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BACTERIAL LUMINESCENCE: IN VIVO AND IN VITRO RESPONSE TO X IRRADIATION

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Bacterial luminescence is suppressed immediately by X rays and recovers promptly thereafter. This effect is similar *in vivo* and *in vitro*, and any disparities are attributed to physicochemical differences between the two sample types. Continuous measurement of dissolved oxygen in samples suggests depletion of oxygen is not responsible for this radiation response. These measurements do show a transient increase of oxygen uptake by bacteria during exposure. Comparisons such as these should be useful in further studies towards identifying the radiosensitive component(s).

Luminescence of *Photobacterium fischeri* is immediately suppressed by X-rays and recovers promptly thereafter[1,2]). The mechanism for this response is unknown. However, it is known that the luminous reactions require oxygen[3]). Further, the oxygen content of cells and tissues is suppressed during irradiation[4,5]). Thus, lowered oxygen tension of luminescent systems during irradiation would explain the suppressed luminescence observed. The re-equilibration of oxygen after exposure would explain recovery of luminescence which is also observed.

As a first step toward understanding the mechanism of luminescence suppression by X-rays, this response has been measured *in vivo* and *in vitro* simultaneously with continuous measurements of dissolved oxygen.

An aluminum irradiation chamber couples a galvanic cell oxygen analyzer and a light guide. The distal end of the latter attaches to a shielded photomultiplier[6,7]). For radiation studies, the chamber is moved opposite to an X-ray beam port.

Whole cells of *Photobacterium fischeri* (ATCC 7744) suspended in buffered saline were used for *in vivo* studies. Cell concentrations were adjusted to 2×10^9 cells per milliliter. For *in vitro* studies, clarified, cell free extracts were prepared from lysed cells. Excess amounts of reduced nicotine adenine dinucleotide (NADH), flavin mononucleotide (FMN) and decanal were added to extract aliquots immediately prior to an experiment. The methods

TABLE 1

Luminescence values for 6 important luminescent characteristics from fig. 1 are compared *in vivo* and *in vitro* for 4 consecutive samples (columns 1–4) on 3 different days (a, b, c); successive determinations of the same characteristic of luminescence vary between 6 and 10%; values are presented as arbitrary luminescence units (1 ALU = 1 × 10^{-8} A); rates are expressed as ALU/sec^{-1}

		in vitro				*in vivo*			
Luminescent characteristic	Experiment	1	2	3	4	1	2	3	4
Initial intensity	a	3.90	3.72	3.70	3.65	2.32	2.35	2.35	2.16
	b	3.30	3.20	3.10	3.22	1.86	2.48	2.36	2.15
	c	3.30	3.25	3.35	3.10	1.91	1.91	1.86	1.72
Maximum suppression rate	a	0.17	0.16	0.19	0.16	0.12	0.12	0.14	0.12
	b	0.14	0.15	0.15	0.15	0.08	0.11	0.10	0.11
	c	0.16	0.16	0.16	0.15	0.11	0.09	0.09	0.08
Total suppression	a	1.59	1.41	1.52	1.42	1.94	1.90	1.95	1.73
	b	1.45	1.34	1.27	1.19	1.89	1.82	1.76	1.59
	c	1.65	1.37	1.71	0.96	1.81	1.74	1.71	1.78
Latent period (in seconds)	a	1	1	1	1	33	36	50	52
	b	1	1	1	1	21	28	28	25
	c	1	1	1	1	10	10	10	10
Maximum recovery rate	a	0.41	0.37	0.36	0.33	0.011	0.015	0.009	0.008
	b	0.31	0.29	0.30	0.31	0.006	0.005	0.006	0.004
	c	0.29	0.27	0.30	0.25	0.011	0.012	0.012	0.011
Final intensity	a	3.90	3.72	3.70	3.65	1.37	1.55	1.49	1.13
	b	3.30	3.22	3.15	3.25	0.89	1.19	1.13	0.95
	c	3.30	3.28	3.25	3.10	1.60	1.58	1.51	1.45

of sample preparation have been reported[8]). Control cell or extract samples emit luminescence essentially constant for 20 min or longer.

When irradiated[9]), luminescence of both cells and extracts is suppressed within seconds, attains a lower, somewhat steady state, and recovers after the exposure. The essential comparisons are shown in table 1 and illustrated in fig. 1.

Luminescence is suppressed faster *in vitro* than *in vivo*. During the latter portion of exposure, *in vitro* luminescence usually recovers slightly while *in vivo* luminescence continues to fall slowly. After exposure, recovery *in vitro* is vigorous and immediate; *in vivo* it is delayed and slow. The slight recovery noted *in vitro* during exposure seems to be related to the overshoot of luminescence after exposure. If recovery does not occur during exposure, no overshoot is observed thereafter (dashed line, fig. 1). The overshoot is

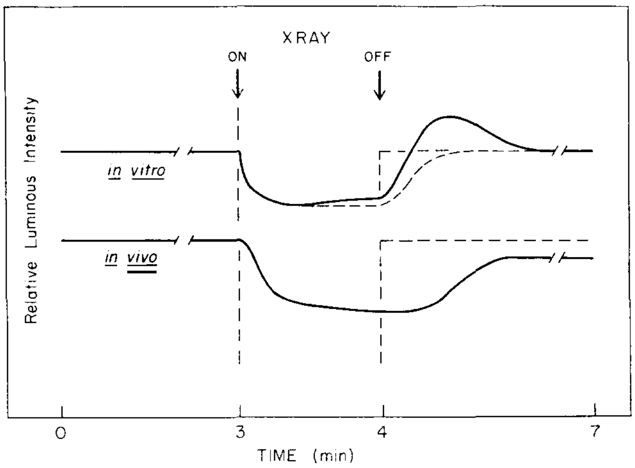

Fig. 1. Comparisons of luminescence *in vivo* and *in vitro* before, during, and after irradiation. At the onset of exposure, luminescence is suppressed faster *in vitro* than *in vivo*. Later during exposure, the *in vitro* system recovers slightly while *in vivo* a slow, continuous fading is observed. Total suppression at the end of an exposure is always greatest in *vivo*. After exposure, recovery *in vitro* begins immediately, is vigorous, and, unlike the *in vivo* system, is complete. If recovery during exposure does not occur *in vitro*, the recovery wave does not follow as indicated by the dashed line.

similar to that noted in cell samples following periods of anoxia[3]). Both systems regain steady state luminescence within 3 or 4 min. Recovery *in vitro* is complete; *in vivo* it is seldom complete.

The differences of response to X-rays by *in vivo* and *in vitro* luminescence are undoubtedly related to the physicochemical differences between the two systems. To explain, the *in vitro* system is devoid of membranes and associated structures, has excess luminescence intermediates (NADH, FMN, and aldehyde), and a high protein concentration. By contrast, *in vivo* system membranes are intact, cytochrome activity is normal, and luminescence intermediates are present in much lower endogenous concentrations. Cells contribute less than 1 % of the total sample volume *in vivo*[2]). This difference alone should alter the chemistry during irradiation, since most of the radiation-induced reactions will occur in saline rather than in the homogeneous protein solution of *in vitro* samples.

One can speculate that intact cell membranes will retard the entry of radiotoxins such as hydrogen peroxide. Data show that suppression of luminescence occurs more slowly *in vivo* than *in vitro*. Excess substrates *in vitro* should reduce the total suppression of luminescence by replacement if these substrates are radiolabile. In fact, table 1 shows less suppression *in vitro* than *in vivo*. Further, these excess substrates should contribute to

TABLE 2

Rates of oxygen uptake before, during, and after irratiation of different solutions

Expt. No.	Solution	Example No.	Dissolved oxygen (mg/l · sec) × 10^{-3} Total oxygen consumption S_1	S_2	S_3	Oxygen consumption by bacteria alone A	B	C	D	(k) Probe sensitivity (A/mg·l) × 10^{-7}
1	Typical	a	1.7	9.1	1.7	—	—	—	—	3.4
	in vitro	b	1.7	8.6	1.7	—	—	—	—	
	samples	c	1.6	8.3	1.6	—	—	—	—	
2	Saline		1.4	3.2	1.4	—	—	—	—	3.9
3	Saline		1.7	3.8	1.7	—	—	—	—	3.4
	Saline + 10^{-3}M	a	1.7	19.2	1.7	—	—	—	—	
	cysteine	b	1.7	21.3	1.7	—	—	—	—	
4	Saline		1.1	3.9	0.8	—	—	—	—	3.6
	Saline +	a	1.1	4.0	0.8	—	—	—	—	
	heat killed	b	1.1	5.3	0.9	—	—	—	—	
	cells	c	1.1	4.4	0.9	—	—	—	—	
5	Saline		1.7	4.1	1.7	—	—	—	—	4.1
	Typical	a	4.6	10.2	4.5	2.9	6.1	2.8	+3.2	
	in vivo	b	4.8	12.3	3.8	3.1	8.2	2.1	+5.1	
	samples									
6	Saline		1.7	3.6	1.7	—	—	—	—	4.2
	Typical		9.2	15.7	9.8	7.5	12.1	8.1	+4.6	
	in vivo									
	samples									
7	Saline		1.6	2.0	1.6	—	—	—	—	5.7
	in vivo		3.8	6.2	3.1	2.2	4.2	1.5	+2.0	
8	Saline		1.4	2.2	1.4	—	—	—	—	3.5
	Typical	a	11.2	12.5	8.6	9.8	10.3	7.2	−0.5	
	in vivo	b	11.2	15.6	10.4	9.8	13.4	9.0	+3.6	
	samples	c	10.4	13.4	9.5	9.0	11.2	8.1	+2.2	

Total oxygen consumption represents oxygen uptake by the probe and cells (S_1 and S_3). In addition, oxygen uptake by radiation-induced reactions occurs during exposure (S_2). A, B, and C represent oxygen uptake by bacteria alone before, during, and after exposure, respectively. The net effect of radiation on cellular oxygen uptake is given under column N and is determined by subtracting A from B.

rapid replacement of luminescence intermediates after exposure. The data support this.

Typical oxygen probe data for several types of solutions are given in table 2 together with probe sensitivities (k) [determined by calibration[7])] for each of eight experiments. Also, for each calibration, data are given for the rate of oxygen consumption by the probe alone, i.e., S_1 and S_3, in cell-free saline. In this medium, S_2 measures the rate of oxygen uptake during irradiation by the probe and by radiation-induced reactions ($P + R$). These values are used to calculate the various rates of oxygen uptake due to cells or solute additions to saline for the various experiments [for example, $A = S_1 - P$, $B = S_2 - (P + R)$].

Probe sensitivities vary somewhat because it is difficult to install the thin polyethylene membranes onto the probes with equal tautness each time. Presumably, a very tightly stretched membrane will allow more rapid diffusion of oxygen to the electrode surfaces than a membrane not so tightly installed.

In table 2, Experiment No. 1, oxygen uptake rates S_1, S_2, and S_3 should be compared with the corresponding values for saline in Experiment No. 2. Oxygen uptake is roughly 3 times greater during exposure (S_2) for *in vitro* samples than for saline.

In Experiment No. 3, the addition of millimolar cysteine substantially increases oxygen uptake in irradiated saline. The addition of other substances also increases oxygen uptake during exposure, but not to such an extent. For example, when *in vivo* samples are heat-killed, oxygen uptake during exposure is only slightly higher than for unadulterated saline (Experiment No. 4).

In Experiments No. 5–8, table 2, typical values of oxyten uptake in *in vivo* samples are given. Comparisons of values for A, B, and C shows the changes of cell oxygen uptake before, during, and after exposure. Generally, oxygen uptake by cells increases during exposure and decreases after exposure. To date, 287 *in vivo* samples have been analyzed. The rate of oxygen uptake by cells was increased during irradiation in 84% of all samples tested; depressed in the remainder. For an example of the latter, compare N, table 2, Experiment No. 8a, with the others. The negative value of N means oxygen uptake was depressed during exposure.

The post-irradiation rate of oxygen uptake by cells was reduced to less than the pre-irradiation rate in 70% of all samples; 20% showed an increase; the remainder showed no change ($A - C$ is positive, $A - C$ is negative, $A - C$ is zero, respectively). Thus, most of the samples showed a transient increase of oxygen uptake; one that disappeared promptly after exposure. Others have reported similar results[4, 5]).

The average standard error for a series of consecutive determinations of the rate of oxygen uptake by untreated cells is approximately 5% ($\bar{x}$ = 0.935 + 0.020 μg O_2/l sec). The estimate for errors in chemical oxygen determinations in complex media is approximately 6% [7]).

The luminescent reaction is independent of oxygen concentration above 0.3 vol% *in vivo* and *in vitro*[3]). In no case during these experiments was oxygen reduced to this level. Thus, the depression of luminescence during X irradiation is not due to measurable anoxia of sample oxygen tension.

These results are particularly significant because they show that some radiation effects appear immediately upon irradiation and recover promptly thereafter. It is also important that these immediate radiation effects result from exposures to low doses. They are first observable within one second at doses less than 100 rad.

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9) General Electric Maxitron 300 operated at 300 kVp and 20 mA; tube target 9 cm from the mid-line of the irradiation chamber, filtration of the X-ray beam consisted of a 4.75 mm beryllium tube window and the walls of the chamber (approximately 1 mm aluminum with 1% copper); half value layer of the X-ray beam passing through the walls of the chamber was 0.75 cm aluminum measured by ferrous sulfate dosimetry ($G = 15.65$ $\varepsilon = 2270$ at 26.8 °C). The dose rate was 6500 rad/min; exposure time was 1min in all experiments.
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