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Ionizing Radiation at Low Doses Induces Inflammatory Reactions in Human Blood

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VICKER, M. G., BULTMANN, H., GLADE, U., AND HÄFKER, T. Ionizing Radiation at Low Doses Induces Inflammatory Reactions in Human Blood. *Radiat. Res.* 128, 251–257 (1991).

Irradiation of whole blood with ^{137}Cs γ rays intensifies the oxidative burst. Oxidant production was used as an indicator of inflammatory cell reactions and was measured by luminol-amplified chemiluminescence after treatment with inflammatory activators including bacteria, the neutrophil taxin formyl-Met-Leu-Phe, the Ca^{2+} ionophore A23187, the detergent saponin, and the tumor promoter phorbol ester. The irradiation response is dose-dependent up to about 100 μGy, is detectable within minutes, persists at least 1 h, and is transmitted intercellularly by a soluble mediator. The response is completely inhibited by Ca^{2+} sequestration in the presence of A23187 or by adenosine, indicating its Ca^{2+} dependency, and by the phospholipase A_2 blocker *p*-bromphenacyl bromide. However, inhibition by the cyclooxygenase blocker aspirin is sporadic or absent. Blood taken after diagnostic examination of lungs with X rays also exhibited intensified chemiluminescence. These reactions implicate a role for specific amplifying mediator pathways, especially metabolites of the arachidonic acid cascade, in the response: "damage and repair" to cells or DNA plays little or no role. Our results provide evidence for a new mechanism of radiation action with possible consequences for the homeostasis of reactions involving inflammation and second messengers in human health and early development.

INTRODUCTION

The mechanisms of the major biological responses to ionizing radiation remain enigmatic, but are still discussed mainly in terms of a single paradigm involving DNA damage (i.e., mutation and cancer) and cell killing. It is assumed that there is a balance struck between the disruption of molecules after collision with energetic particles, the production and scavenging of radicals, and the processes of molecular repair and recovery at the tissue level (*1–6*).

Doses of several grays can modify or destroy sufficient biological molecules of all classes to be immediately life-threatening to humans, while a few milligrays may significantly affect only DNA, yet severely enough to contribute to carcinogenesis and heritable mutations despite the relative efficiency of repair systems. The detection of responses at still lower doses has required highly sensitive controlled observations (e.g., (*7*)). Significantly, though, radiobiological predictions about the effects of radiation at doses encountered in industrial, medical, and low-level accidental exposures rely on extrapolation from effects at high doses. However, the intrinsic uncertainty which remains in regard to the validity of the extrapolation models and the existence of response thresholds at low doses (*1–6*) will continue as long as models are substituted for empirical biological response mechanisms.

Some phenomena, however, are not taken into account in the paradigm of DNA damage, including radical chain reactions and lipid peroxidation in membranes (*8*), oscillatory plasma membrane depolarization (*9*), ion fluxes (*10, 11*), histamine and prostaglandin synthesis (*12*), stress protein synthesis (*13*) similar to oxidative stress by H_2O_2 and heavy metals, loss of enzyme activity (*6*) resembling that after heat shock, specific amplification of genome-integrated oncogene and tumor virus sequences (*14, 15*), and activation of HIV-I retrovirus genes (*16*). Although these observations were made in studies using relatively high doses, they are distinctly reminiscent of reactions to hormones and tumor promoters (*17*), and provide grounds for entertaining a new perspective of biological sensitivity to low-dose radiation. Is it possible that radiation perturbs intercellular communication in addition to its effects on chromosome and lipid membrane integrity?

We have examined this hypothesis in the cellular reactions of whole blood to ionizing radiation using luminol-amplified chemiluminescence, a convenient and efficient indicator of some oxidants produced during the oxidative burst in phagocytes (*18–22*). Physiological activation of inflammation induces plasmalemmal depolarization and the production of second messengers by plasmalemma-associated enzymes, e.g., phospholipase A_2 (PLA2), which generates lysolecithin and fatty acids, including arachidonic acid (ARAC), from lecithin and phosphatidylinositol (*23–27*). Inflammation is mediated partly by the transformation of ARAC into a cascade of potent intercellular agents, including thromboxanes, prostaglandins, and prostacyclins by the cyclooxygenase pathway, and leukotrienes and their

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eicosanoid derivatives by the lipoxygenase pathway (*21, 22, 27*). These metabolites may regulate the activity of protein kinase C (PKC) and, thus, NADPH-oxidase and chemiluminescence. We chose to investigate whole blood because, although its use presents some drawbacks, it offers a nearly intact tissue system in which the interactive behavior of the white cell population might be observed. The results demonstrate that exceptionally low doses of radiation intensify the oxidative burst in activated blood by generating soluble inflammatory mediators and thus provide evidence for a mechanism whereby very low doses might play a significant but hitherto unsuspected role in human health.

MATERIALS AND METHODS

Agents

The concentrations and stock solvents of the chemicals were: 100 μM adenosine in dimethyl sulfoxide (DMSO, spectroscopic grade, Merck, Darmstadt); 400 μM amiloride in DMSO; 100 μM ARAC in ethanol under N_2; 10 μg/ml aspirin in DMSO; 20 μM *p*-bromphenacyl bromide (BPB) in DMSO; 5 μM cytochalasin B (CB) in DMSO; 2 mM ethylene glycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate (EGTA) in Hanks' salt solution buffered with 20 mM Hepes (*N*-[2-hydroxyethyl]piperazine-*N′*-[2-ethanesulfonate]) to pH 7.4 (HHBS); 2 μM formyl-Met-Leu-Phe (fMLP) in DMSO; 20 μM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in DMSO; 1–10 μM monensin in ethanol; 100 μM nordihydroguaiaretic acid in DMSO; 250 μM phenidone in ethanol; 1 μg/ml PLA2 in HHBS; 1 μg/ml phorbol-12-myristate-13-acetate (PMA) in DMSO; 20 μg/ml saponin in HHBS; and 10 μg/ml sphingosine in DMSO. These chemicals were purchased from Sigma (Deisenhofen) and stored at −25°C. A23187 (Boehringer, Mannheim) was used at 2 μM and stored in ethanol at 4°C. *Escherichia coli* B/r was grown in stirred nutrient broth 24 h at 37°C and then centrifuged, washed twice, suspended in water at 10-fold the growth concentration, and autoclaved. Twenty microliters of bacteria was added per ml blood suspension.

Blood and Luminometry

Our procedures were adapted from Kato *et al.* (*28*). Heparinized (10 IU/ml, Sarstedt) venous blood from healthy volunteers was cooled on ice, and used up to 4 h after withdrawal. Blood was diluted 10-fold in HHBS. Final DMSO concentrations were below 1%. All experiments were repeated independently on three or more occasions. The chemiluminescence values presented here are from representative experiments, averaged from three or more replicates, except where stated otherwise.

Diluted blood (0.5 ml) in plastic luminometer or Eppendorf tubes was preincubated 2.5 min in a 37°C water bath before treatment. Chemiluminescence was measured with a Berthold–Lumac M 2010 luminometer (Abimed, Düsseldorf) heating to 37°C. The irradiation response was insensitive to addition of luminol, activator, or DMSO before or after irradiation (not shown). Chemiluminescence was not quenched by intact erythrocytes, because hemolysis by saponin had no effect on chemiluminescence levels. The full response requires both phagocytes and thrombocytes (not shown). The chemiluminescence values are the total light emission obtained by integration over the measurement period.

Irradiation

The blood sample was removed to a plastic cup in the well of a lead block under a 37°C air stream. The irradiator, 9.25×10^6 Bq of ^{137}Cs in a steel cylinder (Amersham–Buchler), was placed under the cup. Average γ-radiation doses, based on standard measurements ($N = 4$, SD = ±12.06%), were obtained from the integrated dose calculated across the sample dimensions in the sample tube.

Blood from patients was obtained from routine samples immediately before and at least 5 min after X irradiation of the thorax and was not cooled on ice. The X-ray dose was derived from the standard calculated heart and lung dose range (150–300 and 300–600 μGy, respectively) and an estimated thorax/body blood volume ratio of 0.25 to yield 56–113 μGy, or an average 84 μGy to the total blood volume. The chemiluminescence value was averaged from three individuals.

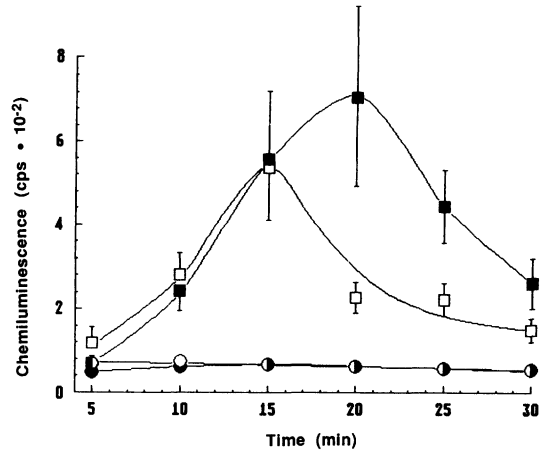

FIG. 1. Chemiluminescence in light counts per second (cps) as a function of the duration of exposure to ^{137}Cs γ rays. Blood was activated with *E. coli* to induce luminol-amplified chemiluminescence during exposure to γ irradiation at 21.7 μGy/min beginning at t_0. The squares and circles indicate blood with and without bacterial activation, respectively. The irradiated samples are indicated by the closed symbols, and bars indicate the SD; $N = 3$ for activated samples and $N = 7$ for the nonactivated controls (where the SD bars are smaller than the size of the symbols).

Diffusion Chamber Assembly

Either irradiated or nonirradiated blood (0.33 ml) was added to each chamber of a filter assembly, consisting of two Lucite blocks each with 0.7-ml horizontal chambers bored from one face and accessible from above. A 13-mm-diameter nitrocellulose filter of 5-μm pore size and 150-μm thickness (Sartorius, Göttingen), sandwiched between a neoprene O-ring and a gasket, was fitted between the two blocks, which were then bolted together. Treated or untreated blood sample pairs were placed on either side of the filter. After 35 min at 37°C, the samples were diluted in HHBS containing luminol and *E. coli*.

RESULTS

Irradiation and Chemiluminescence

Figure 1 demonstrates the biphasic reaction of whole blood to *E. coli,* and thus exemplifies the chemiluminescent response to an inflammatory activator. The ^{137}Cs γ radiation alone induces no reproducible luminol-amplified chemiluminescence. Only after activation does an effect become apparent in the form of an intensified chemiluminescence response. In *E. coli*-activated and irradiated blood the chemiluminescence response typically lags slightly behind that of nonirradiated blood during the first 10 min, but subsequently becomes more intense and prolonged.

Table I shows that blood also responds *in vivo* to a single diagnostic X ray of the thorax, the lowest clinical dose, and that the response to γ irradiation *in vitro* is sensitive to various combinations of activators and inhibitors of the ox-

TABLE I
Chemiluminescence (CL) Responses to Irradiation *in Vitro* and *in Vivo*

				Irradiated			
(a) Agent 1 (CL = 100%)	Agent 2	(b) Agent 1 + agent 2 CL	(c) Control CL	Dose ^{137}Cs γ rays (μGy)	(d) Agent 1 CL	(e) Agent 1 + agent 2 CL	(f): e vs b (b = 100%) CL
PLA2	—	—	77.8	936	−4.2	—	—
fMLP	PLA2	134.6	32.3	936	121.9	190.0	141.2
—	PLA2	31.9	32.3				
PMA	PLA2	96.0	9.1	936	113.6	128.7	134.1
—	PLA2	10.4	9.1				
A23187	CB	31.3	16.2	152	184.7	57.3	183.1
fMLP	amilo	26.2	32.4	585	116.1	34.8	132.8
Saponin	amilo	6.3	16.7	963	164.2	8.1	128.6
Saponin	sphin	60.6	61.8	1365	128.5	80.2	132.3
PMA	sphin	120.4	12.4	936	134.8	167.6	139.2
E. coli	sphin	21.3	5.0	936	122.1	21.0	98.6
E. coli	ARAC	14.1	5.0				
—	ARAC	0.3	5.0				
E. coli	monen	8.3	3.8				
—	monen	−0.8	3.8				
				X rays			
E. coli	—	—	15.9	84	221.6		

Note. Column (a) lists the inflammatory activators ("agent 1") for each experiment. The resultant chemiluminescence, normalized to 100%, is compared to the percentage chemiluminescence cps values for (b) blood treated with agent 1 + inhibitor ("agent 2"), (c) a control with neither agent (subtracted from *all* other values and comparable with the control values in Fig. 1, from which the absolute magnitudes of the effects of agent 1 may be deduced), (d) irradiated blood plus agent 1 or (e) agents 1 + 2. Column (f) relates column (e) with column (b), itself normalized to 100%, for comparison of the chemiluminescence of blood treated with agents 1 + 2 before and after irradiation. Dose was regulated by exposure time. *amilo* is amiloride, *sphin* sphingosine, and *monen* monensin.

idative burst. Like irradiation, neither ARAC (10–100 μM), the Na^+ ionophore monensin (1–10 μM), nor PLA2 activated chemiluminescence if used alone. However, PLA2 intensified the chemiluminescence activated by the neutrophil leukocyte (PMN) taxin fMLP or the tumor promoter PMA, before and especially after irradiation. In one study of isolated PMN (*29*), chemiluminescence was not activated by PLA2 or ARAC, but PMA-activated chemiluminescence was reduced by PLA2, and fMLP-activated chemiluminescence was intensified by PLA2 and ARAC, thus demonstrating the existence of multiple second messenger pathways in the activation of PMN.

The results using activators alone indicate essentially that expression of the irradiation effect depends on the degree of stimulation of PKC by activators of phagocytic or chemotactic pathways. Therefore, by adding selective inhibitors of the pathways involved in stimulating the oxidative burst, we attempted to define a point at which irradiation might effect the chemiluminescence response. Thus CB is reported to potentiate chemotactic stimuli and strongly inhibit phagocytic ones (*21*), and the PKC inhibitor sphingosine (*30*) reduces activation by saponin, *E. coli,* and fMLP (not shown) but intensifies that by PMA. However, like CB, its effects before and after irradiation are equivalent except for blood activated by *E. coli* (compare columns (d) and (f) in Table I). The differences are otherwise insignificant. Amiloride inhibits activation by saponin, suggesting that saponin promotes ion transport rather than damaging membranes and causing leakage. Nor does irradiation itself induce the sort of leakage reported in erythrocytes at doses at least 10^3-fold higher than the highest used here (*11*). Interestingly, ARAC and monensin each inhibited activation by *E. coli.*

These results, too, generally suggest that stimulators or inhibitors of PKC and chemiluminescence modulate the chemiluminescence reaction to irradiation in the same direction, but reveal nothing specific about the reaction in itself. An exception to this finding was that irradiation stimulates chemiluminescence during amiloride inhibition of fMLP, although the absolute effect is extremely small. Irradiation has no influence on chemiluminescence after amiloride treatment alone (not shown). Further investigation of the irradiation response required using inhibitory agents that had little or no effect on PKC activity.

Primary Responses to Irradiation

The Ca^{2+} requirement of the irradiation response was examined by activating chemiluminescence with the Ca^{2+}

TABLE II
Roles of Calcium and PLA2 in the Irradiation Response

(a) Agent 1 (CL = 100%)	Agent 2	(b) Agent 1 + agent 2 CL	(c) Control CL	Irradiated: Dose ^{137}Cs γ rays (μGy)	(d) Agent 1 CL	(e) Agent 1 + agent 2 CL	(f): e vs b (b = 100%) CL
PMA	2°C	85.5	8.0	152	116.6	80.2	93.7
PMA	adeno	143.5	6.4	152	132.8	124.5	86.8
A23187	EGTA	109.9	16.2	780	117.4	112.9	102.7
PMA	BPB	84.7	3.2	152	118.9	88.0	103.9
PMA	aspir	87.4	4.3	152	118.2	96.5	110.3

Note. The results are from one representative experiment in each case except the means of those with BPB ($N = 4$) and aspirin ($N = 5$) are given. For experiments employing A23187: 2 m*M* EGTA was added to all tubes and 3 m*M* Ca^{2+} was added to controls with and without A23187 before irradiation and to others (agent 2 columns) after irradiation, since luminol amplification requires Ca^{2+}. *aspir* is aspirin and *adeno* adenosin. The columns are as in Table I.

ionophore A23187 and with bacteria, which activate independently of Ca^{2+} (*21*). The radiation response was eliminated if, during irradiation, regulation of Ca^{2+} metabolism was disrupted by the presence of adenosine (*31, 32*) or if Ca^{2+} was sequestered by EGTA in the presence of A23187 (Table II). Thus, the irradiation response depends on the availability of Ca^{2+} rather than only on perturbation of the plasmalemma similar to that induced by the ionophore. These effects raise the possibility that a Ca^{2+}-dependent PLA2 (*22*) mediates the irradiation response. It is unlikely that irradiation stimulates either phospholipase C (PLC) or PKC (*33*), both Ca^{2+}-dependent, because chemiluminescence should then be induced independently of a chemical activator, which is not the case. There was no irradiation response in blood irradiated while ice cold, possibly indicating a requirement for active cellular metabolism or membrane fluidity during irradiation to mediate the reaction.

A more specific examination of PLA2 activity required using PMA, which is reported to activate PKC directly, and therefore chemiluminescence, without stimulating preceding second messenger pathways and with no effect on PLA2 (*34*). PMA-activated blood was treated with BPB to inhibit PLA2 (*35, 36*) and then irradiated. BPB completely suppressed the radiation-induced response ($P \ll 0.01$, $N = 4$). The difference between the irradiated and nonirradiated BPB-inhibited samples is not significant. The average effect of the cyclooxygenase inhibitor aspirin (*22*) also was not significant ($N = 5$), but distinct inhibition was evident in two experiments. Both aspirin and BPB reduced PMA-activated chemiluminescence only slightly, an effect which might depend on a derivative stimulation of PLC (*37*). The reported lipoxygenase inhibitors nordihydroguaiaretic acid (*36*) and phenidone (*38*) inhibited all chemiluminescence completely (not shown).

Release of a Soluble Mediator

The reaction following exposure of nonirradiated blood to γ-irradiated blood across a filter, which prevented cellular contact but allowed molecular diffusion, demonstrates that irradiation induces the release of a diffusible agent, which intensifies chemiluminescence in nonirradiated blood (Table III). Analogous results were obtained after replacing irradiation by incubation with *E. coli,* indicating some element of similarity between the soluble factors produced by the two treatments. Some products of ARAC metabolism are among the factors able to induce such activity.

Kinetics of the Irradiation Response

The response to γ irradiation increases monotonically with the dose up to about 100 μGy (Fig. 2A). Irradiation was delivered at about 8400-fold the nominal background rate for humans, considering terrestrial, medical, and internal sources to sum to 1.35 mGy/year (*39*). At the lowest dose used (5.4 μGy: 15 s at 1.30 mGy/h, approximating 35 h at the background rate), roughly one γ-quantum may pass through each phagocyte in 6.5–25 s and each platelet in about 150 s. This indicates that, at 100 μGy, ionization

TABLE III
Both Irradiation and Treatment with Bacteria Induce a Soluble Inflammatory Mediator

Sample	Chamber A	Chamber B	Chemiluminescence chamber B (%)	±SD (%)	Activated (*E. coli*)
1	Irradiated	Irradiated	148.5	12.43	+
2	—	Irradiated	142.4	10.84	+
3	Irradiated	—	141.2	2.94	+
4	*E. coli*	—	129.0	11.92	+
5 (control)	—	—	"100"	9.75	+
6 (control)	—	—	35.9	14.43	–

Note. After incubation with or without γ irradiation, the samples were activated with *E. coli.* The mean chemiluminescence values ($N = 3$) are from chamber B and are percentages of the activated control value normalized to 100%. The nonactivated control value has been subtracted from all values. Blood was treated in Eppendorf tubes 15 min by γ irradiation at 1.41 mGy/h, i.e., 353 μGy, or by incubation with *E. coli.*

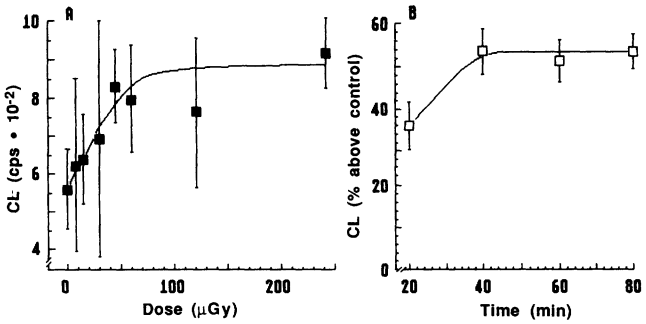

FIG. 2. The effect of dose and the duration of γ-irradiation response. (A) The chemiluminescence (CL) of activated blood varies directly with the γ-irradiation dose after 15–480 s of irradiation at 21.7 μGy/min. Diluted blood plus luminol was incubated in Eppendorf tubes at 37°C, and irradiation ended simultaneously for all samples. After a further 15 min at 37°C, A23187 was added to 2 μ*M* and the chemiluminescence measured 5 min later. (B) The response to a pulse of γ irradiation is persistent. The symbols represent the percentage difference between the chemiluminescence of irradiated blood and that of matched, nonirradiated controls. Diluted blood in Eppendorf tubes was irradiated from t_0 to t_{10} min at 23.5 μGy/min, i.e., 235 μGy, then incubated with nonirradiated controls in a 37°C water bath. The abscissa indicates time after t_0 when the samples were activated with *E. coli* plus saponin. Chemiluminescence was read 20 min later. The symbols in (A) and (B) represent average chemiluminescence values and the bars indicate SD (N = 3).

events in up to about 4% of the cells (*40*) produce the full response. The capability of irradiated blood to intensify the chemiluminescence response upon cell activation persists for at least 1 h after γ irradiation (Fig. 2B) or lung X ray (not shown).

DISCUSSION

Possible Mechanisms of the Response

There are five main results. (1) Low-dose irradiation intensifies chemiluminescence in activated cells in whole blood in a dose-dependent manner. (2) The effect is transmitted intercellularly by a soluble mediator and (3) persists for at least 1 h. (4) The response requires Ca^{2+}, is inhibited by BPB, is unaffected or perhaps poorly inhibited by aspirin, and appears not to primarily activate PKC or PLC. (5) X irradiation induces a response *in vivo,* resembling that to γ irradiation *in vitro.*

One interpretation of these results is that irradiation induces PLA2 and lipoxygenase activity, whose products might be responsible for the effects demonstrated in Table III. However, a role for cyclooxygenase cannot be excluded definitively. The effect of aspirin on the irradiation response is puzzling, but cyclooxygenase may not invariably be stimulated in response to irradiation, which may contribute to variations in the effect of PLA2 and lipoxygenase products in regulating chemiluminescence.

The reported stimulation of chemiluminescence and intracellular Ca^{2+} release by ARAC and some of its metabolites (*29, 41*) contrast with our inability to induce chemiluminescence using ARAC or irradiation alone, and might weaken our interpretation. However, the complex of stimulatory and inhibitory signals released during inflammation depends on feedback and "cross-talk" regulation among the cell types present. Although the formation of leukotrienes and their derivatives requires platelet–phagocyte interactions (*42*), most work has employed single cell types. Different methods might also produce other results; e.g., ARAC-activation of chemiluminescence in PMN reportedly required A23187 (*43*), which we and others (*22*) find itself stimulates chemiluminescence if used above 1 μ*M*. Some leukotrienes and eicosanoids appear to modulate inflammatory responses rather than to activate chemiluminescence directly by stimulating PLC or PKC (*44, 45*).

Precedents for cell stimulation by factors unable to activate chemiluminescence directly include γ-interferon, which induces a maximal reaction to PMA added 12 h later (*46*), and monensin (*47*). Na^+ transport itself is independent of Ca^{2+} influx (*48*) and thus is inadequate alone to activate chemiluminescence directly, as demonstrated above. However, a putative increase in Na^+ transport due to irradiation, although itself insufficient to induce chemiluminescence, is reported to stimulate PLA2 (*37, 38, 48*). This latent effect might explain our finding that irradiation antagonized slightly but consistently the amiloride block of activation by fMLP. Irradiation could augment the supply of a critical component, e.g., Na^+ (*38*), necessary for inducing PLA2 despite the presence of amiloride. However, the inhibition of chemiluminescence activation by monensin might be inconsistent with this suggestion.

Our evidence does not characterize unambiguously the initial radiation "receptor" or the irradiation response. Lysolecithin may play a role (*29*), and important reactions may be modulated by free-radical scavengers like vitamin E and albumin (*21*) and blood constituents, including serum proteins, which adsorb mediators. Changes in cellular redox potential or perturbation of lipid membranes, inducing radical chain reactions (*8*), might affect a critical early process. Altered Na^+ permeability appears to be an interesting candidate. The radiolytic generation of significant amounts of H_2O_2 (*49*) seems unlikely, since only femtomoles may be expected to be produced at low doses. One conceivable target is the iron-heme group of lipoxygenase, cyclooxygenase, and NADPH-oxidase, which may effect lipid peroxidation by producing singlet oxygen through its resonant π-electron structure. The transport of lipoxygenase to the plasmalemma provides a possible basis for the inconsistent effect of aspirin, due to differences in the sources of ARAC available to lipoxygenase and cyclooxygenase (*50*).

Some Consequences of Low Doses of Radiation

Histamine and prostaglandins are released from several tissues after irradiation of humans (*51, 52*) and animals (*12,*

53), but with high intense doses (0.5–300 Gy), making it difficult to compare these results with ours. Thus the death of animals after some treatments might involve massive secondary effects on prostaglandin metabolism. Maximal prostaglandin synthesis occurred within minutes and days after irradiation. The rapid response may support our hypothesis that irradiation directly activates second-messenger systems.

Low- and high-dose responses differ, because the former depends on the stimulation of critical reactions that amplify the response while the latter additionally involves cumulative molecular damage and secondary reactions. For example, an elevated prostaglandin level is viewed as an element of radiation sickness (*54*) and acute tissue injury (*12*). However, such high-dose effects involve complex cell interactions, which are irrelevant to responses at low doses and might well obscure the salient issues of cause and effect.

Thus, being independent of cell and DNA damage and repair, low-dose effects can be explained in the context of the conventional principles of inflammation and second-messenger physiology. Indeed, little molecular damage is expected from low doses, because DNA is a rare target and lipids and proteins are not unique (*7*). However, key physiological processes are sensitive to aberrant or untimely regulatory signals. Caution should be used in interpreting the effects of low and high doses in terms of the same mechanism. For example, the optimal induction of luminol-independent chemiluminescence in lymphocytes and PMN at X-ray doses between 300–700 mGy reported by Barenboim *et al.* (*55*) might depend on the reactions of peroxides produced directly by irradiation. Speculation (*1, 4–6*) about whether low doses might be hormetic or beneficial, e.g., increasing cell proliferation, awaits confirmation, but is fundamentally inconsistent with the results reported here. The primary role in carcinogenesis played by inflammation enhanced cell proliferation should be borne in mind (*56*).

The apparent saturation of the irradiation response at 100 μGy might be due to the proportion of phagocytes and thrombocytes absorbing radiation. Alternatively, the amplificatory and adaptive nature of the arachidonic acid cascade implies that the number of cells directly affected at this dose is sufficient to stimulate the population further and that a radiation pulse might affect cells more efficiently than a continuous exposure at the same cumulative dose. Investigation of the biological mechanisms of irradiation may require attention to the dynamical, nonlinear, and temporal properties of inducible cellular systems.

The regulation of inflammatory reactions by phagocytes and thrombocytes plays a crucial role in the survival of higher organisms, e.g., in diseases involving allergic and rheumatoid-arthritic reactions, heart and circulatory malfunction, thrombosis, and cancer (*56*). Further investigations are needed to answer questions concerning the roles of individual blood cell types, the agents they release, and the consequences of their subsequent effects.

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