In the light of these results, it seems most unfortunate that much of the work on the shape and size of muscle fibre has been carried out on material fixed in formalin.

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RADIOBIOLOGY

Detecting Reversion in *T4rII* Bacteriophage to r^+ induced by Ultra-Violet Irradiation

During investigations into the mechanism of ultra-violet mutagenesis in extracellular phage, the following test was developed for estimating the extent of reversion of bacteriophage *T4B* from *r*II to r^+ induced by ultra-violet irradiation.

All *T4r*II mutants were obtained in this laboratory by ultra-violet irradiation of *T4B*r^+ phage. 12 hr. cultures of *Escherichia coli B* and *K*–12 (λ), obtained in 1958 from E. Freese, were used in assays of *r*II and r^+ phage respectively. Agar media were those of Chase and Doerman[1]; broth media contained, per litre ion-free water, 10 gm. tryptone, 8 gm. sodium chloride, and 1 gm. glucose. Phage were diluted and irradiated in this broth. All assays were performed by the agar layer method of Adams[2]. To detect as few as 20–30 r^+ phage among 5×10^8 *r*II phage the concentration of bacterium *K*–12 (λ) per soft agar tube approached 2×10^9.

A 15-watt 'Sylvania' 17-in. low-pressure germicidal lamp was used to irradiate all samples at a distance of 67 cm. and at 4° C. Phage samples were distributed as two-drop aliquots on glazed white porcelain spot test plates which were agitated during exposure. Irradiated phage were handled in dim light to avoid photoreactivation. Under these conditions a standard inactivation curve, graphed as the logarithm of percentage survivors against ultra-violet dose, was linear to 0·001 per cent survivors; 55 sec. of ultra-violet irradiation resulted in a survival-level of e^{-1}.

Each of 12 *r*II mutant stocks was assayed on *B* and on *K*–12 (λ) to determine accurately the titre of total and of r^+ phage. Triplicate samples of each phage stock were irradiated to various survival-levels as indicated in Table 1. Two to three aliquots of these samples were then assayed on bacterium *K*–12 (λ) to obtain the titre of r^+ phage (background plus ultra-violet-induced mutants). Remaining aliquots of these samples were assayed on bacterium *B* to provide the exact survival-level.

Results are presented in Table 1. Clearly 5 of the 12 mutants revert to r^+ when irradiated with ultra-violet. The extent of reversion ranges from 1·81 $r^+/10^6$ survivors to $4{\cdot}2 \times 10^5$ $r^+/10^6$ survivors. Two other mutants (*UF*54 and *UF*147) may be weakly revertible but cannot be definitely classed as such since the proportion of r^+ phage per 10^6 surviving phage is so low as to make an accurate assay of their number unfeasible by this technique.

Control experiments by Krieg[3] ruled out the possibility that the observed increase in r^+ phage might be due to ultra-violet-induced selection for r^+ phage present in the stocks before irradiation.

This technique for detecting reversion differs from Krieg's[3] in the following points. (1) Ultra-violet-irradiated phage are directly assayed for their r^+ content with no intervening cycle of phage growth. (2) Our average number of lethal hits per phage was considerably lower than Krieg's[3], yet reversion was still demonstrable. (3) Our multiplicities of infection of bacterium *K*–12 (λ) with ultra-violet-irradiated phage during r^+ assay were of necessity less than 1. Krieg's[3] multiplicities of bacterium *B* during the first cycle of ultra-violet-irradiated phage growth were considerably greater than 1, and lead to multiplicity reactivation. The results presented here lead one to surmise that multiplicity reactivation is not a prerequisite occurrence for the ultra-violet-induced reversion of *r*II to r^+ phage.

The possibility is not ruled out that, when present, multiplicity reactivation may of itself lead to the production of revertants.

Different *r*II mutants appear by this simple test to differ appreciably in their ability to be induced to revert by ultra-violet-irradiation. Among the 5 *r*II mutants which could be induced to revert, the extent of reversion ranged from 1·81 to $4{\cdot}2 \times 10^5$ r^+ per 10^6 surviving phage. It is interesting that all those mutants which are most easily reverted by ultra-violet irradiation are also high-frequency spontaneous reverters. The compartmentalization of this small sample into ultra-violet-revertible and ultra-violet-non-revertible is suggestive of some specific mutagenic effect of ultra-violet light upon free phage. Investigations concerning this specific ultra-violet mutagenic effect on free phage, from r^+ to *r*II and from *r*II to r^+, will be published elsewhere.

Table 1. Extent of Reversion of *T4r*II to r^+ induced by Ultra-violet Radiation

Mutant	n where $e^{-n} = S/S_0$	Stock titre $\times 10^9$	r^+_s	r^+_s (S/S_0)	Total r^+ after ultra-violet irradiation	r^+ ultra-violet per 10^9 S	Reversion group
*UF*2	0·60	5·6		533	360	0	*NR*
			970				
*UF*2	9·9	5·6		0·003	0	0	
*UF*54	0·97	4·8		199	290	0·06	*NR*
			470				
*UF*54	6·3	4·8		0·8	0	0	
*UF*147	0·88	2·0		338	630	0·35	*NR*
			805				
*UF*147	5·3	2·0		4·03	1 plaque	—	
*UF*125	0·57	8·3		950	1,180	0	*NR*
			1,670				
*UF*125	7·4	8·3		1·01	2 plaques	—	
*UF*211*A*	1·6	15·0		1,230	1,010	0	*NR*
			6,150				
*UF*211*A*	7·7	15·0		1·35	0	0	
*UF*344*A*	3·4	10·0	10,700	353	950	1·81	*Rw*
*UF*165	1·9	10·0	3,300	495	480	0	*NR*
*UF*114	3·0	2·5	1,010	50·5	300	2·01	*Rw*
*UF*144	13·0	6·0	$1{\cdot}7 \times 10^7$	49·4	$6{\cdot}84 \times 10^3$	$4{\cdot}2 \times 10^5$	*Rs*
*UF*173	5·8	13·0	$1{\cdot}1 \times 10^6$	$3{\cdot}2 \times 10^2$	$3{\cdot}5 \times 10^3$	83	*Rs*
*UF*518*B*	8·0	14·0	$1{\cdot}1 \times 10^7$	$3{\cdot}8 \times 10^3$	$3{\cdot}7 \times 10^4$	$7{\cdot}44 \times 10^3$	*Rs*
*UF*50	0·39	2·1	$0/10^9$	0	0	0	*NR*

n, Average number of ultra-violet 'hits' per phage where $e^{-n} = S/S_0$. S/S_0, percentage of survivors; r^+_s, titre of r^+ phage present per ml; phage stock before irradiation; r^+, ultra-violet, total r^+ after ultra-violet $- r^+ (S/S_0)$. Reversion group: *NR*, non-reverter; *R*, reverter: *s*, strong; *w*, weak.

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