1/10/42 - Test for α growth in presence of 56°C inactivated γ -

-185	.05 cc 24h aerated B → 20 cc broth, aerate at 37° = B_I		
-10	γ heated assay : 10 c	1 - 0 2 - 0	$[\gamma] < 10^2$
-5	α assay : 2×10^6	3 - 180 4 - 193	$[\alpha] = 7.5 \times 10^9$
-3	B_I assay : 2.5×10^4	5 - 63 6 - 83	$[B] = 3.75 \times 10^7$

0 .85 cc B_I + .15 cc [γ 56°] + .02 cc [α : 10] = B_A, at 37

2.75 .85 cc B_I + .15 cc broth + .02 cc [α : 10] = B_B, at 37

5 B_A .05 cc

7.75 B_B .05 cc

	I 20 cc broth :10 A	II 1 cc → 19 cc :10 A	α assay rel.	III 20 cc broth :10 A	IV 1 cc → 19 cc :10 A	α ass. rel.
10	7 - 168 8 - 190		1 (3.6×10^4)			
12				9 - 176 10 - 190		1
14	11 - 12 - Confluent ~1000		6 ~			
16.75				13 - 240 14 - 210 (:50 A)		6
19	61 - 0 62 - 0 (:10 C)					
21		15 - 16 - ~300	35			
23					17 - 18 - ~300	35
25	19 - Total lysis, 20 - isol. colonies -					
30		21 - 22 - ~300	35			
32					23 - 24 - ~300	35
34	25 - 0 26 - 0 (:10 C)					

Comment: α grows normally in presence of 56°C inact. γ -

[α] titer = 7.5 × 10^9 -

[α] input = 1.5×10^7

[α] inf. cent. = 1.45×10^7

PST	Test for γ growth in presence of UV inact. γ -
-150	.05 cc 24h aerated B → 20 cc broth, aerate at 37° = B_I
-10	γ assay (small tube black stopper, 11/7) : 2×10^6 51 - 389, 52 - 305 [γ] = 1.4×10^{10}
-4	B_I assay : 2.5×10^4 1 - 152, 2 - 169 [B] = 8×10^7
-2	.85 cc B_I + .15 cc [UV γ 10 min] = B_γ, at 37° -
0	.02 cc [γ :10] → B_γ
5	.04 cc B_γ → 2 cc broth of tube, prewarmed -

cf. 4m, supernatant : 20 B 3 - 413, 4 - 407

	I: 1cc → 9 cc broth :10 B	II: .1cc → 9.9 cc broth :10 B	rel. assay
8	5 - 127, 6 - 122		1
19	7 - 126, 8 - 121		1
22	9 - 82, 10 - 69 (:20)		1.2
30		11 - 3, 12 - 9	5
50		13 - 41, 14 - 46	34

In B_γ : [γ] input = 2.8×10^7 [B] = 6.8×10^7

[γ] free = 8.2×10^6 = ~ 29 %

[γ] adsorbed = 19.8×10^7 = ~ 71 %

[γ] inf. cent. = 1.25×10^7 $\left(= \gamma \text{ free} + \frac{\gamma \text{ adsorbed}}{4.5} \right)$

Step size = 34 [γ] in tube I : 8m : 2.5×10^4

50m : 8.5×10^5

$$\frac{\gamma_{50^m}}{\gamma_{inf.cent._I} - \gamma_{free_I}} = \frac{8.5 \times 10^5}{8.5 \times 10^3} = 100$$

Comment :

γ growth is small and slow - Incertitude due to unusually high γ titer (confirmed, however, by regular adsorption) - Apparently only a small number of infected bacteria can show as a plaque and give a burst (~ 1/5) - The ratio $\frac{\gamma \text{ inactiv.}}{B}$ is about 10 ; a bacterium should have in average 8 - 8 inact. particles - Possibility of some particles of active γ reaching bacteria not yet infected by inact. γ -

Test for α adsorption on B, [B + γ], [B + UV inact. γ]

PST	
-20	.15 cc 24h aerated B → 20 cc br., aerate at 37° = B_I
-10	α assay : 2×10^6 1 - 219, 2 - 180 [α] = 8×10^9
-3	B_I assay : 2.5×10^6 3 - 215, 4 - 210 [B] = 1.05×10^8
0	.85 cc B_I + .15 cc [γ 1/7] + .02 cc [α:10] = A
1	.85 cc B_I + .15 cc [γ UV 10m] + .02 cc [α:10] = B } at 37°
2	.85 cc B_I + .15 cc [broth] + .02 cc [α:10] = C
4	.02 cc A → 2 cc broth not prewarmed = B_A → 1 cc → cf tube Cf_A
5	.02 cc B → 2 cc broth " " = B_B → 1 cc → cf tube Cf_B } cf. 4m
6	.02 cc C → 2 cc broth " " = B_C → 1 cc → cf tube Cf_C
7	B_A : 50 [col. A] 5 - 95, 6 - 113
8	B_B : 50 " 7 - 118, 8 - 113
9	B_C : 50 " 9 - 184, 10 - 164
11	5m Cf_A : 20 [col. A] 11 - 185, 12 - 200
to	" Cf_B : 20 " 13 - 243, 14 - 245
13.5	" Cf_C : 20 " 15 - 174, 16 - 203

α input = 1.6×10^7

	A	B	C
α free =	$.77 \times 10^7$ (48%)	$.98 \times 10^7$ (60%)	$.76 \times 10^7$ (48%)
α inf. centers =	1×10^7 (62%)	1.15×10^7 (72%)	1.7×10^6 (100%)

Conclusions:

The adsorption of α on B is probably not significantly changed by the presence of γ.

The infective centers are as many as the α input in in absence of γ. In presence of γ only about 20% or less of α adsorbed show as plaques.

PST	Test for α growth in presence of [60 m UV γ] -
150	.05 cc 24 h aerated B → 20 cc broth, aerate at 37° = B_I
0	.85 cc B_I + .15 cc [γ UV 60 m] + .02 cc [α : 10] = $B_{\alpha\gamma}$, at 37°
5	$B_{\alpha\gamma}$ .05 cc →

	I → 20 cc broth	II 1 cc → 19 cc broth	rel. assay
	1 : 10 B	: 10 B	
9	1 - 173 2 - 175		1
14	3 - 4 - confluent (<1000)		5
20		5 - 6 - ~400	45
32		7 - 8 - ~400	45

Comment : α grows as usual -

All α input (see other experiments) shows up as infective centers.

Necessity to repeat the experiment using A as plating strain.

Test for changes in bacteria after action of UV_γ and of $[UV_\gamma + \alpha]$

PST

-150 .85 cc 24h aerated B → 20 cc broth, aerate at 37° = B_I

-3 B_I assay : 2.5×10^4 1 - 83, 2 - 63 $[B] = 3.75 \times 10^7$

0 .85 cc B_I + .15 cc [γ irr. UV 10^m] = B_γ

18 B_γ : 10^3 3 - 25, 4 - 19 (normal colonies).

2 .85 cc B_I + .15 cc [γ irr. UV 10^m] = $B_{\alpha\gamma}$

5 .02 cc [α:10] → $B_{\alpha\gamma}$

14 $B_{\alpha\gamma}$: 10^3 5 - 41, 6 - 24 (irregular colonies, in partial lysis).

After 24 h. the tube $B_{\alpha\gamma}$ shows lysis (almost complete clearing).

Comments.

$[B]$ input $= 3.2 \times 10^7$
$[B]$ colonies in $B_\gamma = 4.4 \times 10^5$
$[B]$ colonies in $B_{\alpha\gamma} = 6.5 \times 10^5$

Bacteria in presence of UV inact. γ do not reproduce any active γ, but are, either lysed or inhibited from reproduction. Moreover, their descendent seem to be still sensitive to α. *
(possibility that even the firstly γ infected bacteria undergo lysis by α without reproducing any active phage).

Different possibilities: 1) B are lysed by UV_γ. (with or without reproducing inact γ)
2) B " inhibited " UV
3) B " " " " and lysed by α (id.).
4) B " " and become carriers of γ UV.

Other possibilities.

* Repeat experiment of α growth in presence of UV γ, sampling until 300 min.

See 1/21 -

Colonies from plates 3 and 4 (B + UV γ) passed on agar slant grow normally - Slant kept in ice-box - [Bγ] labeled.

Colonies from plates 5 and 6 (B + UV γ + α) passed on agar slant give after 24 h only a few colonies of secondary growth. The main area of the slant has undergone [α] lysis. Slant kept in icebox - [Bαγ] labeled.

Comment -
Bacteria grown from B + γ give descendence which is sensitive to α [even if α has acted upon the first generation (not sure, as only a few bacteria of the first gen. had been attacked by α, see 1/21)] The resistance to α is probably only of those bacteria which have been attacked by UV γ - The lysis in the tube Bαγ is probably due to proliferation of the [UV γ infected bacteria], elimination of γ and recovery of sensitivity to α -
Test whether [UV treated B] are still sensitive to γ after division.

Tube B αγ 1/21, containing B + UV γ + α (clear after 24 h, cloudy after 30 h) carried through three passages in broth (dilution more than 10^{-4} each passage) labeled as tube I

Tube Bγ 1/21, containing B + UV γ, cloudy after 24 h, carried through three passages in broth (id.), labeled as tube II.

1/26 - Tube I from third passage in broth inoculated on slant (I), then filtered, and filtrate stored (I filtrate 1/26) -

Tube II from 3d passage in broth inoculated on slants (II) then filtered, and filtrate stored (II filtrate 1/26).

1/27 • Test for acquired resistance after the action of $UV\gamma$ and of $UV\gamma + \alpha$.

	plated with α	γ
$B\alpha\gamma$	0	400
$B\gamma$	250	400
I	0	400
II	250	400

Comment. $B\gamma$) Bacteria B, acted upon by $UV\gamma$ for 18 min, then plated, give a few colonies of bacteria normally sensitive to α and γ (explains why there is lysis in broth with $B + UV\gamma + \alpha$).

$B_{\alpha\gamma}$) Bacteria B, acted upon by $UV\gamma$ and α, to 10 min ~~give~~ then plated, give colonies of bacteria resistant to α and ~~resist.~~ sensit. to γ (why are the first colonies "nibbelt"? 1/21). [Because they had not been acted upon by α (not in excess 1/21).]

I) and II) the same characters have the bacteria developed from the same treatment, but passed 3 times in broth.

$UV\gamma$ doesn't produce resistance in bacteria. [Question: have the bacteria which still develops after action of $UV\gamma$ been infected with $UV\gamma$ at all?] The resistance seems to be produced by growth in presence of active phage only.

The few colonies which develop from $B + UV\gamma$, being sensitive to γ, seem not to be originated by ~~the first~~ some bacteria originally present, ~~which were~~ insusceptible to γ and which therefore didn't adsorb it. One must think that the resistance is induced upon a few bacteria by active γ.

Microscopic observation of bacteria $B + UV\gamma$.

-150	.05 cc 24^h aer. $B \rightarrow$ 20 cc broth, aer. at 37° = B_I
0	.85 cc B_I + .15 cc [$UV\gamma$ 10^{m}], at 37° = B_γ
11	.025 cc B_γ plated, covered with coverslide, and observed. see ~~page~~ map on the opposite side.

Comment. Most bacteria, in presence of $UV\gamma$ in excess, seem to be stopped in their dividing capacity. Some of them (a few percent) undergo a process of clearing and swelling (lysis from without?), some become longer and longer. Exceptionally succeed some bacteria ~~to~~ dividing.