HYDRATED ELECTRONS AND RADIOBIOLOGICAL SENSITISATION

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Recent work has demonstrated conclusively that in water radiolysis, secondary electrons produced along the track of a primary particle exist for a relatively long period of time due to their stabilization by hydration (Hart and Boag, 1962) (Keene, 1962). This can be formally represented by e^- + aqua $\longrightarrow e^-_{aq}$.

Kinetic analysis has shown that the first order conversion of a hydrated electron to a hydrogen atom, $e^-_{aq} \longrightarrow OH^- + H$ is a slow process, the rate constant of which is about $4 \times 10^4 sec.^{-1}$. This implies that the diffusion range of the hydrated electron is appreciable.

There is considerable evidence supporting direct electron scavenging by some materials in either aqueous or alcoholic media (Matheson, 1962). It has been shown that the solvated electron can react extremely rapidly with a variety of aromatic molecules, carbonyl compounds, esters, halogen containing compounds, oxygen etc., some of these reactions proceeding with rate constants of the order of $10^{10}\ M^{-1}\ sec^{-1}$.

Organic electron acceptors appear to fall into main categories:

a) Molecules containing highly polarised regions or electrophilic groups, i.e. chloracetic acid, acetone, ethyl acetate.

b) Molecules possessing a well-defined conjugated system, i.e. Anthracene, Naphthalene, Benzoquinone.

It might be expected that molecules in which these two criteria are combined would be extremely efficient. It should be emphasised that an electron attachment process is more selective than a reaction

involving only neutral free radicals since the efficiency of the latter is less dependent on the polarity of the target molecule and frequently involves an atom abstraction process.

In a biological system, damage resulting from attachment of electrons at a particular site would depend upon the competition of non-damaging processes occurring elsewhere in the system. On the basis of this hypothesis therefore, the magnitude of the biological response would depend upon

a) The distribution of electrophilic centres in the system and

b) The life-time or the diffusion limit of the hydrated electron.

Moderation of a Biological Response by Foreign Molecules.

Interest in the possible importance of e^-_{aq} in the overall mechanism of radiobiological sensitisation was stimulated by the report that N-ethylmaleimide, when present during irradiation, considerably reduced the number of E coli B/r that survived to form a visible colony (Bridges, 1960). The effect was more marked in an anaerobic system, and later work (Bridges, 1962) showed that in some bacteria an anaerobic system was essential to demonstrate the effect. Furthermore, it is perhaps significant that although there are relatively few well established sensitisers, these materials are either known to be, or would be expected to be, efficient electron acceptors, for example,

O_2, H_2O_2, NO, ICH_2COOH, N-ethylmaleimide.

If the phenomenon of radiobiological sensitisation is at all attributable to the reactivity of e^-_{aq}, then it is a consequence of the hypothesis that the sensitising molecule in some way enhances the probability of an electron reacting at the relevant site.

We suggest that an important contributory factor to radiosensitisation is the capture and stabilisation of the electronic charge by the additive molecule. It is inherent in this argument that the negative radical-ion so produced is longer lived than the free hydrated electron thus extending its original sphere of activity. The sensitiser would be

regarded therefore, as an electron carrier and ideally would be a molecule combining a high electron affinity with a structure suitable for delocalisation of the attached electron and thereby stabilisation of, to some extent, the transient radical-ion.

We consider that N.E.M. has the molecular structure consistent with such a prediction. It appears to be a general phenomenon that molecules containing $>C=O$ linkages are very efficient electron scavengers. Furthermore the conjugation of the two carbonyl groups in N.E.M. suggests that the radical-ion initially produced would be a resonating system which could be formally represented as

We suggest that the increase in stability of the radical-ion relative to that of a non-resonating system could be responsible for increasing the likelihood of damage by prolonging the life-time of the electronic charge.

We have confirmed the ability of N.E.M. to sensitise and have made a preliminary search for other sensitisers, being guided by the requirements of the above model.

Experimental

The detailed method of measuring radiosensitivity has already been published, (Dewey, 1963). In brief, a dilute suspension of Serratia marcescens in phosphate buffer, gassed with nitrogen in a lucite container was irradiated with 200 kV X-rays at 2000 rads/min. The suspension was then further diluted as required, aliquots placed in petri dishes and flooded with nutrient agar. Colony counts were made after 48 hours incubation at 25°.

Results and Discussion.

The radiosensitisation effect of N.E.M. on Serratia marcescens is

shown in Table 1. There was no trace of toxicity in unirradiated controls at the levels used, although 0.3mM is near the toxic limit.

TABLE 1.

Material	Concentration (millimolar)	Dose Reduction Factor	Higher Concentration
N-ethylmaleimide	0.03	1.18	Toxic
	0.1	1.74	
	0.3	2.05	
Benzophenone	0.04	1.03	Insoluble
	0.4	1.31	
Diacetyl	0.1	1.00	Not tested
	2.0	1.30	
	10.0.	1.66	

The plot of log survival against dose for Serratia marcescens irradiated in nitrogen is not quite linear (Dewey, 1963) and in addition, the effect of adding sensitisers also causes a slight change in the degree of curvatures. The figures quoted in Table 1. are factors by which doses have to be multiplied so that the points make a best fit on the control curve, any value above 1.1 is significant.

The three other materials containing one or two carbonyl groups in a conjugated system which were selected as potential sensitisers were, - p-benzoquinone, benzophenone, and diacetyl. Benzoquinone is a strong oxidising agent, and believed to be an efficient scavenger, how ever, preliminary experiments showed that this material was toxic at low concentrations. Benzophenone has been shown to react rapidly with e^-_{aq} to form a radical-ion, the life-time of which is appreciable under non--acidic conditions (Adams, Baxendale & Boag, 1963).

The sensitising effect of benzophenone on Serratia marcescens is shown in table 1. Qualitatively the effect was the same as that of N.E.M. although the magnitude of the effect was smaller. The experi-

mental range of benzophenone concentration was limited only by its solubility.

Diacetyl was selected as a model compound in view of its being an example of a pure non-aromatic non-cyclic conjugated di-ene system. It was found to be non-toxic and the resulting radiobiological sensitisation is shown in table 1.

Thus, of three materials selected on the basis of their molecular structures, one was toxic, and two, benzophenone and diacetyl, had a very significant effect on the sensitivity of bacteria to X-rays. On the basis of the above hypothesis it would be predicted that the elimination of conjugated linkages in a molecule containing C=O groupings would remove the sensitising ability. It is perhaps, significant therefore, that although N.E.M. is a sensitiser, succinimide did not sensitise pseudomonas to X-rays (Bridges, 1962).

N-ethylmaleimide is known to react with SH groups and this fact has already been used to formulate theories of its radiobiological action (Bridges, 1960, Howard-Flanders, 1961). Additional effects along these lines are not excluded.

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