1/10/62 - Test for α growth in presence of 56°C inactivated γ -

-185 | .05 cc 24^h 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 - 93 — $[B] = 3.75 \times 10^7$

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

2.75

5 | B_A .05 cc

7.75

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

B_B .05 cc

	I: 20 cc broth	II: 1 cc → 19 cc	α assay rel.	III: 20 cc broth	IV: 1 cc → 19 cc	α ass. rel.
	:10 A	:10 A		:10 A	:10 A	
10	7 - 168, 8 - 190		1 (3.6×10^4)			
12				9 - 175, 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 - , 20 - Total lysis, inf. 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~~ $\times 10^9$ -

[α] input = 1.5×10^7

[α] inf. count = 1.45×10^7

Test for γ growth in presence of UV inact. γ.

PST	
-150	.05 cc 24h aerated B → 20 cc broth, aerate at 37° = B_I
-10	γ assay (small tube block stopper, M/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: 1 cc → 9 cc broth :10 B	II: .1 cc → 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 = 2.8×10^8 [B] = 6.8×10^7

[γ] free = 8.2×10^6 = ~ 29 %

[γ] adsorbed = $19.8\ 10^8$ = ~ 71 %

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

Step size = 24 — [γ] in tube I : 8m : 2.5×10^4

50m : 8.5×10^5

$$\frac{\gamma_{50^m}}{\gamma_{inf.c._I} - \gamma_{free\,I}} = \frac{8.5 \times 10^5}{8.5 \times 10^3} = 100$$

Comment :

γ growth is small and slow – Uncertitude 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 .1 cc 24h aerated B → 20 cc br., aerate at 37° = B_I

-10 α assay : 2×10^6 1 - 219, 2 - 180 $[\alpha] = 8 \times 10^9$

-3 B_I assay : 2.5×10^6 3 - 215, 4 - 210 $[B] = 1.05 \times 10^8$

0 .85 cc B_I + .15 cc [γ 11/1] + .02 cc [α : 10] = A

1 .85 cc B_I + .15 cc [γ UV 10m] + .02 cc [α : 10] = B

2 .85 cc B_I + .15 cc [γ broth] + .02 cc [α : 10] = C

} at 37°

4 .02 cc A → 2 cc broth not prevarmed = B_A → 1 cc → cf tube Cf_A

5 .02 cc B → 2 cc broth " " = B_B → 1 cc → cf tube Cf_B

6 .02 cc C → 2 cc broth " " = B_C → 1 cc → cf tube Cf_C

} cf. 4m

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 Sm 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

α free = (A) $.77 \times 10^7$ (48%) (B) $.98 \times 10^7$ (60%) (C) $.76 \times 10^7$ (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	II	
	→ 20 cc broth	1 cc → 19 cc broth	rel. assay
	110 B	: 10 B	
9	1 - 173 2 - 175		1
14	3, 4 = confluent (<1000)		5
20		5 - ~400 6 -	45
32		7 - ~400 8 -	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_f and of $[UV_f + \alpha]$

PST

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

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

0 .85 cc B_I + .15 cc [f irr. UV 10^m] = B_f

18 B_f : 10^3 $\frac{3}{4} - \frac{25}{19}$ (normal colonies).

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

5 .02 cc [α:10] → $B_{\alpha f}$

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

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

Comments.

$[B]$ input $= 3.2 \times 10^7$

$[B]$ colonies in $B_f = 4.4 \times 10^5$

$[B]$ colonies in $B_{\alpha f} = 6.5 \times 10^5$

Bacteria in presence of UV inact. f do not reproduce any acting f, but are, either lysed or inhibited from reproduction. Moreover, their descendent seem to be still sensitive to α. *

(possibility that even the firstly infected bacteria undergo lysis by α without reproducing any acting phage).

Different possibilities: 1) B are lysed by UV_f. (with or without reproducing virus f)

2) B " inhibited " UV

3) B " " " " and lysed by α (id.).

4) B " " and become carriers of f UV.

Other possibilities.

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

See 1/21 -

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

Colonies from plates 5 and 6 (B + UV y + 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 ice box - [B αy] labeled

Comment -

Bacteria grown from B + y 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 y - The lysis in the tube B αy is probably due to proliferation of the [UV y infected bacteria], elimination of y and recovery of sensitivity to α -

Test whether [UV treated B] are still sensitive to y after division.

Tube B α y 1/21, containing B + UV y + 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 B y 1/21, containing B + UV y, 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 γ and of UV γ + α.

	plated with α	γ
Bαγ	0	400
Bγ	250	400
I	0	400
II	250	400

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

Bαγ) Backeria B, acted upon by UV γ and α, to mini then plated, give colonies of backeria resistant to α and ~~resist.~~ 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 γ doesn't produce resistance in bacteria. [Question: have the bacteria which still develops after action of UV γ been infected with UV γ at all?] The resistance seems to be produced by growth in presence of active phage only.

The few colonies which develops from B + UV γ, being sensitive to γ, seem not to be originated by ~~the few~~ the same bacteria originally present, ~~which were~~ unsusceptible to γ and which therefore didn't adsorb it. One must think that the resistance is induced upon a few backeria by active γ.

Microscopic observation of bacteria B + UV γ.

−150	.05 cc 24 h aer. B → 20 cc broth, aer. at 37° = B_I
0	.85 cc B_I + .15 cc [UV γ $10^{m\mu}$], at 37° = B_γ
11	.025 cc B_γ plated, covered with coverslide, and observed.

see map on the opposite side.

Comment. Most bacteria, in presence of UV γ 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 within?), some become longer and longer. Exceptionally succeed some bacteria in dividing.