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Author
Brown, Philip E.

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Ernest O. Lawrence Radiation Laboratory

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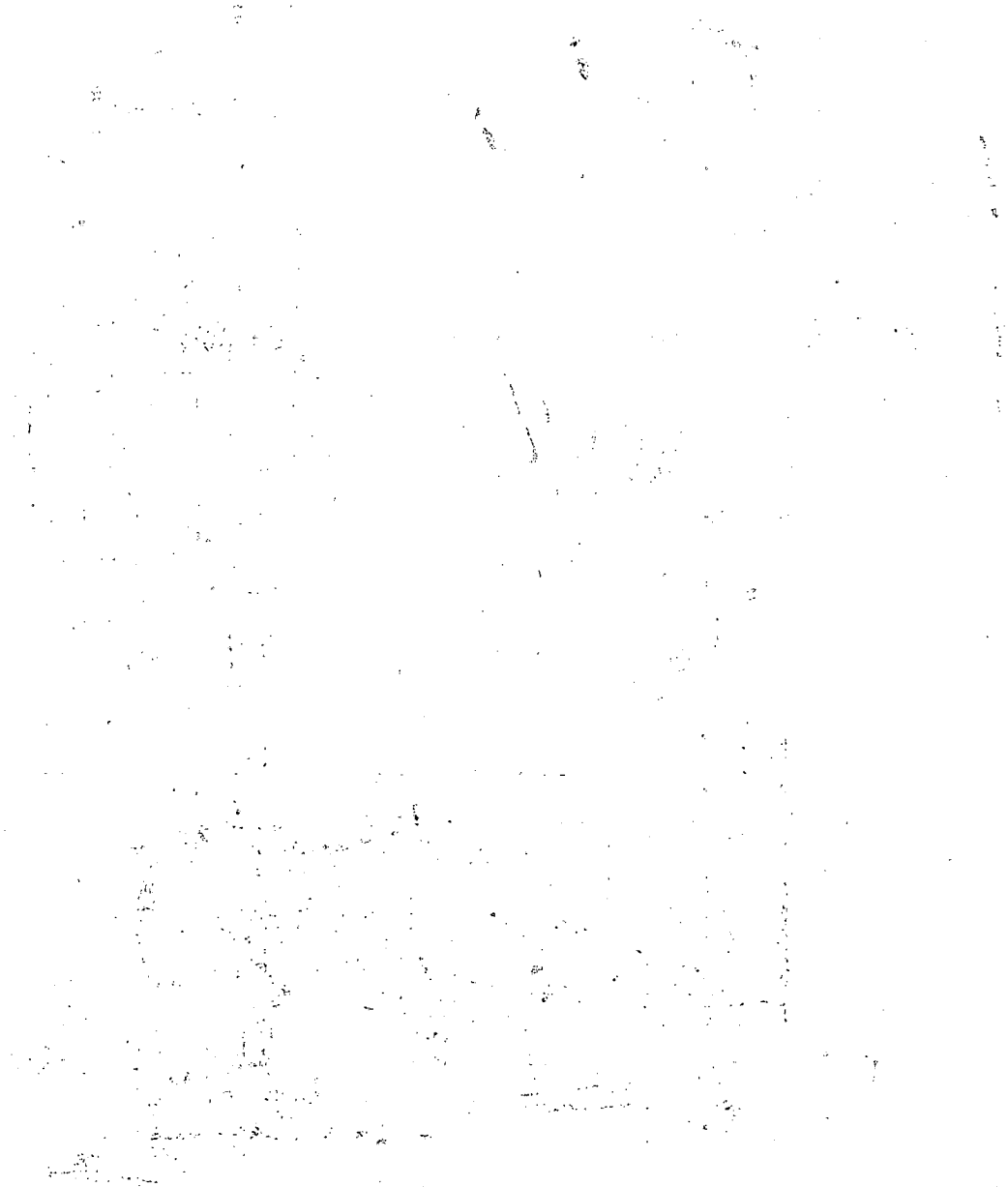

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Among the chemicals which are known to protect living organisms against ionising radiation there is one group, the aminothiols, which is especially effective, but despite numerous papers dealing with the mechanism of action having appeared, there is still no single theory explaining the prophylactic action of these, and other, compounds[1,2,3].

With few exceptions, the most effective aminothiols are those whose structures are closely related to that of cysteamine, $HS\text{-}CH_2CH_2\text{-}NH_2$ [4,5]. The corresponding disulphides are usually equally active, as are compounds which are readily metabolised to this type of compound[6]. Some generalisations which appear from consideration of the structures of these prophylactics are: the amino and thiol groups must not be separated by more than three carbon atoms[5], a free -SH group is required since thioethers are inactive[7], alkylation of the amino group reduces, but does not destroy, activity[5,8]. Superimposed on these requirements are the subtle effects common in pharmacology; thus, although cysteine is a protector, when the amino and thiol groups are interchanged the product, isocysteine, is not only a non-protector but actually sensitises living organisms to the action of radiation[9]. At the present time three theories have been thought to offer reasonable explanations, but none is entirely satisfactory alone[1,3].

The idea that the induction of hypoxia or anoxia was the basis of protection followed from the recognition of the "oxygen effect"--the presence of oxygen during irradiation increases damage but without oxygen natural recovery cannot occur. Another mechanism which has had wide support was that thiols act by destroying the free radicals produced by irradiation[10]. The third hypothesis is that of "mixed disulphide formation"

advanced by Eldjarn and Pihl[11,12]. These workers believe that the thiol groups of enzymes are the radiosensitive sites and argue that protective agents form transient mixed disulphides with the enzyme thiol groups. When a mixed disulphide is attacked by a free radical one of the sulphur atoms is reduced whilst the other is oxidised so that the damage is reduced by roughly one-half.

There are important criticisms of all three theories[1,3], a common weakness being that neither the structural requirements nor the existence of apparently similar compounds which sensitise organisms to radiation can be explained[13]. Evidently we are lacking some general unifying principle which would enable the known facts to be rationalised and to indicate to what extent, if any, the above mechanisms take part. What follows is a suggestion as to what this principle might be.

There is ample evidence to show that DNA is the site of the primary radiation damage in cells[14]. The nature of the damage appears to be single strand breakage followed by deletions and chemical alterations of the bases together with dissociation of histones, if present[15]. The damage has been postulated to be made good by a repair system[16] and evidence for a most efficient repair system in *M. radiodurans* has recently been obtained[17]. The existence of the repair system is the first requirement for the mechanism proposed in this paper.

We can assume that, in order to survive, a dividing cell must successfully replicate a set of nearly normal DNA. We can also agree with Guild[16] that if the repair system has an efficiency of the order of 99%, then small alterations in the velocity of repair could alter the amount of residual damage by a factor of three or more. However, in cases where

the repair system is less efficient, small changes in the repair rate alone have little effect and the relative rates of three processes, damage, repair and replication, become the governing factors. Crudely we may say that so long as the rate of repair is greater than the rates of replication and damage the cell should survive. This criterion breaks down, of course, when the rate of replication is so low as to not replace essential enzymes which are themselves damaged by radiation. The importance of relative rates is borne out by the dependence of the lethal radiation dose on the dose rate[18]. This argument requires that the rates of repair and replication are independent of one another, and there is some support for this in the literature[19]. Thus, control of the three rate processes is required and we now consider how aminothiol prophylactics might bring about this control in the cases of replication and damage; there is insufficient knowledge of the repair process for it to be considered at present.

We first note that survival will be favoured by decreasing both the rate of damage and the rate of replication. There is a simple mechanism which would enable both these requirements to be fulfilled--this is binding to DNA.

If a substance binds to DNA the usual result is that the DNA helix is made more stable. Since DNA replication requires single strand separation[20], the replication rate would be reduced by this increased stability. The mere presence of the binding agent, apart from tending to prevent the original breakage, would also tend to ensure physically that a break in a single strand would not lead to unravelling and consequent secondary damage. There is support for this conclusion in the literature. Thus, histones

inhibit DNA dependent RNA and DNA synthesis[20,21], and deoxynucleoprotein is less sensitive to ionising radiation than DNA alone when acting as a primer for RNA synthesis[22].

A feature common to histones is the presence of a large number of amino groups. This, together with the fact that aliphatic diamines are known to bind strongly to DNA[23], makes it clear that a molecule with two or more amino groups is likely to bind to and stabilise any part of a DNA helix not covered by histone. This conclusion has lead to the discovery that the disulphide forms of the aminothiol protectors also bind strongly to DNA[24], which explains the necessity of both the amino and free thiol groups in these protectors. For binding of this type to be relevant to protection, it is required that the disulphide form of the prophylactic be the active one, but on this point there is controversy in the literature[25]. The conflicting evidence would seem to reflect the ease of reduction of the disulphide (the overall metabolic tendency) and oxidation of the thiol (the in vitro tendency).

Binding to DNA is insufficient in itself to afford protection for the aliphatic diamines, structurally very similar to the protective disulphides, are inactive. Evidently a disulphide link is necessary, and the reason for this may be closely related to the ease of reduction of the disulphide link in living tissue[26]. Certainly cystamine and cadaverine have different effects on RNA polymerase under certain circumstances, for example[27]. Reduction of the -S-S- bond would immediately free the DNA so that replication and repair could take place. This role of the disulphide link is supported by the fact that di(ethylaminoethyl)-sulphide, $EtNH\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2\text{-}NHEt$, is inactive, though it should bind

as well as, and have half the scavenging capacity of, the active diethylcystamine, $EtNH\text{-}CH_2CH_2\text{-}S\text{-}S\text{-}CH_2CH_2NHEt$ [5,9]. With this mechanism in mind, we may proceed to consider to what extent this hypothesis explains the more obvious aspects of prophylactic activity.

One of the most important structural requirements is that the amino and thiol groups should not be separated by more than three carbon atoms[4,5]. The present hypothesis explains this by referring to the work of Mahler and Mehrotra[23] on the effect of a series of normal aliphatic diamines on the melting temperature (stability) of DNA. These workers found that DNA was stabilised only if the number of carbon atoms between the two terminal amino groups was between two and ten. Stabilisation of the helix was maximal with diaminopentane and with diaminodecane had fallen to a very low level. Thus, when ten atoms are present in the chain between two amino groups we may expect very little effect; ten intervening atoms is just the number present in the first non-active aminothiol in its disulphide form, viz., $H_2N\text{-}(CH_2)_4\text{-}S\text{-}S\text{-}(CH_2)_4\text{-}NH_2$.

The work of Mahler and Mehrotra revealed that certain diamines are capable of binding to DNA with a resultant decrease in the stability of the helix. The existence of binding agents which destabilise the helix provides an explanation of the existence of sensitising agents[13]. Destabilisation would lead to an increased rate of replication[28] so that more mistakes arising from primary lesions would be incorporated. Sensitisation by compounds with structures very similar to those of protectors has not been adequately explained previously.

Whether a given diamine binds with stabilisation or destabilisation is dependent on its detailed structure; we can say that the most important

factors governing this are the entropy effects associated with the interaction of the surrounding water with hydrophilic and hydrophobic centres in the bound molecule. Since these effects also govern water solubility, it is not surprising that in a closely related series of protectors such as the L-cysteine ester hydrochlorides there is a good correlation between protective capacity and water solubility[2].

In view of the discovery that histones do, in fact, contain thiol groups[29], it is tempting to modify the hypothesis slightly in order to include Eldjarn and Pihl's theory of mixed disulphide formation. The same result would obtain if the disulphide were formed between the protector and a thiol group of the nucleohistone; a portion of the DNA normally devoid of histone, and therefore a likely position for the development of damage after a primary lesion, would be stabilised by an easily removable binding agent. The attachment of the histone itself to the DNA would also be strengthened. This possible role of the histones might also provide a rationale for the difference between protection in mammals and in bacteria, for the latter are not known to possess histones.

All three of the earlier theories thus remain admissable as contributing mechanisms, though the present hypothesis suggests that a more fundamental mechanism is also required. The proposed hypothesis is summarised below.

Radioprotective aminothiols may act by binding to and stabilising those parts of the DNA helix not covered by histones. This has two effects. Firstly, apart from helping to prevent the primary lesion, the loose ends resulting from single strand rupture are held in place so that secondary damage arising from shortening or chemical alteration is prevented. Secondly, the DNA replication rate is decreased so that a repair

process can deal with alterations before they are replicated. Binding of this type requires that the disulphide form of the protector is the active one and that the disulphide is necessary for ease of removal, so that repair and DNA and RNA synthesis may proceed. This enables certain structural requirements in the aminothiol protextors, and also the existence of radiosensitisers, to be explained.

The mechanism put forward in the above discussion thus appears to rationalise many facets of radioprotection whilst fitting in with current view on DNA structure and function. It is hoped that, in spite of the difficulties which will doubtless be found, the general scheme will prove to be valid and result in further understanding in this field.

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PHILIP E. BROWN

Lawrence Radiation Laboratory
University of California
Berkeley, California

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