MUTAGENIC ACTION OF NON-IONIZING RADIATIONS: ITS IMPLICATION IN RADIATION PROTECTION

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1. INTRODUCTION

Biologic effects of non-ionizing radiations(NIR), mainly microwaves and ultrasonics, has been the topic of two international meetings in 1974(1, 2) and several aspects at cellular and organism level were covered which gave insight into the mechanism of action of NIR on biological systems. Whereas the lethal, mutagenic and carcinogenic effects of UV, near UV and even visible light are known, mutagenic action, if any, of microwave radiation is just not known. Further, mutation is brought about by a sub-lethal damage in the DNA molecule of a cell which gets expressed as the cell progresses through its duplication and this end point is independent of slight temperature variations normally encountered in microwave absorption at low fields. Thus, we have yet another method to prove the existence or not of athermal effects of microwaves. Reversion to prototrophy of a diploid mutant strain of yeast following exposure to a wide spectrum of EM radiation (from cobalt-60 gamma rays to 2450 MHz microwaves) was studied and results are presented.

2. MATERIALS AND METHODS

2.1 Radiation Sources and Dosimetry

Irradiations in the UV (254 nm), near UV (313 nm, 365 nm) and the visible region (480 nm) were carried out in an Aminco-Bowman spectrophotofluorimeter. Energy fluxes incident on the quartz sample holder were determined by actinometry with potassium ferrioxalate system at 254 nm and determining the relative intensities at other wavelengths by the method of rhodamine B in propylal-glycol. The flux values obtained were 1.9 (for 254 nm), 5.8 (for 313 nm), 6.6(for 365 nm)and 9.7 for 480 nm, all in 10^8erg/sq. cm/min. In order to determine the absorbed energy by the cell suspensions, optical densities (10^7cells/ml and 1 cm path length) were measured using a Cary 14 Spectrophotometer. It was 0.23 at 254 nm and about 0.16 at other wavelengths.

A neodymium (Nd) solid state pulsating Laser beam (1.01 μm) was obtained with a laboratory built set-up with filter and lens arranged such that the beam spot at the irradiation plane was of 1.2 cm diameter. Pulse duration was 30 μs and the energy fluence, as measured with a Hadron Model 102C Energy/power meter, was 3.4 J/pulse. For microwave irradiations, a laboratory built 0-200 W equipment at 2450 MHz was used. A 9.4 cm dia. hemispherical horn antenna gave a close field level of 2W/sq. cm at 1.7 cm from the face of the horn for 160 W power level. For low field irradiation, a distance of 14.3 cm was used which gave 25 mW/sq. cm at 60 W power

level. A Narda Model B86B3 Radiation Monitor together with a locally built instrument was used to measure these fields. For ionizing radiation, a cobalt-60 Gamma Cell with an exposure rate of 1.8 kR/min. was used.

2.2 Yeast Culture and Sample Prepatation for Irradiation

A diploid yeast strain BZ34 which requires arginine for its growth was used. Exposure to radiation can induce reversion to arginine-independence by the process of intragenic recombination. These recombinants can be detected by plating the irradiated cells on a medium lacking arginine. Details of culture growth and media are given elsewhere(3). Following exposure, YEPD agar plates were used to determine surviving cells and Arg^- plates to detect radiation induced revertants. A suspension of 10^7 cells/ml in sterile water was chosen for all irradiations with gamma, UV, near UV and visible light irradiations. All irradiations were carried out at room temperature (21 to 24°C) although cell suspensions were stored at about 5 to 10°C until they are taken out for irradiation.

For laser beam irradiations, 2.5×10^8 cells were collected on a 1 cm dia. area of a millipore filter paper by filtering and this paper positioned inside a plastic petri dish was exposed to the horizontal beam. Samples received energy pulses of 2.4 J/pulse at two rates namely (i) at 5s intervals and (ii) at 60s intervals. Following exposure, the cells were re-suspended in water, diluted appropriately and plated. For microwave irradiations, 16 ml of 10^7 cells/ml suspensions was taken in 5 cm dia. plastic petri dishes and positioned vertically below the horn. As the small-tipped thermister probe(of a locally built Clinical Thermometer instrument) could not be used to continuously monitor the temperature rise during irradiation (due to interference by EM field), measurements were made within seconds following the termination of exposure.

3. RESULTS AND DISCUSSION

3.1 Gamma, UV, near UV and visible light radiations

Fig. 1 shows the pooled results of several experiments plotted as the number of induced revertants per million survivors vs irradiation time. No killing of cells was observed for 313, 365, and 480 nm radiations for the exposure range studied and the survival for UV(254 nm) and gamma ray exposures (6 min. & 6 krad) were 90 and 95% respectively. Actually no increase in the reversion frequency was observed for 480 nm radiation (60 min); the experimental points on the fig. were those of samples suspended in 10^{-3}M of ethidium bromide (EB), a chemical which binds to DNA and has an absorption maximum at orange region. Energy absorbed scales are also shown in the fig. for these radiations computed using the optical densities of cell suspensions and irradiated area (4 mm x 5 mm, and 2 ml sample). From the slopes of the lines in the figure, intrinsic efficiency of different radiations were calculated in absorbed energy, erg/cell and given in Table 1. Yeast cell mass of 1.4×10^{-10}g was used to get the value for absorbed gamma energy.

It can be seen that the reversion induction efficiency of UV radiation has decreased by 5 orders of magnitude when compared to ionizing radiations and further decreased by 2 to 3 orders while reaching the black radiation (365 nm). Visible region is not mutagenic.

3.2. Nd Infrared Laser Beam

Pooled results of two experiments are given in Table 2. For exposure to short pulse intervals (5s), 50% of the cells were killed at 48J/sq. cm fluence. But no increase in the reversion frequency was observed. With longer pulse intervals (60s), even killing was negligible for the highest fluence given (120J/sq. cm) indicating large dependence of exposure rate on lethal effect. Like the visible light, this laser is also not mutagenic.

3.3. Microwave Radiation (2450 MHz)

Table 3 summarises the results of the microwave irradiations, exposed both at close and far field positions (acute and chronic exposures). It can be seen that even when the cell temperature is raised to 55°C at which only 37% of cells survived, there was no increase in the reversion frequency, thus clearly demonstrating the non-mutagenic nature of microwaves. Chronically exposed sample did not show even killing leading to the conclusion that the thermal effects are responsible for the lethal effects observed. Thus with sufficient energy densities of microwave fields, only killing of cells is possible and no subtle damage can be caused to DNA to bring about a mutation. This conclusion was predicted by Cleary (4) on theoretical grounds 4 years ago.

IMPLICATION OF THE RESULTS TO RADIATION PROTECTION

Present results indicated that mutagenic action of NIR exists from very low wavelengths to visible light region up to about 450 nm, but quantitation of this effect needs to be made with mammalian cells (either in vivo or in vitro) to have any meaning for evaluating hazards to man. Induction of skin tumors in hairless mice following exposure to 313 nm UV radiation (5) was an investigation in the right direction. As regards wavelengths higher than the visible region(infrared, lasers, radars and microwaves), present results indicate that this region of NIR is not mutagenic and at best, as observed by Webb (6), particularly with extremely high frequency microwaves (80 to 140 GHz), alterations in the metabolic functions of mammalian cells, such as the inhibition of protein or other macromolecular synthesis, can be effected when exposed to weak fields. Thermal effects are only possible in most cases. Thus, the final analysis leads the protection standard setters for NIR, to leave the genetic apparatus of the single cell with its attendant somatic and genetic mutations, but concentrate on non-dividing organized tissue complexes whose functions are mediated by organelles other than chromosomes, for example membranes, and look for functional and pathological changes following exposure.

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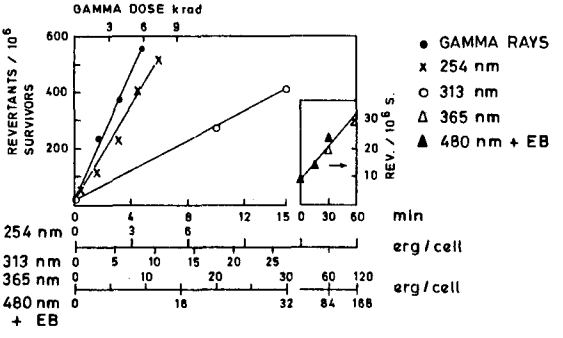

FIGURE 1. Variation of induced reversion frequency with exposure.

Gamma radiation	1.54×10^{-5}	Black light (365 nm)	645
UV (254 nm)	0.92	Visible (480 nm)	--
Near UV (313 nm)	6.5	-- do -- + ethidium bromide	600

TABLE 1. Intrinsic efficiency of different radiations (in absorbed energy, erg/cell) to induce 100 revertants/10^6 survivors.

Pulse interval 5s			Pulse intervals 60s		
Exposure J/sq. cm	% Sur.	Rev. /10^6 S	Exposure J/sq. cm	% Sur.	Rev. /10^6 S
0	100	23	0	100	8
24	87	20	48	94	9
48	50	23	120	97	8

TABLE 2. Survival and reversion frequency of yeast following exposure to laser beam at two pulse rates.

Irradiation conditions		Irradiation time	Terminal temp	% Sur.	Rev. /10^6 S
Acute	Horn output 160W Field 2W/cm^2 at 1.7 cm	0	24° C	100	14.0
		1 min.	55° C	37	14.3
Chronic	Horn output 60 W Field 25 mW/cm^2 at 14.3 cm	0	22° C	100	19.0
		120 min.	no change	100	19.1

TABLE 3: Survival and Reversion frequency of yeast following exposure to microwaves.