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THE DISTINCTION BETWEEN THE ROLES OF O_2 AND OF O_2^- IN BIOLOGICAL RADIODAMAGE

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The enhancement of the biological radiodamage under oxic conditions was attributed also, in part, to the action of superoxide radicals generated from e^-_{aq} and H radicals by oxygen. The confirmation (or exclusion) of such an hypothesis, was made difficult by the inevitable formation of O_2^- radicals in the presence of oxygen. Moreover, the mode of oxygen action was further obscured as the relative contributions of the various radiation effects were not known. Direct and indirect as well as endogenous and exogenous effects are generally contributing, to various extents, to the resulting biological radiodamage. Therefore it seemed necessary to quantitate first the relative contributions of these effects for each test-organism studied. Then, it was important to plan an experimental system in which the different roles of oxygen and of superoxide radicals would be distinguishable.

In the present study the effect of γ-radiation on the survival of T4 bacteriophage and of E. coli B has been investigated. The irradiations of the test-organisms were carried out in phosphate buffer suspensions. The spectrum of the water-radicals was controlled by a careful choice of the appropriate saturating gas and the addition of suitable radical scavengers. All the survival curves exhibited a pronounced shoulder and the inactivation rate constants were evaluated from the linear portions of the dose-response curves.

In the first stage we have examined the effect of high molecular weight radical scavengers on the radiosensitivities of the organisms studied. In the presence of an excess of polyethylene glycol, most of the radicals formed outside the organism are scavenged. Thus the indirect exogenous radiodamage is blocked. In the case of the T4 bacteriophage, the radiosensitivity has been reduced roughly by tenfold. This indicated that the majority of the detectable damage in T4 is due to radicals formed outside the virus. On the other hand, with E. coli B the introduction of the radical scavenger has hardly affected the radiosensitivity. This clearly showed that in the E. coli most of the damage originates endogenously.

In order to estimate the direct and indirect radiation effects we have compared the radiosensitivities in suspensions saturated with either helium or nitrous oxide. In case where the N_2O scavenges most of the hydrated electrons converting them into OH radicals,

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the N_2O - induced enhancement of the radiosensitivity would reflect the relative extent of the indirect effect (if the contribution of the hydrated electrons to the damage is small compared with that of the OH radicals). Our observations have shown that with E. coli as well as with T4 the presence of the nitrous oxide roughly doubled the radiosensitivity. This result is in accord with our conclusion that the hydrated electrons hardly contribute to the radiodamage, and that the effect is predominantly indirect.

In order to ellucidate the roles of oxygen and superoxide radical in radiodamage it was attempted to prevent the oxygen from reacting with the hydrated electrons. This allowed us to study the oxygen effect in the absence of superoxide radicals. For this purpose a mixture 9:1 of $N_2O + O_2$ was used. Under these experimental conditions the high excess of N_2O successfully competes with the oxygen on the hydrated electrons, converting them into OH radicals. Consequently, the effect of molecular oxygen solely could be studied.

Suspensions of T4 and of E. coli have been irradiated under five different sets of experimental conditions. The suspensions were saturated with the following gases: He ; O_2 ; O_2 (+ 0.01M formate); N_2O ; $N_2O + O_2$ 9:1 mixture. The results are shown in Table I.

Table I: Radiosensitivity towards ionizing radiation of T4 bacteriophage and E. coli B: Effects of O_2 , N_2O , and $N_2O + O_2$.

Experimental conditions	G(water radicals)				Inactivation rate constants (Gray^{-1})	
	H	OH	e^-_{aq}	O_2^-	T4	E. coli B
He saturated	.7	2.8	2.8	-	.17	.049
O_2 "	-	2.8	-	3.5	.33	.17
O_2 " (0.01M formate)	-	-	-	6.3	.031	.13
N_2O "	.7	5.6	-	-	.4	.11
N_2O + O_2 saturated	.7	5.6	-	-	.83	.33

These results show that with oxygen and formate, where the superoxide radicals predominate, the radiosensitivity has been considerably reduced, i.e. superoxide radicals play no role in the radiation-induced damage. This conclusion agrees with the fact that the maximal radiosensitivity was observed for the systems saturated N_2O + O_2, i.e. in the presence of oxygen and in the absence of O_2^-. In this case, the enhancement effect of oxygen on radiosensitivity was evident in addition to the effect of the N_2O.

In conclusion, N_2O enhances the biological radiosensitivity by increasing the number of OH radicals which attack the biomolecule. The oxygen subsequently reacts with the targey biomolecules, rendering the damage irrepairable.