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ABSTRACT

The effect of nitric oxide on the sensitivity of Shigella flexneri to x-rays was investigated. Experiments were carried out with oxygen rigorously excluded from the bacteria when they were in contact with the NO. Nitric oxide at a concentration of 15 μM was found to increase the sensitivity of the bacteria to x-rays by a factor of 2. Reasons are given for supposing that NO should affect radiosensitivity in much the same way as oxygen does.

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Oxygen is known to increase the effectiveness of x-rays in killing bacteria[1,2] and cells from many other organisms[3] by a factor of 2 or 3. There have been numerous reports of substances that protect against the effects of irradiation under aerobic conditions,[4] but there does not appear to be any report of a substance which, in complete anoxia, raises the radiosensitivity of bacteria to the level normally associated with full aeration. Before giving the evidence that suggests that nitric oxide may be such a substance, we may consider the factors that first directed attention to this gas.

The observed effect of oxygen on radiosensitivity implies that ionizing radiation produces lesions in the bacteria, some of which are converted to lethal injuries only if oxygen is present. It has been postulated that such a lesion is a radical, probably on a carbon atom in one of the molecules that are essential to cell survival.[5,6] It may be supposed, therefore, that oxygen affects radiosensitivity by virtue of its affinity for carbon radicals. Molecular oxygen owes its reactivity and its affinity for free radicals to the unusual configuration of electron spins in the outer orbitals. Of the 16 electrons in oxygen, 14 fill the $\sigma_g(2p)$ and lower-energy orbitals that form the almost inert structure of molecular nitrogen. The remaining two electrons in molecular oxygen are in two $\pi_g(2p)$ orbitals. There is spectroscopic evidence that these electrons have unpaired spins, a finding which explains the paramagnetic properties of molecular oxygen.[7] The two unpaired electrons make oxygen a bi-radical and give it an affinity for other radicals.[8]

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Another paramagnetic molecule that has an unpaired electron in the $\pi_g(2p)$ orbital is NO, the most stable molecule with an odd number of electrons. Nitric oxide is a stable gas with little tendency to dimerize. It is more soluble than oxygen in water and it does not hydrolyze or dissociate to any great extent. Nitric oxide shows an affinity comparable to that of oxygen for carbon radicals.[8] It combines readily with oxygen to form NO_2, which hydrolyzes in water to form nitric acid, so that NO can be used only in the absence of oxygen. Nitric oxide combines reversibly with haematin[9] and cytochrome oxidase,[10] forming compounds similar to those of CO. That nitric oxide is not toxic in small amounts is shown by the observation that certain bacteria form it in the course of the reduction of nitrites to nitrogen.[11] It would seem from this list of properties that nitric oxide might affect radiosensitivity in much the same way as oxygen does, while at the same time having a contrasting action on cell metabolism.

A preliminary investigation has been made into the effects of nitric oxide on the radiosensitivity of bacteria that were kept free from oxygen while in contact with the gas. The methods used follow those of a previous investigation into the effect of oxygen on radiosensitivity.[2] An overnight broth culture of Shigella flexneri was washed, resuspended, and diluted for use in buffered saline. Samples were placed in a glass irradiation vessel with a glass filter base, through which oxygen-free nitrogen was passed. The suspension was vigorously bubbled with nitrogen for an initial period of equilibration, as well as throughout the course of the irradiation carried out at 25°C with 250-kv x-rays filtered with 1/2 mm Al, at a dose rate of 1500 rads per minute. The top of the irradiation vessel was closed except for a narrow capillary outlet, through which the gas escaped. The suspension was sampled through the same capillary by tilting the vessel. The samples taken before and during the course of the irradiation were plated out on nutrient agar and incubated. The number of bacteria surviving was found from the visible-colony counts. When required, nitric oxide was mixed with oxygen-free nitrogen in amount determined by a flowmeter consisting of a glass tube along which a mercury pellet was pushed by the gas. When an experiment was started, the cell suspension was bubbled with nitrogen for a period of at least 5 minutes at a flow rate of 5 ml per second before nitric oxide was admitted. Samples were withdrawn when required and

were passed from the irradiation vessel into a vial and bubbled with nitrogen to remove the nitric oxide before there was appreciable exposure to air. In addition, control samples were bubbled with nitric oxide for a period comparable to the time of the irradiation, to test the effect of the gas alone upon the survival of the bacteria.

The results are shown in Fig. 1. It was found that 0.8% of nitric oxide in the gas mixture, which gives a concentration of 15 μM, was sufficient to double the sensitivity of the bacteria to radiation, while in the absence of any irradiation, exposure to nitric oxide at a concentration of 20 μM reduced the number of survivors by only 10% in a comparable time. The increase in sensitivity observed with nitric oxide is about the same as that observed with oxygen at a comparable concentration.[2] Further experiments will be needed to test whether oxygen and nitric oxide are comparable, molecule for molecule, in their effect upon the sensitivity of bacteria and other micro-organisms.

It is of interest that the bacteria given nitric oxide in place of oxygen should show the radiosensitivity normally associated with aerobic conditions, while all aerobic metabolism has been halted.

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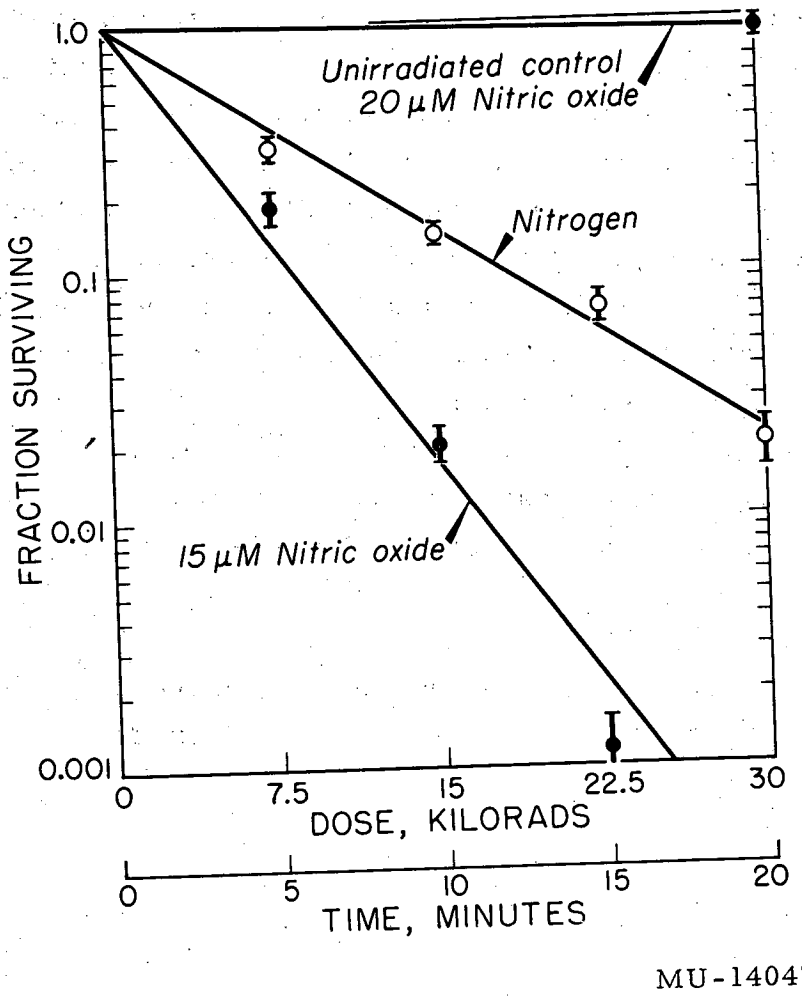

Fig. 1. Survival curves for Shigella flexneri irradiated with 250-kv x-rays while the suspension was bubbled with either nitrogen or nitrogen containing 0.8% nitric oxide. The upper line shows the effect of the nitric oxide mixture on the cells in the absence of any radiation.

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