PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 35 February 15, 1949 Number 2

*EFFECT OF VISIBLE LIGHT ON THE RECOVERY OF STREPTOMYCES GRISEUS CONIDIA FROM ULTRA-VIOLET IRRADIATION INJURY**

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Communicated by M. Demerec, December 5, 1948

It is well known that cells rendered non-viable by ultra-violet or x-irradiation[1, 2, 3] may at times regain their viability if stored under suitable conditions after irradiation. In the case of microorganisms the criterion for viability is usually the ability to form a colony on a solid medium. By recovery is meant the restoration of the ability of an irradiated micro-organism to grow and form a colony.

Little is known about the mechanism of the recovery phenomenon; experimental results reported in the literature have been extremely variable. Moreover, at best only a small percentage of the cells rendered non-viable in an irradiated population recover their viability—that is, the over-all recovery is usually relatively slight.

During a study of antibiotically active mutants in actinomycetes[4] we observed that the per cent survival of ultra-violet irradiated *Streptomyces griseus* ATC3326 (a non-streptomycin producer) conidia increased about 10-fold when irradiated suspensions were stored one or two days following irradiation. So little was known about the recovery phenomenon, with which our observation was obviously connected, and the implications of this phenomenon to genetics, medicine, and cellular physiology seemed so important to us, that an intensive study of recovery from irradiation was initiated.

Since observers have found recovery to take place when irradiated cells are stored in the cold,[3] and since our own first observations were made on suspensions which had been stored in the ice box, the first study was one on effect of temperature. It was soon clear that recovery was not dependent on storage in the cold. However results were extremely variable even in duplicate experiments; for example, one suspension of ultra-violet irradiated spores showed no recovery upon storage at 35°C., while another

suspension prepared from the same lot of spores and irradiated in exactly the same way, showed a 100,000-fold recovery. Some variable factor seemed present in our experiments which overshadowed in importance the effect of temperature *per se* on recovery. Careful consideration was made of variable factors which might have accounted for such tremendous variation. We were using a glass-fronted water bath placed on a table near a window, in which were suspended transparent bottles containing the irradiated spores. The fact that some of the bottles were more directly exposed to light than others suggested that light might be a factor. Moreover, the greatest and most consistent recovery in our preliminary experiments had taken place in suspensions stored in transparent bottles at room temperature on an open shelf exposed to diffuse light from a window. Experiment showed that exposure of ultra-violet irradiated suspensions to light resulted in an increase in survival rate or a recovery of 100,000- to 400,000-fold. Controls kept in the dark (experiments were made between 15°C. to 37°C. only) showed no recovery at all.

The magnitude of the light effect can hardly be overemphasized. The recovery was so much more complete than any previously observed, that we felt we were dealing here with a key factor in the mechanism causing inactivation and recovery from ultra-violet irradiation.

Methods.—The ultra-violet source was a General Electric 15-watt germicidal lamp, 80 per cent of whose ultra-violet radiation was at 2537 Å. The spores of *S. griseus* ATC3326 were suspended in saline or distilled water and irradiated in a thin layer in an open petri dish placed under the ultra-violet source. The suspension was shaken gently during irradiation. Preparation of spores, irradiation, and assay for viable count were otherwise similar to those described previously.[4, 5] Following ultra-violet irradiation, the spore suspensions were placed in glass bottles or test tubes and suspended in a thermostatically controlled glass-fronted water bath. Visible light illumination from various sources as described under individual experiments was directed against the suspension. The light passed through two glass thicknesses, and about $^1/_2$ cm. of water, before reaching the ultra-violet irradiated cells. Filters were used in later experiments as described below. Counts were made of the viable cells in a suspension by plating on nutrient agar and incubating 3 days at 28°C. Ultra-violet treated cells which were to be kept in the dark were placed in a covered can suspended in the water bath.

Effect of Dosage of Ultra-Violet Light on Recovery.—Conidial suspensions were irradiated with ultra-violet at 60 cm. distance from the lamp (intensity about 960 ergs $\times$ min.$^{-1}$ $\times$ mm.$^{-2}$) for periods indicated in table 1. Immediately after irradiation the suspension was divided into two portions, one of which was kept as a dark control, and the other exposed to light from a window about 2 feet away. In this early experiment the

visible light source was not controlled, the suspensions being in the dark at night, and subject to variation in light intensity during the day. However this experiment shows well the consistent recovery which occurred only in the light. Non-ultra-violet treated controls were little affected by visible light, there being if anything a decrease in the count in the light-exposed tubes. In no case did the tubes of ultra-violet treated cells kept in the dark show significant recovery, while in all cases the light-exposed tubes showed recovery varying from 14- to over 72,000-fold according to ultra-violet dosage in this experiment. If the decrease in count of the non-ultra-violet irradiated suspension exposed to light is taken into account it is seen that in the 4-minute experiment the recovery is complete by the fifth day, the count in the ultra-violet irradiated suspension (1.8×10^5) equaling that of the non-ultra-violet irradiated suspension (1.7×10^5).

TABLE 1

EFFECT OF DOSE OF ULTRA-VIOLET IRRADIATION ON RECOVERY IN THE VISIBLE LIGHT. NUMBER OF VIABLE CELLS PER ML. OF SUSPENSION AFTER HOLDING IRRADIATED CELLS IN LIGHT (*L*) AND IN DARK (*D*)

ULTRAVIOLET IRRADIATION, MIN. AND SUBSEQUENT TREATMENT	TIME AT 35° SUBSEQUENT TO ULTRA-VIOLET IRRADIATION						MAXIMUM INCREASE IN SURVIVAL RATE
	0	2 HRS.	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	
0 *D*	2.4×10^6	...	1.5×10^6	1.1×10^6	6.3×10^5	6.3×10^5	3.7×10^5	...
L	2.4×10^6	...	1.5×10^6	9.1×10^5	5.1×10^5	2.9×10^5	1.7×10^5	...
4 *D*	6.2×10^4	...	2.2×10^4	1.3×10^4	1.1×10^4	7.4×10^3	9.7×10^3	...
L	6.2×10^4	...	8.6×10^5	4.8×10^5	2.4×10^5	2.0×10^5	1.8×10^5	14-fold
5 *D*	5.1×10^3	...	4.9×10^3	4.7×10^3	4.4×10^3	4.6×10^3	4.9×10^3	...
L	5.1×10^3	...	7.4×10^5	4.3×10^5	1.7×10^5	9.1×10^4	5.4×10^4	145-fold
6 *D*	60	...	4.1×10^2	15	20	40	55	...
L	60	...	4.9×10^5	2.5×10^5	2.0×10^5	1.6×10^5	1.2×10^5	8,200-fold
7 *D*	15	...	27	15	3	18	20	...
L	15	4.0×10^4	1.5×10^5	3.7×10^4	1.9×10^4	1.5×10^4	9.3×10^3	10,000-fold
8 *D*	<2.5	...	10	20	18	45	40	...
L	<2.5	...	1.6×10^5	7.4×10^4	3.9×10^4	2.7×10^4	1.9×10^4	>64,000-fold
9 *D*	<2.5	...	<2.5	<2.5	<2.5	<2.5	<2.5	...
L	<2.5	...	1.8×10^5	3.6×10^4	2.9×10^4	5.8×10^4	1.6×10^4	>72,000-fold

Effect of Intensity and Duration of Visible Light

Illumination.—A conidial suspension was irradiated with ultra-violet for $1^1/_2$ minutes at 20 cm. distance from the mercury lamp. Immediately after irradiation it was placed in a 28°C. water bath and exposed to as high an intensity of artificial light as was conveniently possible to obtain in our laboratory (two photoflood lamps and light from a projection lantern, all placed about 30 cm. from the cells). Table 2 shows the extent of recovery after various time periods. The temperature of the cell suspension did not rise more than 2 degrees during the illumination. Recovery is proportional to duration of illumination, within limits.

TABLE 2

EFFECT OF DURATION OF VISIBLE LIGHT ILLUMINATION ON RECOVERY

ILLUMINATION TIME, MIN.	VIABLE CELLS PER ML. OF SUSPENSION	RELATIVE INCREASE IN SURVIVAL RATE
0	2.5*	...
10	2.5×10^3	1,000-fold
20	9.2×10^3	3,700-fold
30	1.3×10^5	52,000-fold
40	1.6×10^5	64,000-fold
50	2.0×10^5	80,000-fold
60	5.3×10^5	210,000-fold
145	5.5×10^5	220,000-fold
173	7.7×10^5	310,000-fold
240	8.0×10^5	320,000-fold

* The count of the non-ultra-violet irradiated suspension was $4.2 \times 10,^6$ so that the survival rate at time zero was 6.0×10^{-7}.

TABLE 3

EFFECT OF TEMPERATURE ON RATE OF RECOVERY. ILLUMINATION PERIOD CONSTANT

	VIABLE CELLS PER ML. AT VARIOUS TEMPERATURES				
	20°C.	25°C.	30°C.	35°C.	40°C.
Exp. 1*	9.6×10^3	3.9×10^4	3.6×10^4	1.0×10^5	1.1×10^5
Exp. 2†	...	...	...	...	2.3×10^5
	45°C.	50°C.	55°C.	60°C.	
Exp. 1*	...	...	...	...	
Exp. 2†	2.4×10^5	3.3×10^5	2.9×10^5	2.2×10^5	

* Exp. 1: Count of non-ultra-violet irradiated control was 8.0×10^5 per ml. Count of ultra-violet irradiated suspension before illumination was <10 per ml.

† Exp. 2: Count of non-ultra-violet irradiated control was 2.2×10^6 per ml. Count of ultra-violet irradiated suspension before illumination was <10 per ml.

In another experiment (with different light source) a 3-fold recovery was observed after as little as 2 minutes of illumination, and 810-fold after 4 minutes. An experiment in which the duration of illumination was constant, but the intensity varied, showed that the rapidity of recovery was proportional to intensity, within limits.

Temperature.—In subsequent experiments there was employed a uniform

artificial light source consisting of a slide projection lamp containing a 500-watt Mazda projection bulb. The outer lens of the lamp was placed about 5 cm. from the cells, in order to obtain as intense an illumination as possible. A conidial suspension was irradiated $1^1/_2$ minutes at 20 cm. from the ultra-violet lamp. Table 3 shows the effect of temperature on the rapidity of recovery, the visible light illumination being kept constant at 10 minutes. An independent ultra-violet irradiation was made for each temperature determination; this may partially account for some of the variability in the results. It is seen that the rate of recovery increases with rise in temperature up to about 50°C.

Ultra-violet irradiated suspensions could be kept at 5°C. in the dark for up to 4 hours without interfering with subsequent recovery when illuminated.

The knowledge furnished by the experiments just described enabled us to induce over 100,000-fold recovery with a high degree of reproducibility, by illuminating ultra-violet irradiated suspensions with a light source as described for 20 to 30 minutes at 37°C.

The light source used by us emitted infra-red as well as visible light. Since considerable work has been done on the effect on mutation and chromosomal rearrangements of pre- and posttreatment of x- or ultra-violet irradiated cells with near infra-red,[6, 7, 8] it was of importance to determine the comparative effect on recovery of the infra-red and visible components of our light source. Suspensions illuminated with light in which the infra-red had been eliminated by a filter[9] consisting of a 3.2-cm. deep cell containing 0.5 *N* $CuCl_2$ aqueous solution, recovered almost as much as controls with no filter. This filter absorbs some of the visible red, as well as the infra-red. On the other hand, interposition of a filter consisting of a 3.2-cm. deep cell containing a saturated solution of I_2 in CCl_4, which eliminates most of the visible light and passes the infra-red[8, 9] resulted in no recovery at all. There was moderate recovery when an I_2-CCl_4 filter 1 cm. deep was used, but use of this filter was not a critical test, for a considerable portion of the visible light passed through this filter. These simple experiments do not of course exclude the possibility that infra-red illumination of sufficient intensity will not induce recovery; they do show that the most active component of our light source was the visible light. One of the main features of the infra-red-ultra-violet, or -x-ray studies,[6, 7, 8] is that pretreatment with infra-red has a marked effect on the behavior of cells to subsequent irradiation with ultra-violet or x-rays. We therefore illuminated conidial suspensions of *S. griseus* with visible light before irradiating with ultra-violet. There was no increase whatever in the survival rate on subsequent irradiation with ultra-violet.

The magnitude of the recovery phenomenon made it imperative to make sure that it was not due to some experimental artifact, such as declumping

of clumped cells; and to ascertain whether the effect of visible light was on the menstruum rather than on the cells themselves.

Elimination of clumping and declumping as a factor was shown by experiments where ultra-violet and subsequent visible light irradiation was done on cells which had first been smeared on the surface of nutrient agar plates. Light-induced recovery occurred as usual.

That recovery was not due to a stimulation of germination in cells which had a long lag phase due to ultra-violet irradiation, was shown by the fact that prolonged incubation of plates which had been seeded with irradiated cells never disclosed the presence of slow-growing colonies. The maximum number of colonies was always reached after 3 days of incubation.

There was a possibility that the killing effect of ultra-violet light on *S. griseus* was due chiefly to ozone dissolved in the menstruum from the air, or to peroxides or other compounds formed in the menstruum by the ultra-violet light. If these toxic compounds rendered cells non-viable, then their elimination by decomposition by visible light, might allow cells to germinate and form colonies—i.e., recover.

Numerous experiments were made to detect a possible unusual sensitivity of *S. griseus* to the ultra-violet irradiated menstruum, with negative results. Air from the vicinity of the mercury lamp was bubbled for one hour through a suspension of cells, with no sign of toxicity. Sterile nutrient agar plates were irradiated for one hour, then inoculated with spores with no sign of more than a negligibly lower count than controls. Non-irradiated spores were added to suspensions of irradiated spores to see whether substances given off by irradiated cells might be toxic to non-irradiated cells with negative results. Any toxicity that was observed in these experiments never resulted in more than about 20 per cent killing, whereas ultra-violet irradiated cells under the conditions of our recovery experiments had usually a survival of the order of 1×10^{-6}.

Discussion.—The evidence presented suggests that in visible light we have a factor which uniformly, reproduceably causes the recovery of many of the cells which had been rendered non-viable by ultra-violet irradiation. The action is probably directly on the cells rather than on the menstruum, and there was no evidence of any experimental artifacts being involved. The magnitude of the effect makes it likely that a key factor in the lethal effect of ultra-violet light is being affected by the visible light. Whether or not light-induced recovery bears a relation to other types of recovery previously recorded is difficult to say. All such studies, as well as studies on ultra-violet induced mutation must be evaluated on the basis of whether light-induced recovery has played a part. There can be no doubt that the latter is at least partly responsible in some cases for the notorious variability of ultra-violet-mutation studies.

That the phenomena described here are not confined to actinomycetes

only is suggested by observations in the older literature (summarized in the review by Prat[10]) of the antagonism to ultra-violet light of radiations of other wave lengths. These observations were usually made on cells or tissues irradiated by a mixture of wave lengths as compared to monochromatic irradiations, but consistently showed that the biological effect of ultra-violet light was diminished by simultaneous irradiation with visible or infra-red light. Since such effects were usually slight,[11] these older experiments are hard to evaluate. They, as well as other chemical and physical evidence of antagonism of ultra-violet and other light (also summarized by Prat[10]), suggest the phenomenon may be a general one.

While it is premature to do more than speculate on the mechanism involved in light-induced recovery, the following is suggested as a working hypothesis. Much of the killing effect of ultra-violet light is due to a light-labile alteration of some constituent in the cell. Exposure to visible light restores this altered constituent to its former state.

The powerful action of light on the resuscitation of the ultra-violet treated cell leads us to hope that further study of this phenomenon may yield clues leading to the discovery of factors causing similar recovery from x-irradiation of irradiation from radioactive materials. There is thus the possibility of at least a partial physiotherapy of radiation injury.

Of great importance is the relation of recovery to the mutagenic action of ultra-violet light. Work is in progress on light-induced recovery in the various microbial groups, such as bacteria, yeasts, fungi, and bacteriophage, and on the genetic aspects of light-induced recovery in microorganisms.

Summary.—Illumination with visible light will induce the recovery or the regaining of viability of ultra-violet irradiated conidia of the actinomycete, *S. griseus* ATC 3326. The light-induced recovery phenomenon is reproduceable and uniform and results in as high as a 400,000-fold increase in number of survivors in an ultra-violet irradiated suspension. The characteristics of the phenomenon are described, and its significance discussed.

* This study was aided by a grant from Schenley Laboratories, Inc.

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