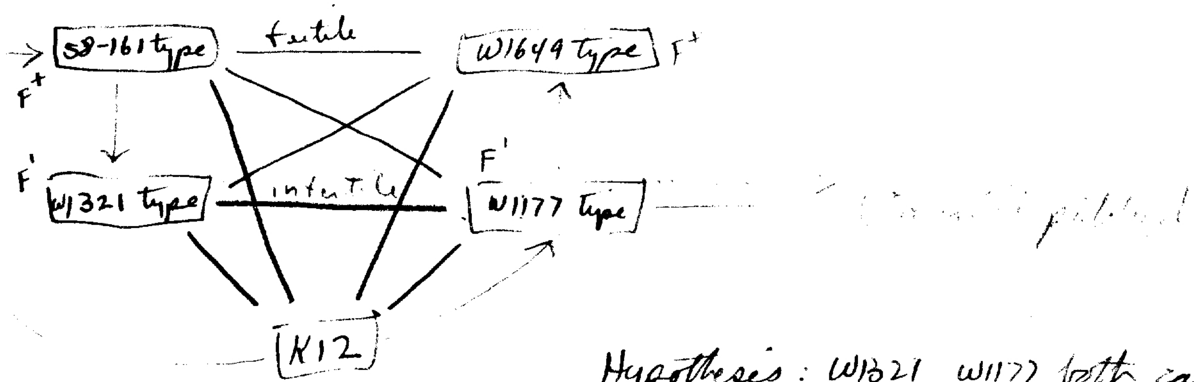
1/10/52. Re-start DAc) agar bottle for spores.

See 894

Incompatibility.

1/4/51.

EML noticed that W1321 (and sublines W1578 from W518) was highly infertile with W1177. Her tests and those of this experiment give following relationships



Hypothesis: W1321, W1177 both carry the same mutant incompatibility allele F'; all others are F⁺ and are freely self- and inter-fertile.

1/3/51. The following crosses were carried out on D/0.

(58-161 × W1177 controls should have been included)

λ is segregating

F' W1321 ×		Yield
F'	W1177	0
F⁺	W1267	+++
F⁺	W1649	+++
F⁺	W 1678 (K12: pol; ser-)	+++

1/5/51. Initiate following crosses: 58-161 × W677

Replating from D/0 to EMSlac, replate each EMS on Slac, D/0

		Yield	SM Lac
1.	Y10 × (W1607 + 58-161)	++	grown together
2	Y10 × W1607 + 58 161	++	Mostly Lac+ S^s / Some Lac- S^R, Lac- S^s, Lac+ S^R / all Lac+ S^s
3	Y10 × W1607	—	———
4	Y10 × 58-161	++	all Lac+ S^s
5	W1607 × (Y10 + W1649)	+++	Mostly Lac - S^R, Some Lac+. Some S^s?
6	" × Y10 + W1649	+++	all Lac -
7	" × Y10	-	+S^s
8	" × W1649	+++	all Lac -

(see over)

This is evidence that F' × F' crosses in presence of F⁺. F' is thus deficient in a "hormone" and "fertile" [illegible] [illegible] [illegible]

(Note Slac was mislabelled EMB!)

Compatibility tests. F? [illegible]

W1607 ×					W1014 × W1015	+
1304	++	1022	+++		×W1649	+
674-680	—	1015	+++			
411	+++	K18Th	—			
-11	—					

[illegible] de [illegible] published!

889: 1 8 [illegible] on EMS Lac

1	+, −
2	0
3	0
4	0
5	+, −
6	−
7	0
8	−

why are controls 2-4 n.g.? 58-161 suspension?

Fertility alteration experiment [illegible]! Strike out exceptional protoplasts to test for [illegible] to use for self-incompatibility expts.

Incompatibilities, addition of tests 889a

1/7/52 W1810 x K12 (SRP) +++ (Mal+ and −)
" x 1177 } D(0) −
" x 1649 } −

1/9/52 Add maltose to part of plate
1/11/52 No effect

1/10/52. Is there a connection between linkage modification and F+?

~~1 W1178 x W1607 : segregation of Mal, S.~~
2 W588 x W1607 " " Lac (cont. plate)
~~3 W1178 x 58-161 Control, seg. Mal, Lac, S.~~
4 W677 x 58-161 " " " "

Lac− > Lac+ Mal− > Mal+

W1178 seems apparently contains segregation of F+

Is λ irrelevant to F+/F−?

1 W1321 x W1027 (F−) } n D(0). } infertile
2 F− x W1267 (F+) } very fertile

A. B.

1 588 x 1607 Lac− > Lac+
2 ~~677~~ 677 x 58-161 Lac− > Lac+
3 W1800 x W1178. Mal+ >> Mal− (∴ BMF− x TLB, F+ f1 shows same ~~abnormal linkage as BMF+ x ...~~)

see 896.

4 1178 x 58-161 Mal+ > Mal− Lac+ > Lac−
5 1178 x W1607 Mal+ > Mal−

Conclusions:

1. Status of W1810 uncertain, check mutations
2. B shows that λ is not directly related to F
3. F+/F− not directly related to abnormal linkage of F.TLB. at least F+ x F− = F+ x F+

[illegible]

1/11/52 W1367 } x 679 SRP } Both fertile, segregating Lac.
W1607 } ∴ 679 is F+

(See over)

W1813 x Y10 +++

W1303 x Y10 +++

679-183 x W1607 ++

Confirms that 679 = F^+

679-680 = F^-

W1033 XX
Hfr.

1/6/52

Harvest 10ml Puamassay tubes to 1ml. Conc xx are, 1ml each ... D(0) plate after ... removed, add 10ml. Dil xx . ... of this ...
No ... before plating.

A.

		Conc	Dil
1	58-161 x W1177	40	3 (+1?)
2	1033 x "	ca 70 (1/2 rare small col)	12
3	58-161 x W1649	50	0
4	1033 x "	17	0
5	58-161 x W1678	~~3~~ 12	0
6	1033 x "	~~3~~ 31	0
7	~~Maas ...~~ W1811 x 1033	0	

These yields on the whole are very low. Repeat.

A' 1/1/51. "Conc" as above. "Dil" = dilute only W1033, do not alter W1077 conc / dil.

1 1033 x 1177 +++
2 " 1649. +++

(no effect of Hfr noted in comp. 58-161 x W1033 x 677.
Re-cover Hfr!)

B. Aerated cultures. Resuspend in 10ml for conc. 1.1/10 for dil. 1ml ...

	Conc	Dil	D(0)
11 W1033 x ~~58-16~~ W1177			
12 58-161 x W1177			
~~13~~			

No yield. Except for rather low yields generally in A, this confirms the sterility of aerated cultures. Try 58-161 x W1177 x 1649 aer and non aer in various combinations to see if it may not act via F^+.

1/9/52

Following cultures grown in Penassay.

1. 58-161 + W1607 + Y10 (100 ml) Sedimented cells and filtered supernatant.
2. 58-161 10 ml
3. Y10 10 ml.

o No titale.
Add "Romaine" 1:1 Penassay. Incr. W1607 11:20 AM – 4:20 PM
(Also incr Y10 in separate culture).

Plate 0 1 2 3 i Y10 on D(o) .2ml 1607; .1ml Y10

1/18/51 No prototrophs in any of the series 0, 1, 2, 3 or when filtrate of 58-161 was used. F+ substance may not be present in filtrate of grown cultures.

1/10/52 Sinterglass U tube, 15 ml Penassay each side.
58-161 + W1607 + Y10 (A) vs W1607 + Y10 (B). 11 AM - 5 30 PM

Plate 6PM on D(o), EMS bac

1/11/52. A ++ B - . 1/12/52. A +++ mostly bact, few –
B —

stimulus is not filtrable!

Do stimulus inherited?
Grow $F^- S^R$ + $F^+ S^S$ together, then inoculate into one broth & select out S^S. Test $F^- S^R$ residue from time to time.

UV Activation of 58-161 for recombination (Hayes)

1/9/52

Harvest 58-161 from 10ml Penassay. Irradiate in saline suspension 100 secs. UV. Incub. Penassay YCx Broth 20 minutes. 11:35 - 12:00. Dilute and cross x W1177 (.1ml each)

Control: 6 prototrophs

UV: ca 200!

This confirms Hayes' claim. Repeat with F⁻ (F⁺ ...) and λ⁻

1/11/52. Repeat similar experiment to "activate" 58-161, W1607, W1248 and ~~W~~ W677.

~~W~~58-161	F^+	A,	A'
W1607	F^-	B	etc.
W1248	λ⁻	C	
W677	F^-	D.	

		EW1649
1	AD	+++
2	A'D	+
3	AD'	±
4	A'D'	+
5	BD	++
6	B'D'	+
7	B'D	+
8	C D	+++
9	C'D	++
~~10~~	~~A E~~	
11	~~A' E~~	
12	~~B E~~	
13	~~B' E~~	

Aggregate of Lac+/- ∴ experiment n.g. unless W1607 is activated by big growth in Penassay! tubes were tele contam. confirmed!

(should be all Lac- ~~some~~) ~~syphers to appear. n.g. est med.~~

~~n.g. est.~~ not also UV < controls.

892a. 1/14/52. Check on BD: effect of yeast extract incubation (as in control for UV) on 1607 F−.

1. W1607 × Y10

2. W1607 (Y.Cx. Broth 20 mins ....) × Y10

A = 58-161 B = W1248 C = Y10

Controls are YCx.

A' UV

A'' ~~dead...~~ saline (cf yexts) control

b.

		D(0) ca:
11	AC	60
12	A'C	0
13	BC	100
14	B'C	10
15	A''C	30

No activation! Dose too high?

(see over)

892 C

1/12/52 ± ① W67 ② W67 uv } × W1649

② was sterile

① gave high yield,

numerous lac+ heterozygotes. [Is W1649 het ?]

① 4 lac+ → all lac v 3 Mal+ S^S
1 Mal− S^R

33 addnl lac + or ? colonies picked from EMS.
16 definitely lac v. Others may be + or v. 6 −.
Replica single colonies. Spot on EMS lac. Bunch / sm EMB / Mal

17 verified lac v. 15 Mal+ } all S^S.
2 Mal +− }

3/27/52. Recheck: W67 × W1177 highly infertile
W67 × W1876 " fertile.

∴ W67 is a very weak F+.

1/10/52

Grow 58-161 A, A' W1607 B, B' Y10 C C' W1649 D D' in 10 ml Penassay I aeration (i antifoam) Harvest aerated 5 in ℓ Unaer to 2".

Harvest and cross in following combinations; on D (O).

A · C	+++
A · D	++
A · C'	++
A · D'	+
A' · C	0
A' · D	+++
A' · C'	0
A' · D'	++
~~B · C'~~	~~0~~
B · C	0
B · D	++++
B · D'	+++
B · C'	0
B' · C	0
B' · D	+++
B' · D'	+++
B' C'	0

(1) $B^a \cdot C^a$ infitile as before

(2) A · C or A · C' futile

(3) ~~A' · C or A' · C'~~ all crosses with D or D' were futile

(4) A' infitile with C or C'. ∴ A' behaves like F^- whereas D' retains F^+ character.

Resistance of D F^+ to F^- behavior on aeration should be verified.

1/13/52 Repeat. A = 58-161 B = ~~W~~ Y10 C = W1649 1 = standing 2 = aerated [3 = oxygenated but A+C from slant inocula did not grow]

A1 B1	+++
A1 B2	++
A2 B1	0
A2 B2	0
A1 C1	+
A1 C2	+
A2 C1	~~+~~ ++
A2 C2	++

This confirms previous study. 58-161 aerated behaves like F^-. Can W1649 be inhibited by oxygen?

1/11/52

1/11 Grow W1607 with 58-161 (and separately) in Permassay.

1/12 (A) Streak out to recover W1607 as Lac- — (B) Inoculate mixed culture, 1:50 into 10 ml tubes of yeast broth to remove bulk of 58-161 11:30 AM – 4 PM.

(C) 1st cultures (before sm selection)

			D(o)	EMS lac	EMB for 58-161
1	58-161	x Y10	~~+++~~		
2	1607	x Y10	0		
3	58-161 + 1607	x Y10	+++	+, −	
#2		x Y10	+++	all +.	

2nd series (4 PM, sm selection)

4	(58-161 + 1607)	x Y10	+++	+, −
5	"	W677.	0	all −
6	1607		6	

3d series (9:30 2d sm selection) 4P12 – 4:30 PM.

7	58-161 + 1607	Y10	+++	+, −
8	"	W677		all −
9	1607	Y10	0	

~~#0~~ 11 AM 1/13/52. 3d sm selection

10	58-161 + 1607	Y10	+++	+, −
~~11~~	~~1607~~	~~Y10~~		

effect of 58-161 on W1607 seems to persist

All 896 also.

A. About 2 lac- : 1 lac+. Collect about 40-60 lac- colonies for reisolated W1607. Inoculate Permassay 1:45 PM. → 1/14 No lac+ in streak. No lac+ detected in streakings of 4, 7. (except 1-5 papillae $< 10^{-3}$).

11	W1607 (58-161 + W1607)	x Y10	+++	+ −
12	W1607	Y10.	0	

F

C. 1/14 Single colonies from restreak of A from Permassay. O = mass culture

PM 1/14.

	D(o)
1	0
2	+++ = W
3	+++
4	+++
5	+++
6	+++
7	+++
0	+++
W1607	0

∴ 6/7 cells were transduced to F+ by growth with F+.

Streak out on Gal EMB. to test heritability from single colonies

F⁻ prototrophs

1/11/52.

1 } F⁻ × F⁻ (S^S) presume of F⁺ { 889-1 ♂ 18 Lac⁻ 15 were S^S
2 } { 889-5 ♂ 13 Lac⁺ 4 were S^S → 3 were Lac⁻ after purn. 1 Lac⁺ (2A)

3 Prototrophs from W1800 × [redacted] W1178

4 " " #58-161 × [redacted] W677

	SRP × W177	W1649
1A	+++	+
B	+++	+
C	+++	+
D	+++	+
2A	+++	+
B	+++	+
C	+++	+
D	+++	+
K-12	+++	+

No F⁻ from presumably F⁻ × F⁻ prototrophs. But see 895 for probable explanation.

Aeration effect: post-aeration

January 14, 1952

Ca. 75 ml 58-161 harvested from Pennassay to 30 ml saline.

4 ml aliquots in each tube 2PM – 4 30 PM 37°.

			A × Y10	B × W1649
1	Refrigerate in saline		3 4	10 11
2	Aerate "	"	1 ✓	20 40
3	" "	D(o)	1 ✓	20 40
4	Oxygenate "	saline	0 –	60 –
5	" "	D(o)	0 ✓	8 ✓
6	" "	Pennassay (1:3)	100	40
7	Initial assay		3 5	2 4
8	Incubate in saline		0 ✓	1 3

A

Antifoam added to each tube.

Results ambiguous owing to low controls.

Repeat Ca 50 ml → ca 2 ml. [× Y10]

1/16/52	1	Initial assay .1 ml		11 50 –
	2	.7 ml + 5 ml D(o)	aerate 1hr.	+++
B	3	" "	incubate.	++

∴ Aeration of washed suspensions is ineffectual.

Repeat aeration effect expt again.

1/18

			1/20	
	1	58-161 × W1177	++	This proves that the aeration effect is related to F (cf 3, 4 which are XX W1177, W1177F+ resp!
C	2	" × 899-5 —	++	
	3	58-161A × W1177	– ✓ 1/22	
	4	" × 899-5 —	+++	
	5			

58-161 str. grown in aerated D(o) + BM or TLB,.

1/19.

			A21	
	1	58-161 × W1177	+ ++ (>100)	Again, the aeration effect was not absolute but correlation with F+ is quite clear.
D	2	58-161A × W1177 A	– ± ? 5 cols	
	3	58-161A × W1177	– ± ? 6 cols.	
	4	58-161A × W1817p A	++ ++	
	5	58-161 × W1817p A	++ +	
	6	58-161 × W1817p	++ ++	
	7	W1607 × ~~W1177A~~ W1817p	++++ +++	
	8	" × W1817pA	++ ++	
	9	58-161 × W1177A	++ ++	

58-161A: aerated in Pennassay from 12N20 – 10A21

C: CO_2 bubbled from ca 6P20 – "

E.	1	58-161	W1177	+++	+++	8. 58-161 W1304	++ 18 cols
	2	" A	W1177	±	2 cols.	9. 58-161A W1304	++ 45 "
	3	" A	W1817	+++	++++		
	4	" C	W1177	±	28 cols.	No effect of ageing	
	5	" C	W1817	+++	+++		
	6	" 4da.	W1177	++	++		
	7	"	W1817	+++	++		

Aeration : air vs N_2 8976

1/24/52

Penassay : 58161 4PM – 10AM.

1 –
2 aerate heavily
0 N_2 bubbled heavily. Growth very poor.
Start agars with 1:20 inocula from ①. 11AM – 4 30 PM.

1 –
2 aerate
3 N_2
4 CO_2 (½ M/10 $NaHCO_3$)
N_2 still inhibits. 4 ÷ 1. 2 >> 1.

Harvest and cross
A W1177
B W1817

1/26/52 all plates sterile!
No apparent source of error

Plate 1/28

1 –
2 aerate (old) strongly
3 " weakly (ca 10 bubbles/minute. little growth stimulation.
4 N_2
5 CO_2

A × W1177
B × W1817

	A 1/24	1/30 A	B	1/30 B	1/24
1	+	+	++		++
2	–	–	+++		+++
3	++	++	++		++
4	+ delayed	+	++		++
5	++ delayed	++	+++		+++

∴ Air not inert gas that causes F+ → F–. Air doesn't seem to influence washed cells, however, according to 8

Oxygenation ; W1649 ; aeration

1/15/52

Grow overnight in Permassay.

A 58-161
B " + Air
C " + O_2
D W1649
E " + O_2
F W1607
G Y10.

+ = 5-20
In general, ++ = 20-100
+++ = 100-600
++++ = >600

		20h.	44h.
1	A D	+	++
2	A E	+	++
3	A G	+	++
4	B D	++	+++
5	B E	++	+++
6	B G	++	+++
7	C D	++	+++
8	C E	++	+++
9	C G	++	+++
10	F D	+	+++
11	F E	-+	+++
12	F G	—	0

No experiment

cells removed ??

Cross delayed.

1/16/52. Repeat test of aeration effect

	A x Y10	B x W1649
1 58-161	++	++
2 58-161 aerated 3		+++

Note divergence of W1649 x with aeration!

1/17/52. A = 58-161 B = 58-161 A C = W1816 D = 1816 A. E = 58-161 48hr F = Y10 "

				48hrs.
1	A	Y10	+++	++
2	B	"	±	5
3	C	"	+++	+++
4	D	"	—	+
5	B	679-680 F+	±	+
6	B	679-183	+++	+++
7	B	W477	—	8
8	B	W588	+++	+++
9	B	W1304	+	+
10	B	W1635	+++	+++
11	B	W1649	+++	++
12	C	"	+	+
13	D	"	-	++
14	E	Y10	++	+
15	E	F	+++	++
16	E	W1649	+	+
17	A	F	+++	++
18	B	F	++ small	++

In these experiments, inhibition by aeration was not absolute. There may be recovery on the plate themselves. The effect parallels the modification of F+ to F-, possibly excepting reaction with W1304 which should be checked in a controlled experiment. Again note possible greater futility of F+ x F- vs. F+ x F+

again 36h. no effect
aeration 58-161 parallels W1816 +++ eff.

1/15/52

1. Washed cells. Mix 58-161 and W-1607 in saline. Incubate 3 - 9 PM. a) Streak out on EMB Lac; b) Inoc. Penassay + sm 10ug/ml. c: λ^+ All XX x Y-10 unless indicated

2. Grow 58-161 + W1321 in Penassay. a&b as above. (a showed background of Lac+ and lambda plaques. 2c λ^S (therefore λ has not been transmitted whereas F+ has).

3. Transmission from lambda-sensitive to lysogenic. W-1655 + W-1607 in Pennassay. Then a&b c: λ^+

4. c: λ^S to sensitive. W-1655 + W-1321....

5. F+ to W-1177. W-588 + W-1177 a & b. (b showed rare + pap. b culture was pure.)

In all cases, Lac- colonies from a) were pooled to make fresh inoculum. Cultures were restreaked to control success of resiolation of F- component. Growth from sm-Penassay in b) was used directly, in each case with only barely detectable Lac+ residuum.

6. Transfer via lambda? 58-161 streaked out on W-1321 on EMB Lac sm. Plaques restreaked. Individual colonies picked and tested for lysogenicity. #1 (out of ca 25) was lysogenic and restreaked, rechecked. Single verified lysogenic colony retained for test of F: (X y-10)

7. Single colonies of W-1816 from stock culture (itself reisolated) streaked on EMB Gal(-). o = stock.

	Tests (x Y-10 exc. 5, x W-1607)		
	a	b	c(= retest pooled Lac- colonies from test streaks of b)
1	−	7 cols. + ?	W1607 cont. −
2	+++	++	+++
3	+++	+++	+++
4	++	+++	+++
5		+++	C: 1 +++ 3 +++ 5 +++ 7 +++ 2 +++ 4 +++ 6 +++ 8 +++ W1177 cont − 4 = W1817 (W1177 F+)
6	−		∴ λ does not transmit F+. Cf. 2.
7	1:+++ 2+++ 3+++ 4+++ 0+++ W-1607 −. F+ of W-1816 is therefore heritable at least 50 cell generations.		

8. Crude supernatant of 58-161. Add sm 10ug/ml. Inoc. W-1607, incubate 1-7 PM. Limited overall growth! Reinoculate loopful to Penassay sm for cross inoculum, and streak out on EMB Lac. (ca 1% Lac+)

9. Whole culture 58-161 inoc. ca 1:20 sm Penassay. Add W-1607 as above.... < .1% Lac+

10. Dense cell suspension 58-161 aerated, as above.... < .1% Lac+

Crosses of sm-selected W-1607 treated component.

8	++
9	+++
10	+++

Is this due to the residual cells? Should be plated out without 2d growth cycle.

Washed cell mixtures are inefficient in transferring F+. λ and F+ are distinct.

Persistence of ~~aeration~~ aeration effects on F+

January 17, 1952.

From various expts. streak out aerated cultures that have shown partial or complete inhibition of F+. Pick single colonies to 1 ml Penassay and test for F (x Y10, W1177 or W1607) by "unwashed" crosses.

A. 898C - B (58-161) 5 cols } all F+
B. 898C - D (W1816) 5 cols. } all F+
C 897 1/18 58-161 5 cols. F+ ~~F~~ F+
D 897 D 58-161 synth. 5 cols. F+
E 897D 58-161 A 1/20 → 1/21 11AM → 4: 1F+ 3 F- 1F± ? E3 F+ E1 F- ✓
F " " C. 4: F+
G Remove. E for iterated aeration 1/21, 2:30 PM → 2F+ 2F - : G1F- ✓ G2F- ✓
H " 1/22 11AM " 3F+ 1-? (contam. on test plate)
I " 1/22 5PM 2F+ 2 - ? "
J " 1/22 830 PM ? cont ? F- "
K " 1/23 9^00 AM F+ (K on suspension x W1177 was ++)

L "
M "
N "

~~= W1830~~

Save E1, G1, G2 for further tests. → later, tested TLB, -
~~a) Re-transduction to F+~~ ∴ n.g.
b) comp. xx W1177, W1817 : by first test, sterile in all combinations! !

~~Fast growth for F - ? Aer 58-161 to aer. Penassay ca 1:100 12^15~~
~~LA Streak out 12^15~~
~~B 3^15 Remove. } Hold in refrigerator~~
~~C 5^15~~

Contaminant or cultures transposed?

No persistent F- found in this series!