RESTORATION INDUCED BY CATALASE IN IRRADIATED MICROORGANISMS

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Working with the bacterial strain *Escherichia coli* K-12 which had been irradiated with heavy doses of ultraviolet light, Monod, Torriani, and Jolit (1949) have recently observed a new type of restoration phenomenon. After irradiation, the rate of survival, estimated by colony counts on agar plates, seemed to increase with the size of plating, that is with the number of cells plated, as though the dead bacteria contributed some restoring factor. This same factor was found in extracts from various organs of the rabbit. After preliminary investigation, these authors identified the restoring factor as catalase. Ferrous sulfate exerted a similar effect but to a lesser degree. In addition, the restoring action was heavily favored by the administration of some visible light, insufficient in itself for giving a notable restoration.

This paper presents results on further development of this study.

I

Material and Methods

1. Bacterial Cultures.—The following bacterial cultures have been utilized:

Escherichia coli, lysogenic strain K-12, obtained from Dr. J. Lederberg, through the courtesy of Dr. J. Monod.

Escherichia coli strain B.

Escherichia coli strain B/r, isolated from strain B by UV irradiation.

B. megatherium, lysogenic strain 899, originally isolated by Den Dooren de Joong, and obtained from Dr. Lwoff.

B. megatherium, non-lysogenic, isolated from the preceding strain by Dr. A. Lwoff.

These cultures were grown at 37°, with aeration, in a synthetic medium 56 developed by Dr. J. Monod, and of the following composition:

KH_2PO_4	13.6 (0.1 M)	for pH 7.4
KOH	approximatively 8 ml. of a 10 N solution	
$(NH_4)_2SO_4$	2.0	
$MgSO_4 \cdot 7H_2O$	0.2	
$CaCl_2$	0.01	
$FeSO_4$	0.0005	
Glucose	2.0 (Sterilized separately)	
H_2O	up to 1000	

For the cultures of *B. megatherium*, this medium was supplemented with various minerals, according to the recommendations of Lwoff, Siminovitch, and Kjeldgaard (1950). For the plating of bacteria after irradiation, these same synthetic media were supplemented with Difco agar at 1.5 per cent, and Gratia's soft agar technique was used. This consists of mixing the sample with 2.5 ml. of synthetic 0.7 per cent melted agar at 48°, and pouring the mixture on a Petri dish.

2. Irradiations.—After 15 hours of incubation at 37°, the culture, which had reached its maximum concentration of 4×10^9 cells per ml., was diluted approximately 1000 times in medium 56, and then irradiated in a thin (2 mm.) layer in an open Petri dish. The UV source was a Gallois mercury vapor lamp of the "high voltage, low pressure" type, whose germicidal action is 95 per cent due to its 2537 A radiation. At the surface of the preparation, the intensity of this radiation, which was always controlled by a suitable photo-electric dosimeter, varied between 1000 and 2000 ergs $mm.^{-2}$ $min.^{-1}$, depending on the experiment.

The x-ray source was a molybdenum anticathode operating under 33 kv. and giving an intensity of 8000 r per minute at the surface of the preparation.

3. Restoration Treatment.—After the irradiation, the bacterial suspension was centrifuged and then resuspended in synthetic medium (so as to eliminate the irradiated medium). It was then immediately inoculated on solid medium in Petri dishes, with and without catalase. Each sample was plated on several dishes: (*a*) with and without catalase (Armour and Co.) freshly prepared aqueous solution at a final concentration of 10^{-5} to 10^{-6} in the soft agar tube;[1] (*b*) with and without visible light before incubation at 37°; the dose of visible light was chosen so as to produce no important photorestoration, and to enhance the action of catalase. After preliminary trial, an exposure of 3500 lux for 2 hours with "daylight type" fluorescent 40 watt tubes was found suitable for this test.

II

Restoration of K-12

Under the preceding experimental conditions, restoration of K-12 by catalase (C.R.) shows the following characteristics.

(*a*) From one day to another, in our experiments at least, large quantitative fluctuations were observed. Occasionally the restoration was feeble, and the ratio of colonies grown with catalase to those grown without did not exceed a few units. On other occasions this ratio was much higher, reaching several hundreds or thousands. The restoration seems to be strictly conditioned by the physiological state of the bacteria at the moment of irradiation (and possibly even after), a state which cannot be easily controlled. One might say that a sort of "aptitude" to C. R. exists for the bacteria.

[1] This crude sample could not be considered as pure catalase, and may have contained some active impurities. Controls on this were not done, but we checked that the same restoring effect was actually given by catalase samples from other origins prepared in England or in France.

(*b*) Under certain rare circumstances, which we have not been able to define, C. R. is observed in the absence of visible light. Even in this case, the results are ameliorated by the addition of visible light (Table I).

(*c*) In general C. R. requires the addition of light. The results of a typical experiment are shown in Table II: catalase in the dark produces very little

TABLE I

Catalase Restoration Experiment on K-12

K-12: 6×10^6 cells per ml.
UV: 1500 ergs. $\times$ mm.$^{-2}$
Assays: 0.1 ml. (6×10^5 cells) plated on synthetic agar.

	Colony counts Without catalase	Colony counts With catalase
Dark	6	900
	6	800
	(Survival: 10^{-5})	
Light		
2 hrs.	45	1460
	42	960
4 hrs.	92	3720
	80	3560

TABLE II

C. R. Experiment on K-12

K-12: 2×10^6 cells per ml.
UV: 2000 ergs $\times$ mm.$^{-2}$
Assays: 0.1 ml. (2×10^5 cells) plated on synthetic agar.

	Colony counts Without catalase	Colony counts With catalase
Dark	0	5
	0	6
Light 2 hrs.	0	1200
	0	1200

effect in contrast to its very pronounced action in the presence of a dose of visible light which, by itself, does not produce any restoration.

(*d*) C. R. depends on the dose of UV light administered, and acts effectively only in a certain range of doses (1200 to 2000 ergs mm.$^{-2}$) corresponding to survival rates of 10^{-4} to 10^{-5}. For low or for high doses the phenomenon diminishes and may become unapparent. This character differentiates in one way

C. R. from photorestoration (P.R.) since the latter exists for all UV doses in such a manner that the two survival curves, with and without P. R., are similar (Kelner, 1949, Novick and Szilard, 1949). The similarity of the two curves has led to the "dose-reduction principle." Such a principle does not appear in C. R.

III

Decrease of Restorability after UV Irradiation

It was of interest to determine how long after UV irradiation the cells conserved their aptitude to restoration. In the case of light (P.R.), Kelner (1949) showed that this aptitude decreased as an exponential function of the time elapsed between the UV irradiation and the restoring treatment with visible

TABLE III

Decrease of Restorability of K-12 after UV Irradiation

Time elapsed at 37° between UV irradiation and addition of catalase	Ratios of restoration			
	Experiment			
	1	2	3	4
hrs.				
0	30	175	500	900
1	47	232	250	400
2	130	26	250	50
4	59	1	80	1
8	1	1	1	1

The ratio of restoration is the ratio: number of colonies after C. R./number of colonies without C. R.

light. This decrease could reflect the exponential disappearance of substances involved in P. R.

In the present case, the preparation of K-12, irradiated with UV at time zero, was immediately placed at 37°. From time to time, a sample was taken and treated (as above) with catalase. The results of 4 experiments are shown in Table III. On the average, the restorability remains constant during the 1st hour; it then decreases, is still noticeable at 2 hours, but tends to disappear at about 4 hours. These times obviously depend on the temperature.

IV

C. R. and Lag Phase

The most efficient doses for C. R. of 1200 to 2000 ergs mm.$^{-2}$ determine a lag phase of about 4 hours in the surviving bacteria; that is, under ordinary culture conditions at 37°, the first divisions begin at the end of 4 hours. Two questions were posed: (*a*) does catalase modify this lag phase (in P.R. there is

often a diminution of this period)? (*b*) does the restoration occur during or after this period? In other words, do the restored cells start dividing at the same time as the survivors?

The first question can be answered by growing the irradiated bacteria in liquid media with and without catalase, and by determining in both cases the moment when cellular division commences. To answer the second question, one must, once this period is passed, determine in addition the growth curves in liquid media by counting the cells capable of forming colonies. If the restoration is completed before the end of the lag phase, the growth rates are the same in both cases. For example, if there are 10 survivors in the absence of catalase and 1000 in its presence, we will pass simultaneously from 10 living cells to 20 in the first tube, and from 1000 to 2000 in the second. In semilogarithmic coordinates, the two growth curves will run parallel, separated by a constant interval which represents the ratio of restoration. If, on the contrary, the restoration takes place later, once the normal survivors have commenced to divide, the colony-forming cells in the second tube will begin by passing from 1000 to 1020 while those of the first tube will double from 10 to 20. The rate of growth will therefore begin to be inferior in the presence of catalase, and the corresponding curve will approach the other.

A culture of K-12 irradiated under normal conditions, with a survival rate of 10^{-4}, was divided into 2 parts: the first, a control *c*, was kept in the dark at 18°; catalase was added to the second, *t*, which was then placed in visible light for 2 hours at 18°. Samples of *c* and *t*, taken at this time and inoculated on agar, gave, after development, the ratio of restoration (as in any usual experiment of the Table II type), which in this case was 100. After a total of 3 hours spent at 18°, the two cultures were diluted in beef broth, the tube *c* 10 times, the tube *t* 1000 times, and then were placed at 37°. Every 30 minutes, a sample was plated on agar, which indicated the number of colony-forming cells at the time of sampling. With these values, the growth curves could be drawn (Fig. 1).

The experiment, repeated 4 times, always gave the same results, that is: (*a*) the catalase treatment does not modify the lag phase; (*b*) on semilogarithmic coordinates the growth curves run parallel, which proves that the restored cells begin to divide at the same time as the normal survivors, with the same division time. The restoration is therefore completed at the end of the latent period.

It is actually possible that it is completed much before the end of this latent period, and one may wonder what is the minimum length of contact necessary between the cells and catalase for maximum restoration.

A suspension of K-12 was irradiated with UV, centrifuged to eliminate the irradiated medium, resuspended in medium 56, and finally divided into 5 fractions, each treated respectively in the following manner:

1. Control, not treated with catalase, plated on medium 56 agar.

2. Catalase added under visible light; after 5 minutes' contact, centrifugation and resuspension in medium without catalase; then exposure to visible light for 1 hour and 55 minutes, and finally plating on medium 56 agar.

3. The same as 2, except that the contact with catalase lasted 30 minutes, and the exposure to visible light without catalase lasted 90 minutes.

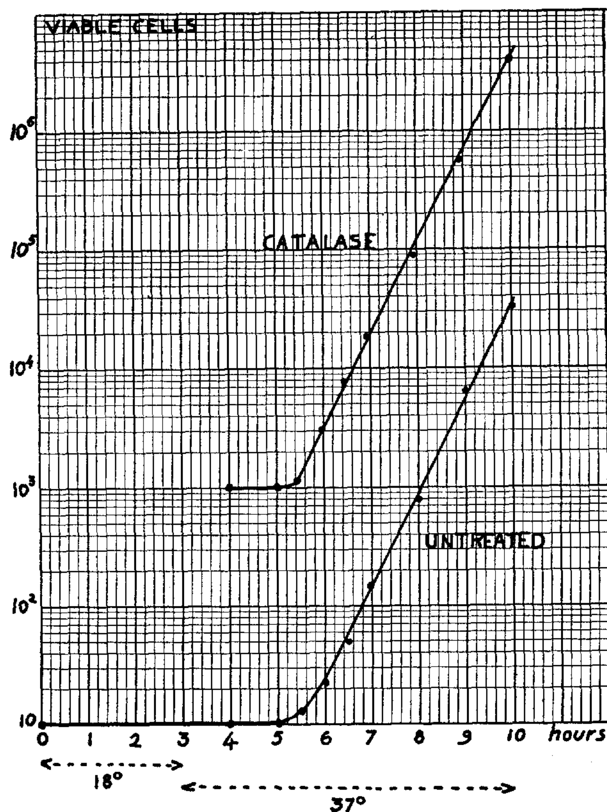

FIG. 1. Growth curves of K-12 irradiated at time zero with UV light, then stored at 18° for 3 hours, and, from then on, at 37°. Untreated, represents the normal survivors; catalase, represents the cells restored by the catalase treatment given during their stay at 18°. In both cases the lag phases and the rates of the division are the same.

4. The same as 3, except that the contact with catalase under visible light lasted 2 hours.

5. Visible light alone (without catalase) for 2 hours.

After incubation, the colony counts gave the following results:

1. Survivors per ml.:	2×10^2			
2. " "	7×10^4	restoration:	350	(C.R.)
3. " "	1.2×10^5	"	600	"
4. " "	1.1×10^5	"	550	"
5. " "	2.4×10^3	"	12	(P.R.)

A contact time of 5 minutes therefore suffices to assure a C.R. more than one-half the maximum possible.

V

No C. R. after X-Irradiation

Just as in the case of visible light, catalase was found incapable of restoring K-12 which had been irradiated with x-rays, the experiment having been conducted in a similar fashion to those with UV rays. The culture was irradiated with doses up to 50,000 r, and then plated on medium 56 agar with and without catalase, with and without visible light. The experiment was carried on under various conditions, the bacteria cultivated in various media, and the doses varied over a large range. In no case did catalase exert any action. It seems possible that no C. R. occurs if the radiolesion is produced by ionizing radiations.

VI

Little or no C. R. in E. coli B and B/r

Experiments identical with those performed with K-12 (section II, Tables I and II) were done with B and B/r—different strains of the same species *Escherichia coli*. Contrary to what might have been expected, strain B, which is very sensitive to the restoring action of visible light, was found to be insensitive to the action of catalase (Table IV). The experiment was repeated several times and always gave the same results; that is, very strong P. R. and no C. R.

As to strain B/r, a very small but not negligible C. R. was found under the same conditions (Table IV). In no case did the restoration ratio exceed a few units.

VII

C. R. in B. megatherium

To what property of K-12 is C. R. linked? If it is its lysogenicity (Lederberg), which we have verified with our strains, the C. R. could show itself in other lysogenic strains such as *B. megatherium* 899.[2] We therefore undertook

[2] Bacteria are called lysogenic when their capacity to form bacteriophages is perpetuated to their progeny without the intervention of exogenous bacteriophages (Lwoff).

TABLE IV

C. R. Experiments on B and B/r

	B		B/r	
	6×10^6 cells per ml. UV: 1100 ergs $\times$ mm.$^{-2}$ Plating 6×10^5 cells per plate.		4×10^6 cells per ml. UV: 2500 ergs $\times$ mm.$^{-2}$ Plating 4×10^5 cells per plate.	
	Colony counts Without catalase	Colony counts With catalase	Colony counts Without catalase	Colony counts With catalase
Dark	25	22	4	8
	20	26	6	7
	Survival 3.7×10^{-5}		Survival 1×10^{-5}	
Light 1 hr.	163	126	70	92
	200	139	83	110
Light 2 hrs.	920	1015	249	754
	1130	1065	251	746

TABLE V

C. R. Experiments on megatherium 899

6×10^5 clusters per ml. of about 10 cells each.
Plating 6×10^4 clusters per plate.

Experiment 1	Colony counts Without catalase	Colony counts With catalase
	UV: 1900 ergs $\times$ mm.$^{-2}$	
Dark	4	5800
	3	5400
Light	2	9000
	3	8000
Experiment 2	UV: 2700 ergs $\times$ mm.$^{-2}$	
Dark	1	11,000
	2	10,000
Light	5	13,000
	4	12,000

the study of C. R. with the latter bacterium, and we found the same characteristics as with K-12; that is: very little effect at low doses; a large restoration in a certain interval of high doses (Table V) in reference to which it should be remarked that the clumping of this bacterium in chains (of about 10 cells on the

average) leads to an overestimation of the C. R. if it is deduced from colony counts; adjuvant action of visible light, inactive in itself at the doses used.

Therefore, the second lysogenic strain of bacteria studied shows itself capable of C. R. It was then of interest to study, as a control experiment, a strain (Mox), derived from the preceding one by A. Lwoff, by repeated passages in oxalated medium, which is no longer lysogenic. A series of experiments carried out simultaneously on lysogenic 899 and on Mox showed: that the non-lysogenic Mox also gives C. R.; that this C. R. is always much less than that shown by 899, and never exceeds a ratio of 10.

In this particular case, the disappearance of the lysogenicity is accompanied by an important decrease in C. R. but not by its disappearance.

VIII

C. R. with a Bacteriophage

Dulbecco (1949) has shown that phages inactivated by UV light are not photorestorable, but become so after absorption on their normal host. Under the influence of visible light, a certain fraction of bacteria infected by inactive phage, become capable of producing active phage. The host therefore plays an essential role in the P. R. of phage owing to certain of its metabolic capabilities. If we alter these aptitudes, P. R. is diminished. This is what is found if instead of UV-irradiating the phage alone, prior to infection, one irradiates the infected bacteria (experiments of the type described by Luria and Latarjet, 1947): not only is the phage inactivated, but the bacteria as well are damaged; they are still capable of phage synthesis but with a diminished yield (Rouyer and Latarjet, 1946). Moreover, the radiolesions of the host hinder greatly the P. R. (Benzer; Dulbecco; Latarjet; unpublished observations). These considerations suggested the study of the effect of catalase on UV-inactivated phage in accordance with three experimental schemes: (*a*) UV irradiation of phage; treatment with catalase; assay of active phage; (*b*) UV irradiation of phage; infection (intact bacteria); treatment with catalase; assay of bacteria yielding active phage; (*c*) infection; UV irradiation of infected bacteria (damaged bacteria); treatment with catalase; assay of bacteria yielding active phage.

In case (*c*) it seemed interesting to compare the results obtained with the same phage T2 on two of its hosts: K-12, itself sensitive to C. R., and B, insensitive to C. R. We did not study the following fourth scheme: UV irradiation of the bacteria; infection with non-irradiated phage; treatment with catalase; assays of bacteria for individual yields of phage. In the case of a lysogenic bacterium such as K-12, this scheme corresponds to a mixed infection with possible interference between the introduced virus T2 and the carried virus λ (Weigle and Delbrück, 1951).

(*a*) No C. R. was found on phage treated before infection. Extracellular phage is no more restorable by catalase than by visible light.

(*b*) When the irradiated phage is treated by catalase while within intact K-12 bacteria (non-irradiated), no C. R. is produced, whereas, corresponding to the results of Dulbecco (1949), P. R. is marked in this case (Table VI). Catalase does not contribute anything to the intact bacteria which would aid

TABLE VI

C. R. Experiment on Phage T2 Irradiated without Its Host

Phage T2: 4×10^8 particles per ml.
UV: 660 ergs $\times$ mm.$^{-2}$
Survivors: 2×10^4 per ml. (survival: 5×10^{-5})
Infection of 10^8 K-12 with 4×10^7 irradiated T2 (single infection)

	Plaque counts on K-12, plating 0.1/10 Without catalase	Plaque counts on K-12, plating 0.1/10 With catalase
Dark	190	212
	208	190
Light	2400	2500
	2600	2500

TABLE VII

C. R. Experiment on Phage T2 Irradiated within Its Host K-12

Infection of K-12 (10^8) by T2 (2×10^7) (single infection).
UV: 2100 ergs $\times$ mm.$^{-2}$ 6 minutes after infection.

	Plaque counts on K-12, plating 0.05 on synthetic agar	
	Without catalase	With catalase
Dark	200	12,000
Light	100	17,000
	100	18,000

in restoring the inactivated phage. This is yet another difference between C. R and P. R.

(*c*) In the third type of experiment, 2×10^7 T2 particles was mixed with 10^8 K-12 bacteria per ml. of medium 56 at 37° (single infection). After 6 minutes' contact, about 60 per cent of the particles are adsorbed by the cells, but their multiplication, strictly speaking, has not yet begun (Latarjet, 1948). The suspension was then given a heavy dose of UV light which inactivated most of the intracellular particles and eliminated practically all the non-adsorbed particles (which conveniently did away with the need for their elimination by centrifugation or antiserum). The irradiated bacteria were immediately plated on medium 56 agar, with and without catalase, in the presence of an excess of intact K-12 cells (the fact that catalase does

not modify the efficiency of plating of T2 on K-12 was verified beforehand). Under these conditions many more plaques were obtained in the presence of catalase than in its absence (Table VII).

In the same experiment carried out using as host strain B, not restorable by catalase, rather than K-12, no C.R. of the phage was found.

These results strongly suggest that within the irradiated "infected cell," the apparent C. R. of the virus is closely related to that of its host. If we keep in mind that, extracellular phage being not restorable, restoration of the phage is secured by some metabolism of the host, it is therefore tempting to consider that C. R. of K-12 involves some damaged systems which this cell may eventually utilize for restoring a virus inactivated by UV light.

The results given in Table VII show that in the absence of catalase, light exercises an effect opposite to that of restoration: more phage is obtained in the darkness than in light, and this result repeats itself constantly, often with even greater clarity. We think this anomaly is a consequence of the lysogenicity of K-12. The infection places T2 in the presence of the carried prophage λ of K-12; the UV irradiation, which induces the reproduction of the latter (Lwoff *et al.*, 1950), initiates a competition of the 2 viruses (Weigle and Delbrück, 1951).

DISCUSSION

Let us summarize first the preceding experimental findings:

(*a*) Catalase, added to certain bacteria sterilized by a heavy UV irradiation, is capable of restoring an important fraction of the cells. This phenomenon (C.R.) occurs distinctly with bacterial strains lysogenic K-12 and lysogenic *megatherium* 899; feebly with a non-lysogenic *megatherium* derived from the former; very weakly with *E. coli* B/r; not at all with *E. coli* B. Catalase also restores the irradiated system "K-12 infected with one phage particle."

(*b*) C. R. is assured by small amounts of catalase placed in contact with the cells for a short period (5 minutes suffices). The action, strictly speaking, is completed before the end of the lag phase since the restored cells begin their growth at the same time as the normal survivors.

(*c*) Full C. R. is produced only with definite UV doses, which, in the case of K-12, leave 10^{-4} to 10^{-5} survivors. It requires most often the addition of visible light.

(*d*) The restorability of the cells is closely linked to their physiological state at the moment of irradiation, as though this state conditioned a sort of aptitude for C. R.; for this reason, results may vary greatly from one experiment to another. At 37° restorability persists for about 2 hours after the UV irradiation, and then drops rapidly.

(*e*) C. R. does not occur after x-irradiation.

Even though these findings are manifestly insufficient to allow comprehension of the essentials of C. R., let us examine a few of them.

1. Site of the Catalase Action.—A few minutes' contact between the bacteria and medium containing catalase at a concentration of the order of 5×10^{-6}, is sufficient to produce C. R. This means either that C. R. results from an almost immediate action of catalase on the bacterial surface, or that the molecule of catalase fixes itself rapidly and solidly on the bacteria, to react from the surface, or to penetrate through the membrane. It is difficult perhaps to envisage the passage of such a voluminous protein molecule through a cellular membrane; but what then about virus particles?

2. Chemical Nature of the Action.—At first hand, one may feel that catalase, which decomposes hydrogen peroxide very actively, acts here by decomposing certain UV-formed peroxides. Other substances, such as ferrous sulfate, which possess this property to a minor extent, produce the same restoring effect with less efficiency (Monod *et al.*, 1949). This decomposing action is not exercised on oxidizing free radicals nor on short lived peroxides produced immediately by the irradiation of the intracellular medium, since the restorability of the cells persists for 1 to 2 hours at 37° after the irradiation, which indicates that the substances involved are very stable.

If the reactions were involved with the first short lived peroxides, we would obtain the same effect by introducing catalase before the UV irradiation (under conditions in which it is not destroyed by the irradiation). This "protection" effect was studied directly on K-12 and on B, either by adding catalase to the suspension before the UV irradiation, or by increasing the concentration of catalase in the bacteria by having them grown on excess of the enzyme. Under these conditions we obtained 2 to 30 times as many survivors *with B as well as with K-12*, owing to this type of protection, without ever finding the ratios of 100 to 1000 observed in C. R. While assuring this protection, catalase is probably blocked by its reactants, and thus is no longer available for the later steps involved in C. R. This would explain why C. R. is produced only by adding the enzyme *after* the UV irradiation.

We feel that catalase acts here on long lived organic peroxides and, in this connection, we may quote an observation made by Wyss *et al.* (1950) wherein it was found that, after UV irradiation, beef broth acquired a mutagenic activity on bacteria. After having eliminated hydrogen peroxide as a possible cause of this activity, these authors attributed it to hypothetical organic peroxides formed by radiation. If catalase was added to the irradiated beef broth, the mutagenic character disappeared. From these results, UV irradiation of beef broth would induce the formation of organic peroxides decomposable by catalase. Our results agree with this idea. In this way, certain chains of "dark reactions," initiated inside the cell by primary photochemical reactions, and leading to mutations or to the blocking of division, would involve, as an intermediate

link, relatively stable organic peroxides. The fact that catalase loses its efficiency after 2 hours at 37°, would mean that at this moment the chain of reactions progresses into further stages insensitive to catalase. Lack of C. R. after x-irradiation would mean that, in the above studied cases, the chain of reactions initiated by ionizing processes, and leading to a blocking of cell division, does not include this stage of organic catalase-sensitive peroxides.

3. Differences between K-12 and B.—If this notion of the chemical nature of the action holds true, why then does such a striking difference in C. R. appear between cells so closely related as *E. coli* K-12 and B, or *megatherium* 899 and Mox? The previously mentioned experiments on the lack of C. R. in cells containing an excess of catalase at the time of irradiation, make it very unlikely that this difference is a function of the cellular concentration of the enzyme. Nevertheless, in our laboratory, Dr. Morin compared the catalase activity of strains K-12 and B by measuring in the Warburg manometer at 37° the volume of oxygen liberated by the bacterial suspension in buffer solution containing H_2O_2 M/200 (method of Fujita and Kodama). If the catalase activity is expressed by the ratio

$$\frac{\text{mm.}^3 \text{ of } 0^2 \text{ liberated in 30 minutes}}{\text{Dry weight of bacteria, in mg.}}$$

the following results were found, as averages of several experiments:

Before irradiation		Immediately after irradiation	
B	K-12	B	K-12
650	880	660	730

In the irradiated samples, the values decreased about 15 per cent after a few hours of incubation. These measurements show that no notable difference in catalase content exists in K-12 and B, and that the habitual UV doses do not destroy appreciably the intracellular enzyme.

The difference between K-12 and B could be due to a difference in the adsorption of catalase. This possibility was not examined.

4. C. R. and Lysogenicity.—Of the five bacteria studied, K-12 and *megatherium* 899 are at the same time the most sensitive to C. R. and lysogenic. In addition, the aptitude to C. R. simulates the aptitude to induction of bacteriophage reproduction in lysogenic bacteria, which also depends strictly on the physiological state of the cells at the moment of irradiation (Lwoff *et al.*, 1950).

We have induced the lysis of K-12 by various doses of UV light in the presence and in the absence of catalase: a culture of K-12 in exponential growth in medium 56 was irradiated with four doses of UV. In each case, one part was then diluted in medium 56, and the other in the same medium containing

catalase at the usual concentration. These two cultures were immediately placed at 37° and irradiated with visible light as in the usual conditions of C. R. Every 30 minutes the optical density of the cultures was measured and the curves of optical density *versus* time drawn (Fig. 2). Fig. 2 shows that the curves are the same in the presence and in the absence of catalase, and that maximum lysis is induced by rather small doses of UV light (600 ergs). Larger doses damage the cell to such an extent that the induced growth of the phage is progressively hindered.

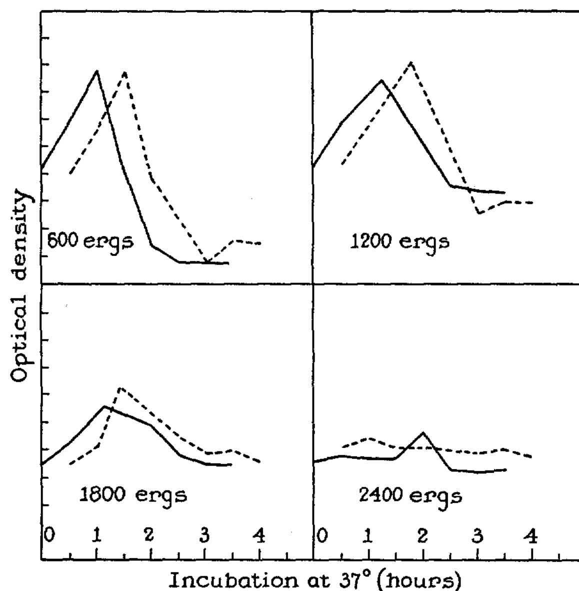

FIG. 2. Lysis induced in K-12 by various doses of UV light given at times zero without (plain curves) and with (dotted curves) further addition of catalase. For the sake of clarification, times zero have been separated by an interval of 30 minutes.

The optical density is scaled from 0 to 10 in arbitrary units. Number 10 corresponds to about 4×10^7 bacterial cells per ml.

UV light kills lysogenic bacteria by two main processes: by inducing lysis and, without lysis, by killing as in non-lysogenic bacteria. The first process predominates at small doses, the second at large doses. One may, therefore, envisage two main processes of restoration: (*a*) by preventing lysis; (*b*) by restoring the non-lysed mortally injured cells.

If C. R. acted uniquely through process *a*, one could understand readily why there seems to be a correlation between it and lysogenicity. (This is not demonstrated in Fig. 2 because, as can be seen in Tables I and II, at best 1 per cent of the irradiated cells are restored.)

However, if this were the case, one should expect that the UV doses at which maximum C. R. occurs would be the same as those which induce maximum lysis. In fact, the former are higher (1200 to 2000 ergs) than the latter, and are doses at which lysis is less pronounced, while the number of non-lysed dead cells is increased. It can be noted in passing, that these doses nonetheless leave some undamaged prophage in most of the irradiated cells, since most of the survivors remain lysogenic.

In this connection, the x-ray results are suggestive. X-rays kill lysogenic bacteria in the same two ways as does UV. In both instances the induction of phage growth and of lysis is very similar and, in particular, is restored by visible light (Latarjet, 1951). On the other hand, killing without lysis differs between x and UV in that it is photorestored only after UV treatment. The absence of C. R. after x-ray treatment suggests, consequently, that its action is not on the lysis type of killing.

We feel, therefore, that catalase restores non-lysed cells, and that, for some unknown reason, this restoration is favored by the presence of the prophage. Thus, after small doses, C. R. would be feeble because most of the killed cells are killed by lysis; after medium doses the number of cells not lysed and still containing active prophage increases, and these cells could be restored by catalase; after higher doses, restorability would disappear because of greater damage caused to the cells, and perhaps also because of inactivation of the prophage. The results obtained with K-12 infected with the phage T2 (section VIII) seem to indicate that catalase restores in K-12 some system which is utilized in phage synthesis.

If the nature and mechanism of C. R. have not been deciphered in this study, it nonetheless remains that the phenomenon observed in the particular case of catalase demonstrates the possible occurrence of chemically induced recoveries in irradiated cells. It becomes easy to visualize how, under certain circumstances, some restoring factor can be contributed to a damaged cell, either from without by the culture medium or by other cells (Delaporte, 1951), or even from within by the cell itself. We are thus led to envisage certain spontaneous recoveries and changes in cellular radiation sensitivity as due to such types of internal after-effects.

SUMMARY

1. *E. coli*, strain K-12, and *B. megatherium* 899, irradiated in strict but still undefined physiological conditions with certain heavy doses of ultraviolet light, are efficiently restored by catalase, which acts on or fixes itself upon the bacteria in a few minutes. This restoration (C. R.), different from photorestoration, is aided by a little visible light.

2. At 37° the restorability lasts for about 2 hours after UV irradiation; the restored cells begin to divide at the same time as the normal survivors.

3. C. R. is not produced after x-irradiation.

4. *B. megatherium* Mox and *E. coli*, strain B/r show little C. R.; *E. coli* strain B shows none. None of these three strains is lysogenic, whereas the two preceding catalase-restorable strains are.

5. Phage production in the system "K-12 infected with T2 phage" is restored by catalase after UV irradiation, whereas phage production in the system "infected B" is not.

6. With K-12, catalase does not prevent the growth of phage and the lysis induced by UV irradiation (Lwoff's phenomenon).

7. Hypotheses are discussed concerning: (*a*) the chemical nature of this action of catalase; (*b*) a possible relation between C. R. and lysogenicity of the sensitive bacteria; (*c*) the consequences of such chemical restorations on the general problem of cell radiosensitivity.

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