PROTEIN SYNTHESIS AND VIABILITY IN X-IRRADIATED *RHODOPSEUDOMONAS SPHEROIDES*

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SUMMARY

In the photosynthetic bacterium *Rhodopseudomonas spheroides* the capacity for induced catalase synthesis is inhibited by low doses of X-rays to the same extent as the ability to form colonies. The inhibition is manifested fully within a few minutes after irradiation.

The presence of catalase during and after irradiation does not modify the effect of X-rays on survival, and the activity of intracellular catalase is not altered by X-ray doses up to 100 kR.

The effects of X-rays on catalase synthesis and survival are potentiated to the same extent by oxygen.

Experiments dealing with kinetics and external factors suggest that X-rays act in an all-or-none manner, early in a sequence of events that culminates in synthesis of the enzyme.

The syntheses of "general cell protein," of proteins associated with bacteriochlorophyll in the chromatophores, and of constitutive catalase are inhibited by X-irradiation to about the same extent as the synthesis of inducible catalase and the ability to form colonies.

The lethal action of X-rays is discussed in terms of the destruction of genetic material that directs the synthesis of proteins.

INTRODUCTION

Various alterations of physiological behavior have been used as criteria of damage to living cells by ionizing radiation. The choice of such criteria is usually influenced by the experimenter's specific interests, the ease of measurement, and the hope that the system chosen may serve as a model to explain cell inactivation. For example, SHERMAN AND CHASE[1] measured the inhibition by X-irradiation of anaerobic CO_2 production in yeast, and BILLEN, STAPLETON AND HOLLAENDER[2] investigated the effects of X-irradiation on respiration in *Escherichia coli*. With regard to more specific biochemical phenomena, BILLEN AND LICHSTEIN[3] measured the induced synthesis of formic hydrogenlyase in *E. coli*, BARON, QUASTLER AND SPIEGELMAN[4] studied the induced formation of β-galactosidase in yeast after high doses of X-rays, and PAULY[5]

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measured the effect of X-irradiation on the capacity for induced synthesis of lysine decarboxylase in *Bacterium cadaveris*. All these workers observed effects only after relatively high doses (above 20 kR) and generally concluded that post-mortem phenomena were being studied.

It will be shown here that in the photosynthetic bacterium *Rhodopseudomonas spheroides*, both protein synthesis and viability are attenuated by X-rays to approximately the same extent ($LD_{50} \approx 2$ kR in aerated suspensions). The effect of X-irradiation on induced catalase synthesis is reported in detail, and the potential significance of this effect as a model for the lethal action of X-rays is discussed in this paper.

MATERIALS AND METHODS

R. spheroides, wild-type strain 2.4.1 and the high-catalase mutant strain CC1 (ref. 6), was cultivated anaerobically in the light as described earlier[7].

Induction of catalase synthesis was initiated either by aeration in darkness (with O_2 acting both as inducer and to provide energy) or by adding H_2O_2 to an illuminated suspension bubbled with helium (with H_2O_2 as inducer and light as energy source). Illumination was provided by a bank of four 100 W, 115 V tungsten lamps operated at 70 V and situated about 8 inches from the cell suspensions. Catalase was assayed iodometrically, as described earlier[8].

Cell protein was determined with the Folin reagent, using the method of LOWRY *et al.*[9]. Bacteriochlorophyll was extracted and assayed by its light-absorption at 775 mμ, using the procedure of COHEN-BAZIRE *et al.*[10]. The absorption spectrum of bacteriochlorophyll *in vivo* differs from that of the extracted pigment, owing to its structural interactions with proteins and other molecules in the bacterial chromatophore. The *in vivo* pigment was estimated as the difference between the absorbancy at 855 mμ (the principal maximum) and that at 830 mμ (an adjacent minimum).

Irradiation techniques

Cell suspensions were irradiated in glass test tubes (20 mm diameter) mounted in a lucite frame 15 cm from the target of a G. E. Maxitron 250 kvp X-ray machine. Gases were introduced through glass tubes inserted into the cell suspensions. The X-ray machine was rotated so that its face was in a vertical plane. The machine was operated at 250 kvp and 30 mA. With 3 mm of added aluminium filtration (hvl 0.34 mm of Cu) the dose rate was 2100 R/min in every experiment but one. In that experiment (see Fig. 1) the dose rate was 2500 R/min.

Plating techniques

Cells were diluted in *M*/15 phosphate buffer (pH 6.8) and surface-plated on tap water agar containing 0.3% yeast extract and 0.2% casein hydrolysate. At least 5 and frequently 10 plates were made for each dilution. This was necessary because of the low doses and high survivals being studied. Colonies were counted after 5 days' incubation at 30°.

RESULTS

Our main result, shown in Fig. 3, was that the capacity of *R. spheroides* for induced enzyme synthesis and its ability to form colonies were inhibited equally by low doses

of X-rays. Before discussing this result we shall, in the next three sections, develop the basis for the experimental procedure and deal with some potential sources of misinterpretation.

The unirradiated system

Catalase synthesis is induced in *R. spheroides* by H_2O_2. In stationary-phase cultures (and undoubtedly in growing cultures also) the rise in enzyme activity upon adding inducer reflects protein synthesis *de novo*, and not a modification of pre-existing protein[11].

Oxygen also serves as an inducer, by promoting the intracellular formation of H_2O_2 (see ref. 12). The energy needed for induced enzyme synthesis can be gained either through respiration or by photosynthesis. If a single dose of H_2O_2 (final concentration about 50 μM) is added to an illuminated stationary-phase culture, catalase synthesis begins after a lag of 7 min and continues for about 30 min. Another such dose of peroxide will then elicit a second pulse of enzyme synthesis, nearly as large as the first and with similar kinetics. In illuminated growing cultures exposed to H_2O_2 the lag is about 4 min and the period of enzyme synthesis is about 15 min. The response of a stationary culture to aeration involves a lag of about 10 min, followed by a synthesis of enzyme that continues for at least an hour if aeration is maintained[12,13].

The synthesis of bacteriochlorophyll keeps pace with growth in an anaerobic culture of *R. spheroides*, provided that the illumination of the cells is constant. A decrease in light intensity causes a period of accelerated bacteriochlorophyll synthesis lasting several hours[10]. The shape of the absorption spectrum of bacteriochlorophyll does not change during this period. Thus the materials with which the pigment is associated in the chromatophore (*e.g.*, proteins) are probably formed at an accelerated rate also.

X-rays, catalase activity, and survival

Related to the main inquiry into the effects of X-rays on survival and on the capacity for catalase synthesis in *R. spheroides*, there exist two other questions: Does X-irradiation alter the activity of catalase that is already present in the cells, and does intracellular catalase protect the cells against the lethal action of X-rays? The answers to both questions will be shown to be negative, within the range of X-ray dose and catalase content prevailing in our experiments.

In cells that are rich in catalase, the catalatic destruction of H_2O_2 added externally is limited by the rate at which H_2O_2 can diffuse into the cells. A catalase assay of intact cells based on destruction of H_2O_2 will then measure the permeation of H_2O_2 rather than the catalase activity[8]. Any change in permeability (*e.g.*, induced by irradiation) will be reflected in the assay. In toluenized cells[8] the permeability barrier is removed and the assay reflects catalase activity. Table I shows the H_2O_2-destroying activity of intact and of toluenized cells of high-catalase mutant *R. spheroides*, exposed to doses of X-rays up to 100 kR. Neither the permeation of H_2O_2 (activity of intact cells) nor the catalase activity (toluenized cells) is altered by these doses of X-rays.

The survival curves of Fig. 1 show that the lethal effect of X-rays on *R. spheroides* is not lessened by the presence of catalase during or after irradiation. From Fig. 1a it can be seen that the high-catalase mutant (catalase content 35 μg/mg dry cell mass)

is slightly more sensitive to X-irradiation than the wild type (0.02 μg catalase/mg). Fig. 1b shows that wild-type cells preinduced to a catalase content of 0.75 μg/mg have the same sensitivity as uninduced wild-type cells. Thus catalase should be regarded, in the experiments to be described, as nothing more than a specific inducible enzyme.

The failure of catalase to protect cells against X-irradiation, reported by ENGEL AND ADLER for *Escherichia coli*[14], is established here for much higher levels of catalase in *R. spheroides*. The lethal effects of X-rays and of H_2O_2 on high-catalase mutant and wild type *R. spheroides* will be reported in detail elsewhere.

TABLE I

EFFECT OF X-RAYS ON THE PERMEABILITY TOWARD H_2O_2 AND ON THE CATALASE ACTIVITY OF HIGH-CATALASE MUTANT *R. spheroides*

A suspension of high-catalase mutant *R. spheroides* (0.04 mg dry cell mass/ml) was irradiated and then exposed to 0.01 *M* H_2O_2 for 15 sec; the initial and final H_2O_2 concentrations were measured[8]. The slow destruction of H_2O_2 by intact cells reflects the rate of permeation of peroxide into the cells. The more rapid destruction by a suspension of toluenized cells reflects the activity of intracellular catalase.

X-ray dose (kR)	*Initial $[H_2O_2]$/Final $[H_2O_2]$*	
	Intact cells	*Toluenized cells*
0	1.13	24
30	1.11	28
60	1.11	24
100	1.11	28

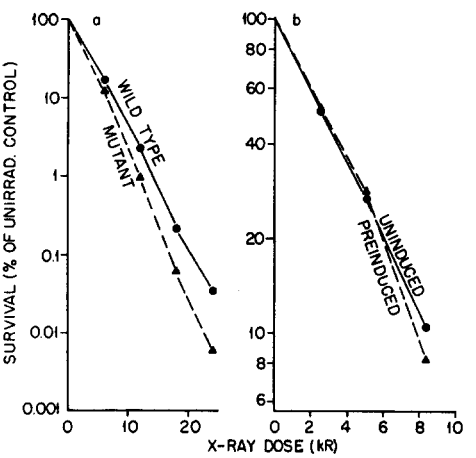

Fig. 1. Survival of X-irradiated *R. spheroides* cells differing in their catalase content. (a) Wild-type and high-catalase mutant cultures were diluted 100-fold in *M*/15 phosphate buffer (pH 6.8) and aerated during irradiation by stirring a shallow layer of culture in a petri dish. Samples were diluted and plated for assay of viability after successive increments of irradiation (dose rate 2500 R/min). The catalase content of the wild type was 0.02 μg/mg dry cell mass; that of the mutant was 35 μg/mg (b) Wild-type cultures were diluted 4-fold in 0.01 *M* phosphate buffer (pH 6.8), held in test tubes, and bubbled with air during irradiation (dose rate 2100 R/min). An uninduced culture (catalase content 0.02 μg/mg) was compared with a culture in which a catalase content of 0.75 μg/mg had been induced by adding H_2O_2. The catalase content did not rise appreciably in either culture during irradiation.

Induced synthesis: choice of an end point

Fig. 2 shows the results of two experiments in which stationary-phase cultures of wild-type *R. spheroides* were irradiated and then tested for their ability to form induced catalase. The procedure (see figure legend) involved induction by H_2O_2 in one experiment and by air in the other. Fig. 2 shows that X-irradiation reduced the extent of induced catalase synthesis in *R. spheroides* without altering the time-course of this process. At least for the first hour, whether the inducer was air or H_2O_2

the newly formed catalase in an irradiated suspension was a constant fraction of that in the unirradiated control. This behavior was observed with X-ray doses up to 40 kR, and provided a basis for choosing an end point in subsequent experiments. When

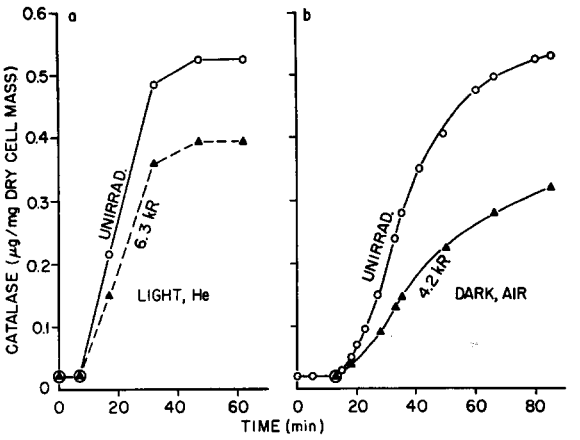

Fig. 2. Kinetics of induced catalase synthesis in unirradiated and X-irradiated wild-type *R. spheroides*. (a) A stationary-phase culture was diluted 4-fold into phosphate buffer (0.01 *M*, pH 6.8) and irradiated (6.3 kR) under an atmosphere of He. H_2O_2 was then added at time zero to a concentration of 50 μM and the suspension was incubated in the light under He. The unirradiated control culture was treated in the same way but was shielded from the X-ray beam. (b) A culture, diluted as stated in (a), was aerated (starting at $t = 0$) and given 4.2 kR of X-rays (from $t = 2$ to $t = 4$ min). Aeration was continued in darkness, and the catalase content was assayed from time to time.

H_2O_2 was added as inducer, the catalase formed during the ensuing 50 min was taken as the end point. With air as inducer the end point was the catalase formed during the first 30 min of aeration.

The effect of X-rays on catalase synthesis was found to be a little greater (see later) when nutrients were present during and after irradiation. In all subsequent experiments using stationary-phase cultures, unless otherwise specified, the cultures were diluted 4-fold with phosphate buffer containing 0.3% yeast extract and 0.2% casein hydrolysate before irradiation.

Inhibition of survival and enzyme synthesis

Using the protocol of the last two paragraphs, with stationary-phase cultures of wild-type *R. spheroides*, we measured the effect of X-irradiation on induced catalase synthesis and on survival. Results are shown in Fig. 3. At low doses the ability to form colonies and the ability to synthesize catalase are equally sensitive to X-irradiation. The inhibition of catalase synthesis is immediate, being expressed during the first few minutes after irradiation. Both manifestations of radiation damage show greater sensitivity in the presence of oxygen, with 2.5 kR in an aerated suspension equivalent to 10 kR in a suspension bubbled with He. The shapes of the curves yield an extrapolation number* less than 3; microscopic examination of the suspensions revealed that about half of the cells were single and half existed as pairs. We offer no explanation for the threshold in the presence of air and its absence in the presence of helium. In some instances involving low doses given under helium (*e.g.*, the last row of Table II), the inhibition of catalase synthesis was observed to be greater than the decrease in survival. But at doses for which the survival was 30% or less, the effect on survival always exceeded the effect on catalase synthesis. At high doses the survival curves

* This number is obtained by extrapolating the exponential part of a survival curve to zero dose and recording the intercept on the "survival" axis.

remain exponential, whereas the curves for induced enzyme synthesis become progressively less steep. This effect will be discussed later in connection with other aspects of the behavior of this system, and in relation to earlier literature on X-ray inhibition of enzyme synthesis.

The response vs. the time at which the system is irradiated

Figs. 2 and 3 pertain to experiments in which the cells were irradiated just before or just after the inducer was introduced. With air as inducer, the irradiation was of course more effective when applied after the induction had been initiated, because of the "oxygen effect" (see Fig. 3). With H_2O_2 as inducer, in suspensions bubbled with He, it made no difference whether the irradiation or the H_2O_2 was delivered first, provided that the irradiation ended before the newly formed catalase began to appear. But if the irradiation was postponed further (with either air or H_2O_2 as inducer) its effectiveness in suppressing enzyme synthesis diminished abruptly. This change occurred precisely when the period of irradiation began to encroach on the period of rising enzyme activity (Fig. 4). It appears that once the apparatus for enzyme synthesis has been mobilized, the system is less sensitive to irradiation. The effect of irradiation on survival showed no corresponding change. In a broad way, the study of induced enzyme synthesis is comparable to the study of survival in a synchronized culture: a chain of events is started at a precise time in the entire population; the system can then be irradiated at different times and the response noted.

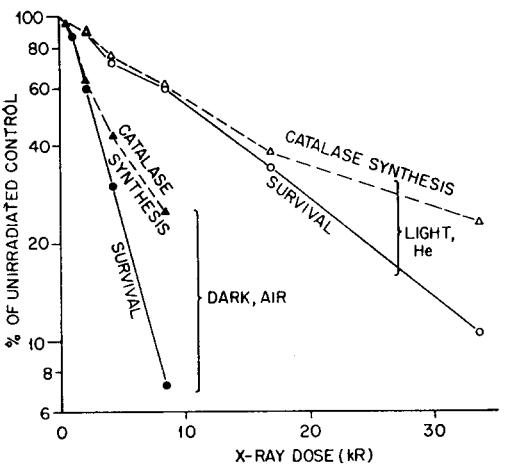

Fig. 3. Effect of X-irradiation on viability and induced catalase synthesis in wild-type *R. spheroides*. Experimental protocol as in Fig. 2, except that the cells were diluted into phosphate buffer containing 0.3% yeast extract and 0.2% casein hydrolysate. Samples were plated for assay of viability immediately after irradiation.

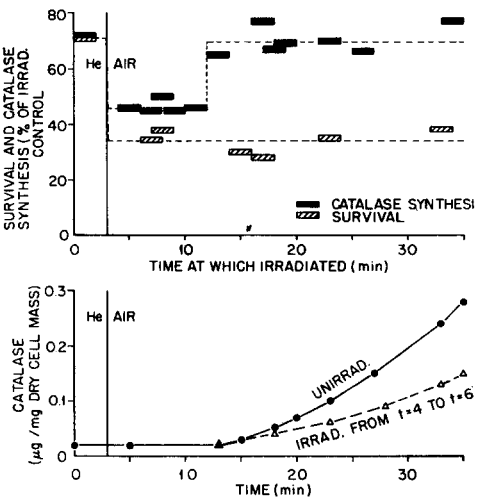

Fig. 4. Effects of 4.2 kR of X-rays (dose rate 2100 R/min) delivered at various times during the induction of catalase synthesis in wild-type *R. spheroides*. Stationary-phase cultures were diluted as described for Fig. 3 and bubbled with helium; the He was replaced with air (at $t = 3$ min in the figure) to initiate the induction of catalase synthesis. Aeration in the dark was continued and catalase was assayed from time to time. For cells irradiated after $t = 10$ min, the increment of catalase during the first 30 min following irradiation was compared with its counterpart in an unirradiated control. For cells irradiated before $t = 10$ min, the comparison was based on synthesis from $t = 0$ to $t = 30$ min. Fig. 4b shows the time-course of catalase synthesis in two suspensions.

Factors modifying the response to irradiation

In a growing culture of *R. spheroides* the sensitivity of induced catalase synthesis to X-irradiation becomes progressively greater. Concomitantly the effect of X-rays on survival decreases slightly as the culture grows. These effects are shown in Table II, representing an experiment in which growing cultures were irradiated under helium, given H_2O_2, and allowed to continue growing anaerobically in the light. In a young culture (first row), the synthesis of catalase after irradiation amounted to 81% of that in an unirradiated control. This culture and its companion control culture were given a second dose of H_2O_2 (50 μM). The resulting second burst of catalase synthesis showed the effect of irradiation to a greater degree: second burst (irradiated) = 51% of second burst (control).

The effect of irradiation on induced catalase synthesis could also be modified slightly by adjusting external factors. By decreasing the amount of H_2O_2 (inducer) or the light intensity (energy source) so as to reduce the response of the unirradiated system by a factor of 4, the sensitivity toward X-rays could be elevated slightly.

TABLE II

EFFECT OF CULTURE AGE ON THE X-RAY SENSITIVITY OF WILD-TYPE *R. spheroides*

Growing cultures were irradiated (12.6 kR) under an atmosphere of helium. H_2O_2 was then added to each culture, to a concentration of 50 μM, and the cultures allowed to continue growing anaerobically in the light. After 1 h the synthesis of catalase, induced by H_2O_2, was determined. Samples were plated for assay of viability immediately after irradiation.

% of maximum growth	$\left[\frac{\text{Viable cells (irrad.)}}{\text{Viable cells (control)}}\right]$	$\left[\frac{\Delta \text{ Catalase (irrad.)}}{\Delta \text{ Catalase (control)}}\right]$
21	0.28	0.81
37	0.32	0.61
61	0.34	0.48
95	0.40	0.34
100 (Stationary)	0.44	0.32

TABLE III

EFFECTS OF NUTRIENTS* AND OF CHLORAMPHENICOL ON THE X-RAY INHIBITION OF INDUCED CATALASE SYNTHESIS IN WILD-TYPE *R. spheroides*

Stationary-phase cultures were diluted 4-fold with phosphate buffer (0.01 *M*, pH 6.8), with or without added nutrients. The diluted cultures were given 8.4 kR of X-rays under an atmosphere of helium; H_2O_2 was then added to a concentration of 50 μM and the suspensions bubbled with He in the light. Catalase was assayed initially and 50 min after the addition of H_2O_2. Chloramphenicol was administered after irradiation but before the addition of H_2O_2.

Experiment	± Nutrients*	Chloramphenicol (μg/ml)	Δ Catalase control**	Δ Catalase irradiated**	$\left[\frac{\Delta \text{ Catalase (irrad.)}}{\Delta \text{ Catalase (control)}}\right]$
1	—	0	0.55	0.38	0.69
	+	0	0.51	0.29	0.57
2	+	0	0.43	0.22	0.51
	+	0.2	0.24	0.125	0.52
	+	1.0	0.096	0.050	0.52

* 0.3% yeast extract and 0.2% casein hydrolysate.
** Increments of catalase in μg/mg of dry cell mass.

The sensitivity could also be increased, as mentioned earlier, by adding nutrients (yeast extract and casein hydrolysate) to a stationary-phase culture. When these factors were manipulated simultaneously, the response (Δ catalase, irradiated cells/ Δ catalase, control) was changed, in one experiment, from 0.65 to 0.5. Inhibition of catalase synthesis with chloramphenicol did not alter the response to irradiation. The effects of nutrient and of chloramphenicol are shown in Table III.

None of these factors, aside from culture age, altered the effect of irradiation on survival. Preliminary attempts to promote recovery by holding the suspensions in various conditions (at several temperatures, with and without nutrients and/or energy sources) after irradiation were negative.

Survival and synthesis of various proteins in irradiated cells

In *R. spheroides* the syntheses of many proteins show the same order of sensitivity toward X-irradiation as does the synthesis of inducible catalase. This was established for "general protein" (Folin assay), for chromatophore proteins, and for the constitutive catalase of the high-catalase mutant. The results of three pertinent experiments are summarized in Table IV. In these experiments, growing cultures (about 40% of maximum growth at the time of irradiation) were given 12.6 kR of X-rays under helium and then allowed to continue growing in the light. Samples were plated for assay of viability immediately after irradiation. Samples were withdrawn at 1-h intervals for the assay of catalase, general protein, and bacteriochlorophyll. The tabulated values, representing percent of the unirradiated controls, are based on increments

TABLE IV

EFFECTS OF X-RAYS ON VARIOUS SYNTHETIC ACTIVITIES IN WILD-TYPE AND HIGH-CATALASE MUTANT *R. spheroides*

See text for experimental details.

Expt.	*Strain*	*Percent of unirradiated control*					
		Viable cells	*General protein synthesis*	*Catalase synthesis*		*Bacteriochlorophyll synthesis*	
				Induced	*Constitutive*	*In vivo spectrum*	*Extracted pigment*
1	Wild type	37	53	56	—	—	—
2	Mutant strain CC1	36	44	—	46	—	—
3	Wild type	51	62	—	—	54	70

during the first 2-h period after irradiation. In the first experiment wild-type cells were aerated after irradiation, and the synthesis of general protein (Folin assay) was followed as well as catalase synthesis induced by the aeration. The second experiment shows general protein synthesis and the synthesis of constitutive catalase in the high-catalase mutant. In the third experiment, the suspensions of wild-type cells were kept in an atmosphere of He and the light intensity was reduced 4-fold after irradiation. The decrease in illumination engendered an accelerated synthesis of bacteriochlorophyll and a slowing of total protein synthesis. The development of the *in vivo* absorption spectrum of bacteriochlorophyll was inhibited by irradiation to the same extent as general protein synthesis and as survival. The synthesis of bacteriochlorophyll itself (measured as the extracted pigment) showed a lesser response to

irradiation. We infer that the *in vivo* spectrum reflects the presence of chromatophore proteins as well as bacteriochlorophyll, and that synthesis of these proteins is inhibited by irradiation to the extent indicated by the absorption spectrum.

These experiments show that the failure of induced catalase synthesis in irradiated *R. spheroides* is representative of a large class of synthetic failures.

DISCUSSION

The foregoing details give some indications of how irradiation inhibits protein synthesis in *R. spheroides*.

The experiments involving external factors (see Table III) were undertaken with the hope that by introducing a new rate-limiting factor in the over-all process of induced catalase synthesis, we might alter the sensitivity to X-rays. Suppose that the over-all process includes two steps, one of which is much more sensitive than the other to X-irradiation:

$$\ldots\ldots \rightarrow A \rightarrow B \rightarrow \ldots\ldots\ldots \rightarrow X \rightarrow Y \rightarrow \ldots\ldots$$

(sensitive) (insensitive)

Assume that these steps can be slowed by irradiation without being eliminated in an all-or-none manner. Then if, under certain environmental circumstances, the step $A \rightarrow B$ is strongly rate-limiting for the over-all process, the system will be sensitive to X-rays. But if $X \rightarrow Y$ is rate-limiting, the system will be insensitive. Thus by shifting the locus of rate-limitation (*e.g.*, from $A \rightarrow B$ to $X \rightarrow Y$), one could alter the response to X-rays. These considerations do not hold if the X-rays can act only in an all-or-none manner, *i.e.*, by eliminating entire catalase-forming systems or leaving them intact. In that case the effect of X-rays will not depend on which step is rate-limiting. We have attempted to impose new rate-limiting conditions in four ways: by restricting the amount of inducer, of energy, and of nutrients, and by adding chloramphenicol. In no case was the sensitivity to X-rays changed markedly. It is probable, therefore, that entire enzyme-synthesizing sequences, or units, are eliminated by X-irradiation in an all-or-none manner.

In stationary-phase cultures the effect of X-rays on induced catalase synthesis has two noteworthy features that are illustrated in Figs. 3 and 4. Fig. 3 shows, in the flattening of the dose-effect curves, that the cells have a residual radiation-insensitive ability to make induced catalase. Fig. 4 shows that as soon as the machinery for synthesizing induced catalase has been fully mobilized, the system is less sensitive to irradiation. Thus it is the formation of this machinery, and not its operation, that is particularly sensitive to X-rays. The formation of the fully functioning enzyme-synthesizing machinery can of course involve many events: the expression of a gene, the action of the inducer, etc. Before the addition of inducer, precursors of the enzyme-forming machinery may exist in different stages of completion. The full, normal operation of this system will be prevented when X-rays eliminate one or more of the necessary events. A residual capacity for enzyme synthesis, more resistant to X-irradiation, will then remain if the cells can form a limited amount of the enzyme-synthesizing machinery by drawing upon precursors that are already present. This capacity could account for the flattening of the dose-effect curves for induced catalase synthesis (Fig. 3). The effects illustrated in Figs. 3 and 4 suggest, then, that X-rays act for

the most part early in the sequence of events culminating in induced enzyme synthesis.

Observations made with growing cultures (see Tables II and IV) have established that in *R. spheroides* the syntheses of many proteins, including inducible catalase, are inhibited by X-rays to about the same extent as survival. Induced catalase synthesis appears less sensitive in growing cultures than in stationary-phase cultures, but only if the system is tested in a certain way (by adding a single dose of H_2O_2). In contrast, X-rays inhibit survival more in a growing culture than in a stationary-phase culture.

With this background one can explain, at least in part, the failure of some investigators[3–5] to observe an inhibition of protein synthesis comparable to the inhibition of survival. The exclusive use of high doses would have led us to conclude that survival is by far the more sensitive criterion of radiation damage (see Fig. 3). The use of young, growing cultures can also give the impression that protein synthesis is less sensitive to X-rays than is survival (Table II). The greater loss of viability in growing cultures, as compared to stationary-phase cultures, is far more pronounced in *E. coli*[15] than in *R. spheroides*. Finally it is possible that precursors of the protein-forming machinery, which provide a basis for radiation-insensitive protein synthesis, are more abundant in other species than in *R. spheroides*.

The experiments of McFALL, PARDEE AND STENT[16], using *E. coli* cells in which the DNA (but not the RNA) contained $^{32}I'$, showed that the disruption of DNA molecules leads to a decline in viability and a comparable loss in the ability of the cells to form β-galactosidase. Here the failure to survive and the failure to synthesize an inducible enzyme are both related to an act in which genetic material is destroyed. McFALL AND MAGASANIK[17] point out that thymine-starvation and accumulation of catabolites, and not ^{32}P decay, could have caused the effects observed in these experiments. This criticism is less serious in the experiments of RILEY *et al.*[18], in which the DNA was labeled by mating a strain of *E. coli* containing ^{32}P with an unlabeled strain. The latter experiments, however, did not include a comparison between loss of viability and loss of enzyme-forming capacity. Other experiments in which the ultraviolet action spectrum for loss of enzyme synthesis and colony formation have been determined also suggest that genetic material is the radiation-sensitive part of the mechanism of enzyme synthesis[19].

The essence of our experiments is that in *R. spheroides*, protein synthesis shows an immediate response to X-irradiation, comparable to the loss of viability. The slackening of protein synthesis is seen long before an appreciable number of cells could have divided (the generation time in a growing culture is about 5 h). The major effect of irradiation appears to be exerted in an all-or-none manner, early in the sequence of events that depends ultimately on genetic information and that culminates in the synthesis of protein. Thus we are led to an hypothesis that X-irradiation is effective primarily in destroying the genetic apparatus, and that the failure to survive and to synthesize protein are related consequences of this destruction. The loss of half of the protein-forming capacity in a population of cells would seem sufficient to account for a failure of half of the cells to form colonies.

Nevertheless, it is possible that the inhibition of protein synthesis and the loss of viability represent completely different actions of X-rays. At worst we are dealing with a well-defined biochemical phenomenon (induced catalase synthesis) that shows an immediate response to X-irradiation, that can be turned on (with inducer) and

regulated (with light as the sole source of energy) easily, and that gives results more quickly than does the measurement of survival. At best, we have a system that may be dissected to reveal the principal cause of radiation injury in a bacterium.

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