Technical Papers

Preprotection of Mice against X-Irradiation Mortality by Sodium Nitrite[1]

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It is generally recognized that ionizing radiations elicit the formation of highly reactive oxidants (considered to be OH and O_2H radicals, and H_2O_2) in aqueous media containing dissolved oxygen (*1–3*). There are indeed reasons to believe that many of the observed biological effects of ionizing radiations are a consequence of the chemical reactivity of these oxidants. The studies of Barron *et al.* (*4, 5*) have shown that moderate doses of x-rays can inactivate certain cellular enzyme systems by virtue of oxidation of essential sulfydryl groups in these enzymes. The demonstration by Dowdy and his co-workers (*6*) of the protective effect of anoxic anoxia against lethal doses of x-rays in rats constitutes additional evidence for the role of radiochemical reactions involving oxidants in irradiation injury. Hypoxia secondary to methemoglobinemia has been invoked as a possible explanation for the protection afforded by *p*-aminopropiophenone against 800 r in CF_1 mice (*7*). One may interpret also the radiation protection afforded by preirradiation administration of cysteine (*8*) and glutathione (*9*) as being due to the reducing (i.e., antioxidant) properties of these compounds rather than to the presence of sulfydryl groups per se. In a series of experiments designed to screen a number of possible protective agents, sodium nitrite, a reducing agent which can also elicit methemoglobinemia, was reported to be ineffective when administered prior to irradiation of mice with 800 r (*10*).

In the course of studies on the modification of radiosensitivity in animals, based on considerations to be discussed later, a protective effect against x-ray–induced mortality of mice was observed when sodium nitrite ($NaNO_2$) was administered prior to x-irradiation. The present preliminary report describes the results obtained.

LAf_1 mice of both sexes, approximately 6 weeks old, and weighing 20–25 g, were used throughout. The animals were allowed free access to food (Purina laboratory chow) and to tap water at all times. Control and experimental animals were irradiated simultaneously, caged together in groups of 5 each, and otherwise treated similarly. In all experiments the control and experimental groups were matched with respect to age, sex, and body weight.

The irradiation source was a Westinghouse Therapy Unit. The radiation factors were 250 kvp; 15 ma; 0.5 mm Cu plus 1-mm Al filter; HVL, 1.5 mm Cu; target-to-skin distance, 100 cm; dosage rate, 25 r/min, as measured with a Victoreen r-meter placed in air at the position of the mice. Each radiation dose was delivered in a single exposure. During irradiation the mice were contained in individual, perforated lusteroid centrifuge tubes, placed radially on a circular wooden turntable platform rotated at 3.5 rpm to assure uniformity of radiation dosage.

Sodium nitrite (CP) was dissolved in *M*/15 phosphate buffer pH 7.2 to a final concentration of 5 mg $NaNO_2$/ml. Either 0.5 or 0.25 ml of this solution was administered intraperitoneally approximately ½ hr before irradiation. The control irradiated groups received equivalent amounts of phosphate buffer. Daily weight changes and survival up to 30 days postirradiation were used as criteria for evaluation of protective effect.

A summary of the results of the three separate experiments is presented in Table 1. The data reveal that preirradiation administration of $NaNO_2$ reduces markedly the mortality of mice resulting from a single-dose whole-body irradiation. Of the control group of 19 mice exposed to 600 r in Expt. I, 84% were dead at the conclusion of the 30-day period, whereas none of the 16 nitrite-treated animals, receiving 100–125 mg $NaNO_2$/kg body weight, were dead. Definite, but less marked, protection was obtained also in the experimental group which had received 1.25 mg $NaNO_2$ (equivalent to 62 mg/kg). In Expt. III, 100% of the control group of mice exposed to 750 r were dead at 30 days, whereas only 22% of the nitrite-treated group (2.5 mg $NaNO_2$/mouse) had succumbed. These differences are statistically highly significant.

The weight curves represent averages of daily individual body weights expressed as percentages of preradiation weight. It is apparent from Fig. 1 that the $NaNO_2$-treated animals receiving 600 r whole-body x-irradiation exhibited a maximum weight loss of only 7% by the seventh day postirradiation, after which a steady increase in body weight was observed, whereas the control group showed a precipitous weight loss starting approximately 8 days postirradiation, and continuing to a maximum weight loss of 25% on the fourteenth day. Mortality was heaviest during this period. A similar differential in weight loss between the $NaNO_2$-treated experimental group and the control group, all receiving 750 r, is shown in Fig. 2.

The previously observed failure of sodium nitrite to protect against irradiation (*10*) may be ascribed to differences in the strain of mice used or, more

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[2] With the technical assistance of M. Ellis.

TABLE 1

EFFECT OF PRETREATMENT WITH SODIUM NITRITE ON MORTALITY OF MICE AFTER TOTAL-BODY X-IRRADIATION

Expt. No.	No. mice	Treatment	X-ray dosage (r)	Survival at 30 days postirradiation		P
				Number	Percentage	
I	16	2.5 Mg $NaNO_2$	600	16	100	< 0.000001
	19	Phosphate buffer	600	3	16	
	6	2.5 Mg $NaNO_2$	None	5*	83	
I	18	1.25 Mg $NaNO_2$	600	9	50	0.03
	18	Phosphate buffer	600	3	17	
II	18	2.5 Mg $NaNO_2$	600	17	94.5	< 0.000001
	20	Phosphate buffer	600	5	25	
	5	2.5 Mg $NaNO_2$	None	5	100	
III	9	2.5 Mg $NaNO_2$	750	7	78	< 0.001
	10	Phosphate buffer	750	0	0	

* One animal died 30 min after injection.

likely, to the large dose of irradiation employed. The dose of 800 r used by these workers, expressed as percentage of the LD_{50} under the conditions employed, appears to exceed the dose limit at which protection is observed.

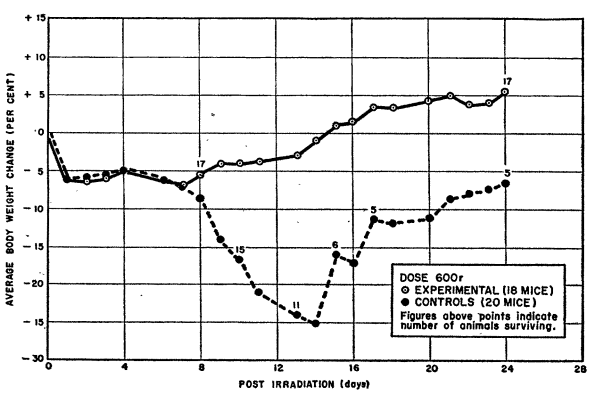

FIG. 1. Effect of sodium nitrite pretreatment on body weight of mice exposed to 600 r.

Feinstein *et al.* (*11*) have presented experimental data which indicate that the activity of mouse liver catalase is decreased after intraperitoneal injection of H_2O_2 and after whole-body x-irradiation. They concluded that "H_2O_2 may play a significant role in radiation toxicity." Although the role of the enzyme catalase as a limiting factor in protection against radiation injury in mammals has not been established conclusively, an attempt to account for the action of $NaNO_2$ in modifying the radiosensitivity of mice leads to some interesting speculations when viewed in the light of the results of Chance's studies on catalase kinetics. Chance (*12*) has shown that the reaction of catalase with H_2O_2 involves first the formation of a catalase-H_2O_2 complex. Once formed, however, this complex does not decompose spontaneously under ordinary circumstances but is relatively stable unless collision and reaction with a donor molecule occur. It is of the greatest interest, so far as the present data are concerned, that the decomposition of the catalase-H_2O_2 complex is greatly accelerated by sodium nitrite, ethanol, methanol, or sodium formate (*13, 14*). Chance's detailed investigations clearly indicate that a second order reaction occurs between the catalase-H_2O_2 complex and a donor molecule, resulting in the coupled oxidation of the donor. In addition, the catalases specifically bring about the oxidation of primary and secondary alcohols and related structures. It is of interest to note here (as has Chance) that the structure of nitrous acid resembles that of a secondary alcohol.

If one assumed, then, that catalase activity leading to the destruction of H_2O_2 (and possibly of organic peroxides) is a limiting factor in radiation injury in the mammal, it seems not unreasonable to anticipate that an accelerated decomposition of H_2O_2, in the presence of an excess of suitable donors, would lead to protection against the effects of ionizing radiation. It is proposed, therefore, that a mechanism such as this may be involved in the protective effect against x-irradiation here observed with $NaNO_2$ pretreatment of mice. That this hypothesis may be valid is suggested by recent experimental data reported by Hollaender *et al.* (*15*), who have found that the lethal

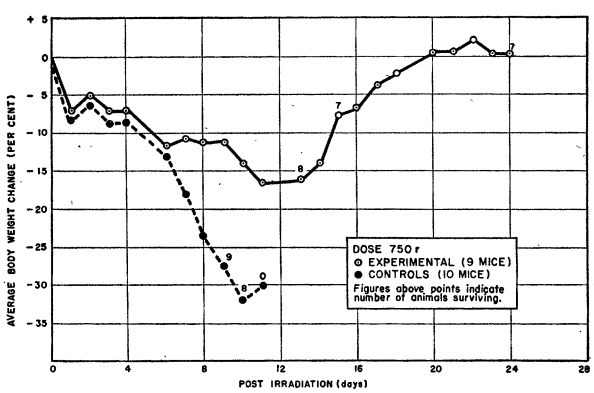

FIG. 2. Effect of sodium nitrite pretreatment on body weight of mice exposed to 750 r.

effect of x-rays on bacterial cells was reduced significantly by sodium formate, ethanol, and glycols. On the basis of the present discussion, these compounds would be expected to accelerate the decomposition of H_2O_2 by catalase and thus prevent the accumulation of deleterious concentrations of this oxidant.

The possibility that the radiation protection afforded by sodium nitrite may be mediated through methemoglobin formation is not excluded. This explanation is open to question, however, since the degree of methemoglobinemia induced by the doses of sodium nitrite used by Rust *et al.* (*10*) (100 mg/kg), which failed to protect, was comparable to that produced by doses of *p*-aminopropiophenone which afforded definite protection (*7*). Studies directed toward the elucidation of this question are in progress.

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A Simple Stage-mounted Micromanipulator

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Transplanting inclusions of a few cubic microns volume between living cells is one of the primary objectives of this laboratory. In planning procedures for making such transfers it became apparent that a micromanipulator having the following characteristics was essential:

1) Control in three dimensions to a tolerance of about 1 μ.

2) A range of 0.4 mm in each dimension, movement being essentially rectilinear.

3) Syringe intake and output with a volume control tolerance of 1×10^{-12} ml.

4) Operation under oil immersion with phase contrast objectives.

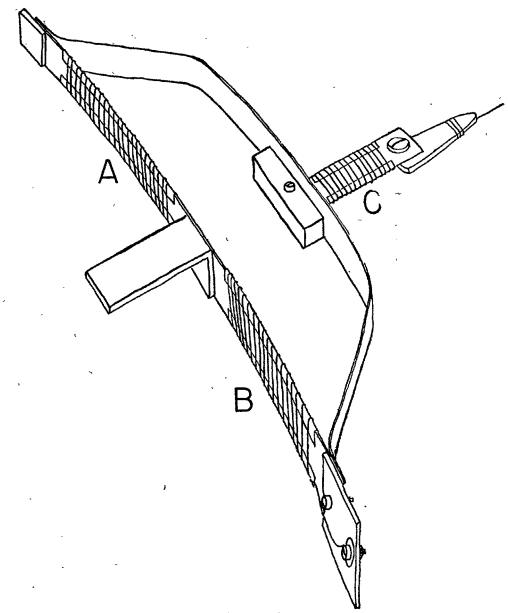

FIG. 1.

The principle utilized in the design of this instrument is that of differential thermal expansion using electrically heated bimetallic elements as motion sources. The foregoing principle has not previously been employed in micromanipulative equipment according to the literature available to us. For its designed function this model has certain advantages over the pneumatic or strictly mechanical manipulators. The primary advantage enjoyed by this manipulator is its small dimensions, which permit it to be mounted on the stage of the microscope. Remote control is effected electrically.

Hypodermic action is obtained by electrically heating a hollow glass needle whose effective volume change depends upon the internal volume of the needle used, the coefficient of expansion of the filling liquid, the operating temperature range of the heater, and the portion of the total volume heated.

Construction of an appropriate needle requires some practice but becomes a simple procedure. The needles used in our study are made from Pyrex glass tubing, and are 2–3 cm in length, with one end closed and the other end drawn out to an internal diameter of approximately 1 μ. The walls are relatively thin, giving a total external diameter of approximately 2 μ. The needle is completely filled with freshly distilled water, which has very little dissolved gas in it. The low dissolved gas component prevents separation of gas bubbles from the liquid with which the needle is filled. Such gas bubbles give a "mushy" effect in volume control. When heat is applied to the needle, the liquid expands and ejects a proportional part of its volume; the reverse occurs upon cooling. On occasion, volume control has been stable down to approximately 1 μ^3 (1×10^{-12} ml).

The organisms being used in our experiments are extremely thermosensitive, yet the heat produced by this