Method: W3703 1 ml + W6- 0.1 ml + 5 ml peasyag.

Incubate 24 hrs. at 32°C.

Spread on MLac + M

Replica plate it on M-6lac seeded 2979 on it.

Result: No F+, and no Hfr was observed.
### Table: Experiments on F<sup>R</sup> to F<sub>G</sub>

<table>
<thead>
<tr>
<th>Date</th>
<th>28/06</th>
<th>1959</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Does F&lt;sup&gt;R&lt;/sup&gt; keep F&lt;sub&gt;G&lt;/sub&gt; for a while?</th>
</tr>
</thead>
</table>

**Experimenter's Comment:**

**Method:**

1. **Cultural setup:** Grow overnight culture in 5 ml of medium.
2. **Ratio of mix:** 1:1.
3. **Experimental condition for F<sup>R</sup> reaction:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Untreated</th>
<th>Treated by Sm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>2.</td>
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</tbody>
</table>

**Shaked:** 3 hrs. at 37°C on rotator.

**McGly F<sup>G</sup>**

- **M F<sup>R</sup>**
- **X**
- **F<sub>G</sub>**
- **O**
- **I**
- **N**

**Results:**

- **Recombination:** 3994 (F- G<sub>604</sub> G<sub>804</sub>)
- **3086 (F- F<sup>R</sup>)**
- **Infection:** 3086 + 3994

**Next Step:**

- **Purify:** 4526 + 4526 + 3086 on Blue Sm.
- **Test rec. compatibility by replica method.** (See next p. 176)

- **Treated on MGal**
- **Treated on MGal Sm.**
- **After creation by Sm.**
- **Treated on MGal**
- **Treated on MGal Sm.**

- **Sm.:**
  - **37°C 3 hr. incubation.**
  - **After addition of Sm.**

- **Treated on MGal Sm.**

**Possibilities:**

1. Sm-treated MG<sub>8537</sub> still can transfer F under the pressure of Sm.
2. F<sup>R</sup> can carry F<sub>G</sub> for a while, and possess F<sup>G</sup> character for a while. But it cannot continue these traits.
Continued from former page

Method: 1] 104 and 105 (4534-3036) were streaked on Black Sm.
       2] Replica plate on Mlac B, mixed 072028; and one fertile colony is shown out.

Control. 4534 x 3036
Master plate

4534 x 4526
Master plate

Possibilities:
1] Phoretic mutant of F- (Host range mutant).
2] Spontaneous mutant of FR to Hfr.
   (This seems unlikely, because there is no recombination in No. 3; see p. 76c).

Next step:
1] Repeat this experiment with control.
2] If it is host range mutant of F- it must be non-infective to F- and F-.
Rough estimation of Hoehr's range hypothesis.

1. Mix 4526 Fb with 4526.
2. Incubate for 5 days in a petri dish.

3. Make spot test on M6al. x2979.

If black spot is obtained, it may be a Hoehr's range mutant of Fb.

Result and conclusion: 4526 Fb + 4526 does not show black spot. Fb (Fb+) is still very rare. It is still possible to isolate re-isolate Fb.
27/4 1959

Experiment conditions: 3 0.1 = 0.1 : 5mL

Result: F+  F-
1. A#2, Wk, *r, c, are infective and give low fertile, probably F+ (See back pages).
2. A#2, Wk, *r, c, are sensitive to antibiotic treatment.

#5

C  A0

MacB
X3828

MacB
X3828
Detection of recombinants from double F+ strain.

F+ x F-

# 2 was used. 

<table>
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<td>1959</td>
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<td>F+</td>
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</tr>
<tr>
<td>Method</td>
<td>1. Seed # 2 on Blac. Sin. (x 10^7 x 10^7, 0.1 mL) + (10^8 x 10^7, 0.1 mL) x 2.</td>
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<tr>
<td></td>
<td>2. Replica plate on Mgal. Seeded 2979. 0.1 tt.</td>
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<td></td>
<td>3. Look for Co colonies.</td>
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<table>
<thead>
<tr>
<th># of colonies tested</th>
<th># of colonies tested (on Mgal 2979)</th>
<th>Hfr Colonies</th>
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<tbody>
<tr>
<td>657</td>
<td>510</td>
<td>450</td>
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<tr>
<td>437</td>
<td>427</td>
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<td>48 44</td>
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</table>

These two colonies are F- (See below).

Possibility: 1. spontaneous reversion of F+ or F- to F- (A F-)

Result: F+ was not obtained (negative result was obtained)

Conclusion: F+ and F- lose any allelism and cannot recombine; they are very close with each other or does not make agamos.
an example: Replicate plating method gives clear spots in this case.
Isolate Hfr from W3208, unsuccessful result.

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### Purpose:
It is necessary to get Hfr.

### Method:
1. Take W3208 from stab culture and purify it on Blood.
2. Cross-hybrid the 10 colonies. (X2979 on M6A)
3. Pick Hfr and treat by AO and see if it is susceptible to AO or not.
   
### Method for infection:

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<tbody>
<tr>
<td>1</td>
<td>W0F0, or W3208 X2979</td>
</tr>
<tr>
<td>2</td>
<td>M = 6ml</td>
</tr>
<tr>
<td>3</td>
<td>1ml</td>
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<tr>
<td>4</td>
<td>x 2979</td>
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</table>

### Result:
This strain is infective to F-: F0 (not Hfr)

### Ratio of infection:

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<tbody>
<tr>
<td>Hfr X F-</td>
<td>3086</td>
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<tr>
<td>15/23 X 100 = 65.2 (%) &lt;= (see backpage)</td>
<td></td>
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</table>

### Control:

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<tbody>
<tr>
<td>F0 X 3086</td>
<td>17/21 X 100 = 81.0 (%)</td>
</tr>
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</table>

### Next step:
Treat W3208 with AO, and look for Hfr or use lysophilysed culture for inoculation (no mutation of Hfr).
Comparison of infectivity of Fg to Fg
characterization of Host range mutant of F8. 4526 F, H.

Purpose: Confirm this is Host range mutant of F8, and the trait is inherited directly determined.

1. Infect FGH to W3735 (F^R M) and isolate W3735 FGH.
   - Mix 1:1 in passage (1:10 ; 1:1; 1:1) W3735 FGH.
   - Purify it on B6al and replica plate it onto Blood agar, which soaking in W3735, or W4526.
   - Pick W3735 (F^R) and cross back with W4526, make

Purpose: Put markers into F^R mutant to differentiate between F^R mutants.

2. Isolate Gal- from W4526
   - HFT-6 → W4526
     - 0.2 ml 1 ml overnight culture. Keep this on horn.
     - Spread it B-D, and incubate it overnight.


Result: Infective (very efficient) 100% (2/2).
3828 F7 → Y10.

1st trial:
1. Mix them 1:1.
2. Incubate it overnight.
3. Streak it on Blac. and pick Lac+ Test H2LbLb.

Result: Strong competition was observed. 3828 became selective advantage over Y10.
Unsuccessful all F- try again.

2nd trial:
1. Mix them 3828:Y10 2:1
2. Incubate it overnight. Count colonies.
3. Cross brush Lac+(Y10) against 3828 on Blac B.

Ratio: 33/6 (See back page).
3828 F7 was transferred to Y10.
F7 only gene F, not F3.
Result: they are not $F_3^+$ and $H_+ \cdot F_3 = \text{ not } F^-$. 

Retest: 1, 2, 3. In each retest:

1
2
3
4
5
6
7
8
9
10

Synthesis.
Method

1. Mix them 1:1
2. Incubate it overnight at 32°C
3. Purify it on blue and test lac+ on sex-complementility.

Result:

W60G was obtained after mixed culture with 3828F2 and W6-.

Is it 3828F2 on F+?

Result

4/36 = 5% on MMxy

3828F2 × W6

1 2 3 4 5 6 7 8 9 10
### Infection of F+ and F- to W4528

**U.W. MICROBIAL GENETICS**

#### Method for Selection of More Infective F+:

1. **1st Batch:**
   - Inoculate overnight culture in 5 ml. ca. 10^8 cells/ml.
   - Repeat this process.

2. **2nd Batch:**
   - From 1st experiment: The infectivity of F+ was still not enough. Try selection again using W4528.

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<tr>
<td>8/61</td>
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#### Purpose:
Comparison of the infectivity of F+ mutants.

#### 1st Comparison:

- **W6 F- x W4528**: 0/35 (0%); compare rate of infection.
- **W6 F+ x W4528**: F+ selected on Bgal. (See back page)

#### 2nd Comparison:

- Spontaneous revertants of F+ (non-selected F+) to W4528 were not successful.
- 0/32: 0% rate of infection; all F-.

---

**Note:**
- The selection was done for 10 times: W6 F+.
- From 8/61's experiment, the infectivity of F+ was still not enough. Try selection again using W4528.
m Hhal
x 2979.

2nd trials of infection of F4 to W 4528
Unsuccessful:
May be some reason.
Treat W4552 (3086 Fr.)
with Ao, and reinfect F₁ to it.
Test the possibility of Φ₄ type F'.

Method of treatment: Usual method.

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</table>

Purpose and principle:

3086 Fr. would transfer their F to F⁻. But the infected cell shows low fertility after infection. Then, what factor was left behind at the time of F transfer?

If a factor which determines Hfr character existed in a cell treated with F, one type of strain, reinfection of F₁ to F⁻ obtained after treatment of the F⁻ apprises since Hfr or F⁻ mutant with high frequency.

Hfr

Result: Rate of F⁻ = 0.1% (See back page.)

1st run was not successful.

Use usual method for infection.

2nd run was successful, gradual F⁻. Rate of infection: 0/16 = 0% (See below)

Why?

Conclusion: F₁ x 3086 F⁻ gives phi F⁵, not F⁻.

Reinfection of F₁:

3086 Fr. ± not simple F⁻.

This hypothesis looks incompatible to the result. (3086 F⁻ x W6) gives P₄.

1st run: X2979 on MXyl.

2nd run: X2979 on MXyl.

w x 8552 F₅
**U.W. MICROBIAL GENETICS**

Unsuccessful: compare infectivity of F' of W3642F+ to F'.

Take control (W6) F+ MH5 GAL2 lacY

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<tbody>
<tr>
<td>1</td>
<td>Exp. design:</td>
<td>W3642F+</td>
<td>$\times$</td>
<td>3086</td>
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<td></td>
<td>Select on DBalGal.</td>
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<td>2</td>
<td></td>
<td>W6</td>
<td>$\times$</td>
<td>3086</td>
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<td></td>
<td>Replicate this in MH5 gal</td>
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<td>3</td>
<td>Method:</td>
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<td>4</td>
<td>Result:</td>
<td>W3642F+</td>
<td>$\times$</td>
<td>3086</td>
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<td>Control.</td>
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<td>W6</td>
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<td>$\Sigma$</td>
<td>3442</td>
<td>$\times$</td>
<td>3086</td>
<td></td>
<td></td>
<td>$\Sigma$</td>
<td>W6</td>
<td>$\times$</td>
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Further experiment:
Use 3033 for F receptor.
Agglutination of F' strains.

Starting point: W4243 F+ showed agglutination, but F- was not (A+ sugar- sk).

Young culture: 2 hrs. show no distinct difference between them.

Experiment:

1. Incubate

<table>
<thead>
<tr>
<th>Hfr2 F-</th>
<th>Hfr4 F-</th>
<th>Hfr5 F-</th>
<th>F-</th>
<th>F+</th>
</tr>
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<tbody>
<tr>
<td>0321</td>
<td>0331</td>
<td>WLFp</td>
<td>0536</td>
<td>026R</td>
</tr>
<tr>
<td>W6F2</td>
<td>W4331</td>
<td>WLFP</td>
<td>086Fc</td>
<td>WL-31K</td>
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and incubate them for overnight. All cultures were obtained from stock collection.

In overnight culture, agglutination was not observed.

2. Incubate 0.2 ml of the overnight culture into 5 ml phaenex broth and shake it on rotator at 37°C for 2 hrs. 12:00 AM - 12:31 AM.

Result: Agglutination was not observed in Hfr, F', F+ and F- after 2 hrs. 4 hrs.

Conclusion: Agglutination of F' cells are sometimes observed, but not always does it.

Presumably, it is influenced by experimental conditions sensitively.
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**Principle:**

Control:

- W6 × W4526 FR
- W6 × W3086
- W6 × W4526 Fg⁻ (I)
- W6 × W4526 Fg⁻ (II)
- W6 × W4526 Fg⁻ (III)

**Ratio:**

- 0/26
- 22/28
- 10/31
- 10/29
- 11/27

**Mixed culture:**

- Overnight: W6 1 ml., 8 ml.
- Repeated 3 sec.
- Purify on blood agar.
2nd trial on W4583

Isolation of F3 from F2+

Possibility. Method for detection of F3 with many F- colonies.

Principle:

\[ \mathcal{F}^+_{\text{M}^+ \text{Lac}{-}} \times \mathcal{F}^{-\text{M}^+ \text{Lac}{-}} \rightarrow \mathcal{F}^{-\text{M}^+ \text{Lac}{-}} \]

\[ \mathcal{F}^{-\text{M}^+ \text{Lac}{-}} \times \mathcal{F}^{-\text{M}^+ \text{Lac}{-}} \rightarrow \mathcal{F}^{-\text{M}^+ \text{Lac}{-}} \]

Method:

1. Treat \( \mathcal{W}3828 \mathcal{F}_2 \) with AO (20% solution for 30 minutes, 5°C, 30 minutes).
2. Select the colony on Mlac. End of the system.
3. Crossbreed the treated \( \mathcal{W}3828 \mathcal{F}_2 \) against and then seeded on Mlac.

\[ \mathcal{W}3086 \text{ Mlac} \]

Results: 51/100, 51% increase.

4. Inoculate three females into primary.
5. Make spot test on Mlac. Use control of \( \mathcal{F}_2 \): \( \mathcal{W}3876 \); F- \( \mathcal{W}3828 \)

Result: All of the females are all \( \mathcal{F}_3 \), not F-.

Refer to page 95.
ao treated (w2527f3) w4088

State I (Hlf lae): 10 on Mlac 3086
State II (Lo lae): 32

Tot on 3/9 or F-: in Mlac 7/86

Mlac 2086

1816 3086
1816 3086
1816+3086

Mlac 7/86
7/86 1 1 1
1816 3086
32 32 32 32 32

1816 3086
1816+3086

3086

1816
1816+3086

1816
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1816+3086

1816
1816
1816

1816+3086
## U.W. MICROBIAL GENETICS

**Cross:** F<sub>2</sub> x F<sub>4</sub>  
**Select:** A<sub>1</sub> and A<sub>2</sub>  
**Date:** 20/3/1959

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1. Purify W<sub>6</sub>F<sub>2</sub>, W<sub>6</sub>F<sub>4</sub>, W<sub>3</sub>086 on Blac.
2. Make overnight culture of them.
3. Add 0.2 ml of the culture into pen (5 ml) and incubate 4 hrs.
4. Mix W<sub>6</sub>F<sub>2</sub>, W<sub>6</sub>F<sub>4</sub>, W<sub>3</sub>086 and shake it for 2 hrs. at 37°C.
   - W<sub>6</sub>F<sub>2</sub> × W<sub>6</sub>F<sub>4</sub> × W<sub>3</sub>086
   - W<sub>6</sub>F<sub>2</sub> × W<sub>3</sub>086
   - W<sub>6</sub>F<sub>4</sub> × W<sub>3</sub>086
5. Seed it on Blac Bac. and incubate them.
   - Initial dilution: 10<sup>-3</sup>x10<sup>-1</sup> /plate.
6. Replicate on the 1/4 A<sub>1</sub> seeded W<sub>2</sub>779 and W<sub>4</sub>5-50 (A<sub>1</sub>, F<sub>1</sub>) (0.3 ml/plate).

Result: Utilization of W<sub>6</sub>F<sub>2</sub> in transfer of A<sub>1</sub> is very low as Pate said; therefore it is very hard to kill it for F<sub>1</sub> or not. However, rest of infection under this condition was confirmed.

### Control F<sub>2</sub> × W3086

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<th>5</th>
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<tbody>
<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>241</td>
<td>178</td>
<td>174</td>
<td>152</td>
<td>152</td>
</tr>
<tr>
<td>Total no. of colonies</td>
<td>10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
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### Control F<sub>4</sub> × W3086

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<tbody>
<tr>
<td>F&lt;sub&gt;4&lt;/sub&gt;</td>
<td>269</td>
<td>154</td>
<td>152</td>
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<td>152</td>
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<tr>
<td>Total no. of colonies</td>
<td>10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
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### F<sub>4</sub> x F<sub>2</sub> × W3086

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<tr>
<td>F&lt;sub&gt;4&lt;/sub&gt;</td>
<td>444</td>
<td>77</td>
<td>304</td>
<td>344</td>
<td>259</td>
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<tr>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>77 (F&lt;sub&gt;2&lt;/sub&gt;)01 x F&lt;sub&gt;4&lt;/sub&gt;</td>
<td>717 (F&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>66 (F&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>45 (F&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>43 (F&lt;sub&gt;2&lt;/sub&gt;)</td>
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<td>Total no. of colonies</td>
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<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
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# Effect of U.V. on Infectivity of F-

1959

## Experimental Conditions:
1. Purify all intact or 15 ml of 5% sucrose before use.
2. Use young culture (45° C for 18 hours) in culture tubes.

## Principle:
- W4534 (M+Gal + \( L^{5} \) F-)
- W3637 (M- \( s^{5} \) \( L^{5} \) F-)
- W3104 (Gal+ \( L^{5} \) F-)

### Method:
- Survival of F-: 0.1 ml W4534 UV + W3637 \( 1 ml \times 10^{10} \) to \( 1 ml \times 10^{11} \) in culture tubes.
- Spot on M60/C.

### Table:

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Colony Count</th>
<th>Plate</th>
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### Survival Test:
- Sec. 0, 5, 10, 15 on M60/C, UV.
- Test for Infectivity.

### Test:
- 0, 10, 15 on M60/C, UV.
Infection of F' by killed cell.


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### Principle:

- 4534 F' H only Gp
- 3637 F' H Gp
- 3104 F' Golu Gp

### Method:

1. Treat w/5.1 % and w/5.2 % with various agents.

2. Add 1 ml of F' donor parent overnight growth culture to 1 ml of EtoH, and Cdc3, Cdc4.

3. Add 0.1 ml of 8% (usualy 0.1 ml/100 ml) at room temp, 1.5 min.

4. Expel the treated agent by centrifugation or by sonication.

Cdc3 & Cdc4 is adsorbed onto plastic Petri's disk, therefore it is easily removable by this procedure.

5. EtoH is removed by centrifugation (once).

6. Son is removed by centrifugation (twice).

#### Next step:

- Check various concentration of Sm, and various time of treatment.
**Microbial Genetics**

**Confirmation: Infection of F₈ to F⁻ by Streptomycin-killed cell**

(24 hrs treatment) at 37°C

### Principle:
- W₄₅₃₄ M Only F₈
- W₃₀₈₆ M Only F⁻
- W₃₉₉₄ M Only S₅ᵣ H₅

### Method:
1. Add 0.2 ml of Sm to:
   - Overnight culture of W₄₅₃₄ (Ca 1.0⁸ cells/ml), 2 ml, phenol.
   - Control: non-addition of Sm. 2 ml.

2. Incubate at overnight, at 37°C.

3. Wash twice with broth (2 ml) 5 min., centrifugation.

4. Spot on MGal streaked W₃₀₈₆ + W₃₉₉₄, on it.

### Survival Test

- W₄₅₃₄ treated by 8m (comparative control) x10 = 2.46
- Untreated

### Infectivity of F₈

- W₄₅₃₄ on MGal

**Conclusion:** Sm-killed cells still have some F-infesting ability to F⁻, but it decreases with about < 1 ~ 0.1%.
Transferring ability to infect F\(^1\) to F\(^-\) after killing by Sm.

(Date: 28/VI 1959)

(Short time treatment:)

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<tbody>
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<td>1</td>
<td>200U</td>
<td>y 200</td>
<td>0.2ml Sm solm</td>
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<td>1/2hr</td>
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<tr>
<td>2</td>
<td>200U</td>
<td>x 100</td>
<td>0.1ml Sm solm</td>
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<td>1/2hr</td>
<td>2hr</td>
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**System:**

W7904 F\(6^+\) x W4534 F

W2985 F\(A^-\) x W4576

**Method:**

1. Add 5u 200U/ml. 100u/ml. separately.
2. Incubate them for 1/2 hr. 2 hr.

**Survival test:**

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<th>Time</th>
<th>30 min</th>
<th>100U</th>
<th>200U</th>
<th>2 hr</th>
<th>200U</th>
<th>2 hr</th>
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**Infectivity test:**

W472 B Fe

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<th>200</th>
<th>0%</th>
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Black

2425

3106

3/25

3/106

on H3 0.3

on H30 0.3

on H30 Sm