Translated from *Uspekhi Khimii*, 54, 1559-1579 (1985) U.D.C. 539.12+541.14+541.51+541.64+543.42+577.3

Kinetics of the Radiation-induced and Photochemical Oxidation of Biomacromolecules in Aqueous Solutions

I.I.Sapezhinskii

The sequence of radiation-induced photochemical reactions of proteins, nucleic acids, and other biological species has been investigated: the primary stages in the formation of free macroradicals and other active products and their reactions leading to the modification of the primary structure of the biopolymers. Simplified versions of kinetic schemes which describe satisfactorily the experimental data are discussed. The redistribution of the initial damage-induced defects in protein-DNA-other cell components systems has been subjected to a formal kinetic analysis.
The bibliography includes 129 references.

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I. INTRODUCTION

The question of the primary mechanisms of the action of light and ionising radiation on the principal biochemical components of the cell is one of the essential questions in radiobiology and destructive photobiology. As early as the middle 1950's, Academician N.M.Emanuel', who was engaged in the study of the kinetics of liquid-phase oxidation reactions, put forward a broad programme for the study of radiation-induced and photochemical reactions of proteins, nucleic acids, and lipids. Extremely simplified and fairly naive ideas about these processes were current at that time. This programme involved the study of the degradation of biomacromolecules by methods which were new at the time: EPR, pulse radiolysis, and flash photolysis. This review includes certain results obtained in the course of the execution of this programme.

The sequence of the effects of optical and ionising radiation on living organisms can be subdivided very arbitrarily and with much simplification into three stages. The first stage includes the entire set of the physicochemical primary processes from the instant of irradiation up to the formation of relatively stable products of the radiation-induced and photochemical modifications of the primary structure of the biopolymer. The second stage is molecular-biological and in it numerous enzymes systems for the repair of DNA molecules begin to operate, which leads to both the elimination of the modified sections of the nucleic acids and to the appearance of additional DNA structural defects. Finally, the third stage includes further processes which entail damage of the genetic material in the cells, organs, and tissues and this leads to the distortion of metabolism, the breakdown of the regulatory systems, and finally to biological effects. This review has a bearing only on the first, physicochemical stage—the action of radiation on proteins, DNA, and other components of the cell.

Proteins perform catalytic, transport, protective, immune, supporting and other functions in the living cell, forming part of the composition of all the cell organelles and their content amounts of 50% and above of the dry weight of living organisms. This is why, on exposure to light and ionising radiation, proteins bear the brunt of the radiation-induced stress and constitute in this sense the principal "chemical target" of the action of radiation. At the same time DNA macromolecules are the main "biological target", because damage to such molecules entails in most cases biological effects.

Radiation-induced and photochemical reactions of macromolecules involve numerous and varied reactions of free radicals, electronically excited states of the macromolecules, peroxy-radicals, peroxides, and other active species. Protein molecules are the most complex macromolecules with numerous groups which are modified by radiation. The discovery of all such changes is a task for the future and this applies, albeit to a lesser extent, to DNA.

During the last 20 years, there has been a qualitative leap forward in the understanding of the mechanisms of the radiation-induced and photochemical degradation of biopolymers. The use of EPR, pulse radiolysis, and flash photolysis makes it possible to discover tens of different intermediates participating in the degradation of proteins and nucleic acids: these are the active products of the radiolysis of water, numerous types of free macromolecules and peroxy-radicals, electronically excited states of aromatic groups of proteins and DNA bases, the radical-ions derived from the macromolecules, photoinitiators and radiosensitisers, singlet oxygen, and various peroxides. Naturally, the contribution of a particular species to the overall degradation process is determined by its yield and the kinetic characteristics of the possible competing reactions. The first aim of the present review was therefore a kinetic examination of the most probable processes and the simplest of the possible kinetic schemes which describe satisfactorily the experimental data.

In the living cell proteins compete with other molecules, including DNA, for the active species produced by photolysis and radiolysis and the second aim of the review was therefore a formal-kinetic examination of the redistribution of the initial damage-induced defects in multicomponent systems at concentrations comparable to those within the cell.

The inclusion in a single article of results concerning different influences on the macromolecules by ionising radiation, ultraviolet (u.v.) light, as well as light in the presence of photosensitisers is not surprising because many products of these reactions are the same and the kinetics of their behaviour are also similar. An exception is apparently the effect of u.v. light on DNA, in which the main photoproducts (pyrimidine dimers) are formed directly from the singlet and triplet electronically excited state without the participation of other intermediates.

II. THE YIELDS OF RADIATION-INDUCED AND PHOTOCHEMICAL REACTIONS OF PROTEINS AND NUCLEIC ACIDS

The study of radiation-induced or photochemical reactions of biomacromolecules has a history of almost 100 years. The total number of studies exceeded 10 000 long ago. Conferences are being held regularly and reviews devoted to the primary photochemical and radiation-induced reactions of proteins and DNA both in solutions and at the level of living cells are being published.[1-34]

Among the many thousands of applications, only a few contain quantitative data suitable for the construction of the possible reaction mechanisms—these are data concerning the yields of the modified groups, the disappearance of the initial groups, and the dependence of the observed effects on a broad spectrum of experimental conditions: the irradiation dose rate, the concentrations of the macromolecules, oxygen, and various additives, and temperature. Very few studies have been devoted specifically to the radiation-induced and photochemical oxidation of macromolecules, which is apparently associated with the rapidly increasing set of intermediates and final oxidation products. The lack of interest in the photochemical oxidation of biopolymers has been promoted also by the fact that certain photobiological effects of u.v. light are independent on the oxygen concentration, although photochemical oxidation reactions do naturally occur under these conditions.

Table 1. The radiation-chemical yields in the absorption of oxygen by solutions of nucleic acids (molecules/100 eV) at different temperatures and irradiation dose rates.[36]

Substance	c, M	0.4 Gr s^{-1}; 20 °C	0.04 Gr s^{-1}		
			5° C	20° C	50° C
Single-stranded DNA	$7\cdot10^{-4}$	6.8	—	6.6	—
	$4\cdot10^{-3}$	7.5	8.3	8.6	9.2
Double-stranded DNA	$7\cdot10^{-4}$	4.6	—	4.4	—
	$4\cdot10^{-3}$	—	4.7	—	—
RNA	$7\cdot10^{-4}$	4.0	5.0	5.4	6.6
Poly(U)	$2\cdot10^{-4}$	14.6*	17	—	—
	10^{-3}	21	—	—	—

*At 5 °C.

The principal overall characteristic of the oxidation processes is the rate of absorption of oxygen on irradiation—the radiation-chemical or quantum yield in the consumption of oxygen. Unfortunately there are few quantitative data for these quantities, although the methods for the determination of the rate of oxidation are extremely simple and the first studies on the absorption of oxygen using the Warburg apparatus were published about 60 years ago.[35]

Table 1 presents the radiation-chemical yields in the absorption of oxygen by solutions of natural nucleic acids and synthetic polyuridylic acid [poly(U)].[36] Evidently the yields for single-stranded DNA vary from 6.8 to 9.2 O_2 molecules per 100 eV depending on the experimental conditions. For double-stranded DNA, the yields are somewhat smaller (4.4–4.7). The yields in the case of RNA are 4–6.6 O_2 molecules per 100 eV. The maximum yields have been obtained for poly(U) (up to 21). Some increase of the yield with decrease of the dose rate and with increase of the temperature of single-stranded DNA, RNA, and poly(U) is striking. These results have been explained[36] by the occurrence of chain reactions with short chains and a scheme has been published for the reactions of free radicals derived from nucleic acids, the confirmation of which requires additional data. The radiation-chemical yields for the absorption of oxygen by proteins are in the range 2–4 O_2 molecules per 100 eV.[37]

Table 2 presents the possible participants in the radiation-induced and photochemical reactions of proteins (B) and nucleic acids (D). The yield in the absorption of oxygen in the radiation-induced oxidation of proteins and nucleic acids are comparable. The quantum yields of the sensitised and unsensitised photochemical oxidation of proteins are fairly high owing to the presence in the proteins of the readily oxidisable tryptophan, tyrosine, cysteine, cystine, methionine, and histidine residues. The quantum yields in the photochemical oxidation of DNA are extremely low. If it is assumed that the breaks in DNA arise in reactions involving free radicals, then the yield of the photochemical oxidation of DNA is of the order of 10^{-4}% according to Zavilgelsky et al.[32]

The active products of the radiolysis of water (Table 2, Nos. 3–7) are obligatory participants of the radiation-induced oxidation reactions. The contribution of each can be discovered with the aid of relatively selective acceptors. For hydrated electrons, this is nitric oxide, while for hydroxy-radicals the acceptors are alcohols and sugars. The superoxide radical-ion (O_2^-) or the hydroperoxy-radical are apparently involved in all types of oxidation of macromolecules. Singlet oxygen is almost always formed in sensitised photochemical oxidation, but its formation under influences of other types is problematical.[36]

Other inorganic and organic radicals may be formed in the photolysis and radiolysis of the additives introduced and they can interact fairly effectively with macromolecules. Low-molecular-weight peroxy-radicals react effectively with proteins and less so with DNA.[39-51]

Photosensitisers in singlet and triplet electronically excited states participate in the sensitised photochemical oxidation of biopolymers and the reaction mechanisms can be extremely varied.[38]

The free radicals derived from proteins and nucleic acids are formed under all types of influences. It is noteworthy that the very concept of "free macro-radicals" is fairly arbitrary, because irradiation results in the formation of a large set of radical species of different types. For proteins, these are the radical-anion and the free radical obtained from the peptide unit and the lysine, arginine, phenylalanine, tyrosine, cysteine, cystine, methionine, aspartic acid, glutamic acid, and proline free radicals.[52-60] Other types of radicals have also been suggested. For DNA, these are the thymine free radical and radical-anion, the guanine radical-cations, and free peroxy-radicals.[61-64] The study of the reactions of single-stranded DNA by pulse radiolysis led to the detection of cytosine, guanine, and adenine radicals.[65] Apparently the overall yield of protein and DNA free radicals, when these are present at high concentrations, can reach six radicals/100 eV under the conditions of complete trapping of the water radiolysis product. The quantum yields in the

formation of radicals when DNA and proteins are acted upon by ultraviolet light have not yet been measured; at a low temperature radicals are formed as a result of two-quantum reactions[66,67] and the concept of quantum yield is extremely relative. Protein and DNA peroxy-radicals are formed under all types of influences, but their yields are unknown. The electronically excited states of the protein and DNA groups naturally arise on exposure to u.v. light and the yields of the triplet states apparently reach several per cent. On sensitisation, there is a possibility of the population of the triplet levels by energy transfer from suitable sensitiser levels. In radiolysis the yields of the excited states in solutions of biopolymers are low[68] and it has been suggested that they are formed as a result of the Vavilov–Cherenkov radiation.[69–71]

Table 2. The radiation-chemical (molecules/100 eV) and quantum (%) yields in the oxidation or formation of particular products of the reactions of proteins (B) or DNA (D).[1–71]

No.	Initial, intermediate, or final products of the reactions of proteins and DNA	RIR	PSR	PCR
1	$O_2 + B \rightarrow \dot{R}_B$	2—4	1—10	0.2—2
2	$O_2 + D \rightarrow R_D$	4—8	+	+
3	$\dot{O}H$	2.7	—	—
4	$\dot{e}$	2.7	+—	+
5	$\dot{H}$	0.6	+—	+—
6	H_2O_2	0.9	+—	+—
7	O_2^-, $H\dot{O}_2$	+	+	+
8	$^1O_2$	?	0—100	?
9	$\dot{R}_{inorg}$	+	+	+
10	$\dot{R}_{org}$	+	+	+
11	$R\dot{O}_2$	+	+	+
12	^1S	—	+	—
13	^3S	—	+	—
14	$\dot{R}_B$	3	+	+
15	$\dot{R}_D$	3	+	+
16	$R\dot{O}_{2B}$	+	+	+
17	$R\dot{O}_{2D}$	+	+	+
18	^1B	?	—	+
19	^3B	?	+—	+
20	^1D	?	+—	+—
21	^3D	?	+	+
22	π	1	+	+
23	Single breaks in DNA	0.5—1	+	+
24	Double breaks in DNA	0.01	+	+
25	Breaks in the peptide bonds in proteins	+	+	+
26	Decomposition of DNA bases	1—2	+	+
27	Decomposition of aminoacid residues of protein	2	1—3%	1%
28	Cyclobutane dimers in DNA	?	+	1—4%
29	Cross-links	+	+	+
30	DNA–protein cross-links	+	+	+
31	Inactivation of DNA	+	+	+
32	Inactivation of enzymes	0.01—3	0.1—10%	0.1—10%

Notation: RIR = radiation-induced reactions, PSR = photosensitised reactions, and PCR = photochemical reactions of protein and DNA macromolecules; the "+" sign refers to reactions with species whose participation in the processes has been reliably established, the "+-" sign refers to their possible participation, the "-" sign shows that they are not involved, and the "?" indicates their tentative involvement; $\dot{R}_{inorg}$ and $\dot{R}_{org}$ are the free radicals arising in photolysis or in radiolysis in the presence of inorganic or organic additives, ^1S and ^3S are the singlet and triplet electronically excited states of the photosensitisers, $^1O_2$ is singlet oxygen, $\dot{R}_B$ are protein free radicals of various types, $\dot{R}_D$ are DNA radicals, $R\dot{O}_{2B}$ and $R\dot{O}_{2D}$ are protein and DNA peroxy-radicals, ^1B and ^3B are the singlet and triplet electronically excited states of the aromatic groups of the protein, ^1D and ^3D are the corresponding states of the DNA bases, and π are stable and unstable peroxides derived from the macromolecules.

Unstable and stable peroxides (Table 2, No.22) are formed under all types of influences on the macromolecules in the presence of oxygen; the radiation-chemical yield in their formation, amounting to about one molecule/100 eV, is apparently significantly too low because of the occurrence of subsequent rapid reactions.

Table 2 (Nos.23–30) presents certain recorded changes in the macromolecule on exposure to light and ionising radiation. They are numerous and apart from these there are apparently also others hitherto undiscovered because the overall yield in the absorption of oxygen is greater than the sum of the yields of the known damage-induced defects.

Single and double breaks on γ-irradiation, the formation of cyclobutane dimers on u.v. irradiation, and the formation of "protein–DNA" cross-links under all types of influences can be included in the biologically significant damage to DNA. It is believed that the last type of damage is least well repaired by the numerous enzyme cell repair systems.

Table 2 shows that there are numerous data concerning the radiation-induced oxidation and other reactions of proteins and DNA. It is also seen that in many cases there are no quantitative data. This so far precludes the determination of a relatively complete picture of the reactions.

III. THE RATE CONSTANTS FOR THE POSSIBLE FREE-RADICAL REACTIONS INVOLVING BIOPOLYMERS

The rate constants for the free-radical reactions are obtained by pulse methods or are calculated from the dependence of the rate of the process on the concentration of the substrate, oxygen, or various added acceptors. It is noteworthy that the very concept of the rate constants for reactions involving protein and DNA macromolecules is fairly arbitrary. The effective rate constant for the reaction of a macromolecule with any species includes the sum of the partial rate constants for the reactions through individual groups of the polymer with these species. For DNA molecutes the rate constants were calculated either in relation to the concentration of the monomeric units or in relation to some fragment of the macromolecule or the entire polymer molecule—all these possibilities are used in the literature and the data must be recalculated.

The presence of large errors in the constants calculated using fairly complex kinetic schemes and with incomplete allowance for competing reactions is significant. In the general case many rate constants are correct within the limits of an order of magnitude. The Tables below present the averaged rate constants for proteins, DNA, lipids, and other substances, taken and recalculated from reviews quoted in previous Sections. Preference has been given to data obtained from the concentration variations. In the case of proteins data are quoted for the "average" protein with a molecular weight of $(2–4) \times 10^4$ and for DNA data are presented for molecules with a molecular weight of 4×10^4. It is noteworthy that the rate constants are similar to the diffusion constants and vary little with molecular weight. The situation is more complex for lipid membranes which can incorporate hundreds of a wide variety of chemical compounds; phosphoglycerides, glycolipids, and cholesterol. Data referring to the most reactive unsaturated lipids are quoted for such membranes.

Table 3 presents estimates of the rate constants for bimolecular reactions of the active water radiolysis products and singlet oxygen with proteins, DNA, lipids, cysteamine (one of the best known radioprotectors), phenolic inhibitors of free-radical processes, typical nitro-compounds (radiosensitisers), and oxygen. The constants have been adjusted

to 25 °C. Evidently the hydrated electron and the hydroxy-radical interact effectively with all the substances enumerated above and the rate constants are highest for proteins and lowest for lipids. The radical-anion O_2^- is significantly less reactive, reacting sluggishly with proteins containing no metal atoms; in the use of the iron- and copper-containing proteins the rate constants reach 10^7 litre mol^{-1} s^{-1}. Cystamine, phenols, and nitro-compounds react effectively with superoxide. Singlet oxygen reacts fastest with proteins and phenols, while its reactions with DNA and lipids are relatively ineffective.

Table 3. The estimated rate constants for the reaction of active radiolysis and photolysis products with proteins, DNA, lipids, cysteamine, phenolic inhibitors of free radical processes, nitro-compound radiosensitisers, and oxygen (litre mol^{-1} s^{-1}). [11,12,25,26,31,32,37]

Substance	$\bar{e}$	·OH	O_2^-	$^1O_2$
Proteins	$5\cdot10^{10}$	$(1—6)\cdot10^{10}$	$<10^3$	$10^8—10^{10}$
DNA	$2\cdot10^{10}$	$(1—6)\cdot10^{10}$	$<10^3$	$<10^4$
Lipids	10^6	10^7	$<10^3$	$10^3—10^5$
Cysteamine	$2\cdot10^{10}$	$2\cdot10^{10}$	10^6	—
Phenols	10^9	10^{10}	$10^6—10^7$	$10^5—10^7$
Nitro-compounds	$2\cdot10^{10}$	10^9	$10^7—10^9$	—
Oxygen	$2\cdot10^{10}$	$4\cdot10^{9}$*	—	—

*At pH 11.

Table 4. The estimated rate constants for reactions of various free radicals and peroxides with certain substances (litre mol^{-1} s^{-1}). [11,14,22,25,37,38]

Substance	$\dot{R}_B$	$\dot{R}_D$	$\dot{R}$	$R\dot{O}_2$	$\dot{R}_H$	Π_1	Π_2
Proteins	$10^6—10^8$	$10^6—10^8$	$10^8—10^{10}$	$10^{-1}—10^3$	10^9	$10^{-3}—10^{-2}$	—
DNA	$10^3—10^4$	$10^3—10^4$	$10^3—10^4$	$10^{-2}—10^1$	10^{10}	—	—
Lipids	$<10^3$	$<10^3$	$10^6—10^7$	$10^{-2}—10^1$	—	—	—
Protectors	$10^7—10^8$	$10^7—10^8$	$10^7—10^8$	—	—	1—10	—
Phenols	$10^4—10^6$	$10^4—10^6$	$10^5—10^7$	$10^4—10^6$	—	0.1—5	—
Nitro-compounds	10^9	10^{10}	$10^7—10^8$	—	—	—	—
Oxygen	$10^8—10^9$	$10^8—10^9$	$10^6—10^9$	—	—	—	—
k	$10^1—10^4$	$10^3—10^6$	$10^6—10^8$	$10^4—10^8$	$10^6—10^8$	$10^{-1}—10^{-5}$	$10^{-1}—10^{-5}$

Notation: $\dot{R}$ are low-molecular-weight organic radicals, $R\dot{O}_2$ are peroxy-radicals, $\dot{R}_H$ are sensitiser free radicals, Π_1 are protein peroxodes, Π_2 are DNA peroxides, k are the rate constants for the destruction of the corresponding radicals in first- or second-order reactions (s^{-1}).

In a complex multicomponent system the primary damage induced both by water radiolysis products and by the direct action of radiation quanta and charged particles leads to secondary reactions, both intramolecular and intermolecular. Numerous reactions of free radicals and a redistribution of the initial defects among different components take place. In order to represent the entire picture of the redistribution of damage-induced defects, it is necessary to have data for the interaction of the majority of different types of free radicals with one another and also for their reactions with cell components.

Table 4 presents the rate constants for the reactions of protein, DNA, and lipid free radicals, peroxy-radicals, and protein and DNA peroxides with various substances. The estimated rate constants, obtained as a rule by competitive methods, are presented. The wide range of these values is due to the different chemical natures of the radicals. Proteins, cysteamine, inhibitors, aromatic nitro-compounds, and oxygen exhibit a high reactivity in relation to all the active species. The interaction of lipids and DNA with various radicals is characterised by lower rate constants. It is also seen that there are virtually no data for the reactions of protein and DNA peroxides. The rate constants will be used below in Section VIII in the examination of the formal kinetics of the distribution of damaged-induced defects in "protein–DNA" and "protein–lipids" systems.

IV. KINETICS OF THE REACTIONS OF FREE MACRORADICALS IN SOLUTION

There exists an extensive literature on the reactions and transformations of free radicals analogous to those formed in proteins and nucleic acids. [55,60,63,67] These are primarily reactions involving the abstraction of a hydrogen atom, which may result in the migration of the free valence "within" the protein globule or the double helix of DNA. Reactions of this type probably lead to the protection of macromolecules by protectors of the type of cysteine and cysteamine. Free radicals can subsequently add to molecules with double bonds and to aromatic groups. In addition the free radicals can undergo decomposition, isomerisation, recombination, and disproportionation reactions. Finally one may also mention oxidation-reduction reactions, for example, with variable-valence metal ions.

Protein and nucleic acid macromolecules can be regarded as miniature chemical reactors with a complex geometry and internal dynamics, in which rapid free radical processes take place. The rates of these reactions are limited, on the one hand, by the reactivity of particular radicals in relation to groups in close proximity and, on the other hand, by the rapid and slow movements "within" the macromolecules.

The main bulk of data for the reactions and transformations of protein and nucleic acid free radicals have been obtained in experiments by the EPR method on dry specimens and frozen solutions at low temperatures. The kinetic characteristics of the behaviour of radicals in solutions cannot be estimated from data for the radiolysis and photolysis in the solid phase. This is because of the unusual nature of the processes involving the accumulation and destruction of macroradicals in solid specimens for which the kinetic curves cannot be described within the framework of the usual ideas concerning reactions in the liquid and gaseous phases and it is necessary to introduce the concept of the polychromaticity of the rate constants, the rate constant distribution functions, and activation energies; a non-uniform distribution of radicals and the occurrence of reactions at the temperatures of the structural transitions in the matrices are characteristic of the solid phase. [55,60,63,67,72–76]

Certain data for the kinetics of the accumulation and destruction of protein free radicals have been obtained for aqueous protein solutions by EPR, pulse radiolysis, and flash photolysis. The main difficulty in comparing data obtained by different methods consists in the fact that apparently all the radicals except those which give rise to broad lines are recorded by EPR, while flash photolysis and pulse radiolysis record only the radicals which give rise to absorption bands

outside the region of absorption by the macromolecules, i.e. above 300 nm. In the main these are the cystinyl and tryptophanyl free radicals.

Kinetic curves have been obtained[77-80] for the accumulation and destruction of protein free radicals on u.v. irradiation of their solutions. It was found that there are two stages in the destruction of free radicals: one rapid stage with times of the order of 10^{-2}–10^{-1} s and another, slow stage with times in the range 1–20 s. The disappearance of free radicals formally follows a kinetic first-order reaction equation and bimolecular components have not been found. It has been shown for serum albumin that the rate constant for the slow stage depends only slightly on temperature when the latter is varied from 10° to 40 °C. The characteristics of the destruction of free radicals do not differ greatly for different proteins. It has been found that the contribution of the slow component is proportional to the content of the alpha-helix in the proteins, which made it possible to employ this correlation for the determination of the content of the helix in a series of protein-containing species.[80] It also follows from the data quoted above[77-80] that the rate of accumulation of free radicals is higher than the rate of their destruction; the slow component is hardly manifested. This constitutes direct indication that the radicals are effectively destroyed by the radiation.

The kinetics of the destruction of free radicals in solutions of egg albumin have been studied by pulse radiolysis.[81] The kinetic curves for the destruction of the free radicals can be described by a set of exponential components with effective rate constants of 440, 8 and 0.4 s^{-1} when the experiments were carried out with the solution saturated by N_2O (the hydroxy-radicals react) and with approximately the same values on irradiation in an atmosphere of nitrogen. Analogous results have been obtained also for other proteins.[82-91]

The time scale of the reactions of the DNA free radicals in solutions at room temperature is extremely wide, ranging from microseconds to seconds.[65,92] A study[92] demonstrating the liberation of the K^+ and H^+ ions on irradiation of the potassium salt of polyuridylic acid in solution is of interest. This effect is treated as one involving the rupture of the poly(U) chain, entailing an increase in the degree of dissociation. It has been shown by pulse radiolysis with conductimetric measurements that the kinetics of the liberation of the ions are described by an equation for a first-order reaction with a rate constant of 0.8 s^{-1}. It has been suggested that the breaks in poly(U) are produced as a result of the decomposition of the poly(U) radicals formed when the polymer is attacked by hydroxy-radicals. The destruction of the macroradicals in first- and not second-order reactions is not very surprising, since analogous effects have been observed also in other systems. The free valence probably "moves" along the macromolecule, leaving behind a trail of transformations and the duration of this movement is rate-limiting. Next there are two possibilities: either there is a reaction between two macroradicals or a low-molecular-weight radical is initially split off from the macroradical and reacts with a polymeric radical. It is easy to show that the required disproportionation rate constant should not be less than 10^7–10^{10} litre $mole^{-1}$ s^{-1} and only then is it possible to account for the exponential character of the destruction of the free radicals. Data for the formation of lysozyme dimers on irradiation have been obtained[91] by pulse radiolysis (with recording of light scattering), the dimerisation rate constant amounting to 10^6 litre $mole^{-1}$ s^{-1}. If it is assumed that the formation of the dimers is the result of the recombination of the lysozyme macroradicals, then the above quantity must be regarded as the lower limit to the rate constants for the recombination of the macroradicals. This quantity is sufficient to explain the presence of slow components, but is too low to account for the rapid exponential components. The macroradicals most probably decompose with elimination of low-molecular-weight radicals and a cross-disproportionation. In the case of peroxy-macroradicals there is a possibility that the latter decompose with formation of O_2^-.

There exists yet another explanation of the exponential kinetic curves for the destruction of radicals—it involves the immediate formation of a pair of radicals on one macromolecule separated by a fairly short distance. This situation may obtain on irradiation of proteins with u.v.light.

In the presence of oxygen in the system, peroxy-macroradicals are formed. It is difficult to investigate them by direct methods, but there exists an extensive literature on the reactions and transformations of low-molecular-weight peroxy-radicals whose structures are similar to those of the radicals derived from the protein and DNA groups (see, for example, Timofeev-Resovskii et al.[25] and Kayushin et al.[60]).

The construction of a possible mechanism of the oxidation processes is the aim of a quantitative or at least a semi-quantitative description of the experimental data within the framework of a simplified kinetic scheme. The proposed schemes must explain the yields of the oxidation and product formation reactions and the kinetics of the decomposition of the groups of the macromolecules and of the accumulation of the modified products. The formulae obtained must be consistent with the dependence of the rate of oxidation on the dose rate, temperature, the concentrations of the substrate oxidised, oxygen, and various additives, pH, ionic strength, viscosity, etc.

A simplified kinetic scheme for the secondary free-radical reactions involving biomacromolecules is presented below.[78-98]

Scheme 1

$$B \xrightarrow{h\nu,\ O} \dot{R}(\dot{R},\ \dot{R}^+,\ R^-),\ O_2^-(H\dot{O}_2), \qquad (I)$$

$$\dot{R} \xrightarrow{k_1} \ldots P_i, \qquad (II)$$

$$\dot{R} + O_2 \xrightleftharpoons{k_2} R\dot{O}_2, \qquad (III)$$

$$R\dot{O}_2 + O_2^- \rightarrow \Pi + O_2, \qquad (IV)$$

$$R\dot{O}_2 \rightarrow P_i + O_2^- + H^+, \qquad (V)$$

$$2R\dot{O}_2 \rightarrow P_i + O_2, \qquad (VI)$$

$$2O_2^- \xrightarrow{2H^+} H_2O_2 + O_2, \qquad (VII)$$

$$R\dot{O}_2 + B \rightarrow \Pi + \dot{R}(R\dot{O}_2 \cdots HR \rightarrow \Pi + \dot{R}), \qquad (VIII)$$

$$\dot{R} + O_2^- \rightarrow \Pi, \qquad (IX)$$

$$R^- + O_2 \rightarrow \dot{P}_i + O_2^-, \qquad (X)$$

$$\Pi \rightarrow P_i, \qquad (XI)$$

where B is a biomacromolecule, $\dot{R}$ is a generalised representation of many types of macroradicals, RO_2 are peroxy-macroradicals, Π are stable or labile peroxides, and P_i is a generalised representation of the reaction products.

In the kinetic scheme considered, reaction (I) represents the primary processes involving the formation of free radicals of different types and the O_2^- radical-anion in the system. Reactions (II) and (III) are competing reactions of free macroradicals, while peroxy-macroradicals are formed in reaction (III). Reaction (IV) represents the cross-disproportionation of peroxy-radicals and superoxide radical-anions. It is noteworthy that the participation of O_2^- in the radiation-induced and photochemical oxidation of peptides and proteins has been discovered by the addition of superoxide dismutase, $O_2^- + O_2^- \xrightarrow{2H^+} H_2O_2 + O_2$.[93,94] The rate constant for reaction (IV) is about 10^9 litre $mole^{-1}$ s^{-1}. A process involving the additional formation of superoxides, namely reaction (V), has been proposed to account for data for the radiation-induced oxidation of proteins and DNA.[36,37] Reactions (VI) and (VII) are the usual radical disproportionation reactions.

Reaction (VIII) has been introduced to explain the relatively high yields in the absorption of oxygen, which exceed the yields in the formation of radicals when proteins and DNA interact with hydroxy-radicals. It has been suggested that this reaction proceeds via an intramolecular mechanism. Reactions (IX) and (X) are possible. It is noteworthy that reaction (IX) can proceed also in the presence of a deficiency of oxygen. Finally, the peroxides formed in reaction (IV) can decompose with formation of a wide variety of products.

The rates of the radiation-induced oxidation of proteins and DNA depend only slightly on temperature, which is consistent with Scheme 1, and only reaction (VIII) can have an activation energy greater than 10 kcal mole^{-1}, while the remaining reactions are characterised by lower activation energies. Within the framework of Scheme 1, the yield of peroxides and the absorption of oxygen should depend only insignificantly on the irradiation dose rate and should be determined by the competition between reactions (IV) and (VII), which has in fact been observed experimentally.[14,38,76,83,93-98]

For many compounds (serum albumins, trypsin, glycyltryptophan), the dependence of the rate of photochemical and radiation-induced oxidation on the oxygen concentration is described by the formula[95-98]

$$y = \frac{\omega}{\omega_{p=1}} = \frac{p(a_1 + 1)}{a_1 + p} , \qquad (1)$$

where ω is the rate of oxidation at a given oxygen concentration, $\omega_{p=1}$ the rate of oxidation in air, p the relative concentration of oxygen, and a_1 a parameter whose value is in the range 0.01–0.1. Within the framework of Scheme 1, the parameter a_1 is given by

$$a_1 = \frac{k_1}{k_2 O_2^{air}} ,$$

If it is assumed that $k_1 = 10^3$ s^{-1} and $k_2 = 10^8$ litre mole^{-1} s^{-1} (Table 4) and that the concentration of oxygen in water exposed to air at 25 °C is 2.6×10^{-4} M, then the calculated parameter a_1 is 0.04, a value which is comparable to that found experimentally.[95,98]

V. THE PRIMARY PROCESSES IN THE RADIATION-INDUCED OXIDATION

The formation of the hydroxy-radical, the hydrated electron, and the hydrogen atom when water is exposed to ionised radiation is the result of a series of physical and chemical processes which we shall not consider here. Free radicals are formed initially in tracks, spurs, and blobs and their distribution in space is extremely non-uniform. This non-uniformity and the inhomogeneity of the initiation should be taken into account in some way, but we do not possess the relevant experimental data. We shall therefore assume that the formation of the active water radiolysis products is fairly uniform.

The rate constants for the reactions of macromolecules with $\dot{H}$, $\dot{O}H$, and $\bar{e}$, presented in Section III, are close to the diffusion-dependent values. This actually implies that at protein and DNA concentrations of the order of 10^{-4} M the active radiolysis products are captured by these substrates; at concentrations of the order of 10^{-5} M there is no point in taking into account the cross reactions of the radiolysis products, since the rates of their reactions with biopolymers are much higher. In the presence of oxygen, the hydrated electron and the hydrogen atom do not participate in the oxidation process, but superoxides and hydroxy-radicals remain active:

$$2\dot{O}H \rightarrow H_2O_2 ,$$
$$2O_2^{\bar{}} \xrightarrow{2H^+} H_2O_2 + O_2 ,$$
$$O_2^{\bar{}} + \dot{O}H \xrightarrow{H^+} H_2O + O_2 .$$

It is easy to show that the third reaction makes the greatest contribution to the destruction of hydroxy-radicals. In order to simplify the kinetic calculations, it was useful to introduce formally a linear destruction reaction [reaction (XIII)] of hydroxy-radicals which are destroyed both in the reactions described above and in the interactions with impurities.

Taking into account these considerations, a simplified scheme for the primary processes involving the formation of macroradicals when biopolymers (B) are exposed to radiation is presented below:[92-103]

Scheme 2

$$H_2O \xrightarrow{h\nu} \dot{O}H, \bar{e} , \qquad (XII)$$

$$\dot{O}H \xrightarrow{k_3} H_2O_2, H_2O , \qquad (XIII)$$

$$\dot{O}H + B \xrightarrow{k_4} \dot{R} , \qquad (XIV)$$

$$\bar{e} + B \rightarrow R^{\bar{}} , \qquad (XV)$$

$$\bar{e} + O_2 \rightarrow O_2^{\bar{}} . \qquad (XVI)$$

At a fairly high oxygen concentration reaction (XV) may be disregarded and we obtain the following expression for the dependence of the relative rate of oxidation z on the concentration of the substance oxidised c:

$$z = \frac{\omega}{\omega_\infty} = \frac{c}{a_2 + c} , \qquad (2)$$

where $a_2 = k_3/k_4$, ω is the rate of oxidation, and ω_∞ the rate of oxidation at $c \rightarrow \infty$. Relations of this type have been known for a long time.[99] They describe satisfactorily the concentration variation for many low-molecular-weight substances and synthetic and natural polymers, including proteins and DNA. Since the rate constant k_3 is not elementary, relations of type (2) do not carry significant information about the oxidation process, but deviations from them in the radiolysis of aminoacids, sugars, and other substances are of interest.[100,101]

The unusual dependence of the gamma-chemiluminescence of solutions of glycyltryptophan on the peptide and oxygen concentrations made it necessary to consider the hypothetical $H\dot{O}_3$ species[96]—the acid analogue of the ozonide radial-ion $O_3^{\bar{}}$.

The influence of hundreds of substances on the radiation-induced reactions of proteins and nucleic acids have been investigated. Such studies had in the main two aims—the elucidation of the mechanisms of the radiation-induced reactions of macromolecules and the establishment of rapid tests for the preliminary testing and selection of potential protectors and sensitisers. The introduction of a fourth initial component into a system containing water, the polymer, and oxygen greatly complicates the system and makes it difficult to describe the experimental data within the framework of simple kinetic schemes. In many cases, it is possible to take the fourth substance into account. The acceptor introduced can interact with all types of active species involved in the radiation-induced oxidation: the active products of the radiolysis of water, the free radicals of the biopolymer, the peroxy-radicals, and the peroxides; the reactions of the acceptor entail the appearance in the system of new radicals

derived from the acceptor, which can also react with macromolecules. The introduction of the acceptor (A) leads to a whole set of reactions:

$$\dot{O}H + A \rightarrow \dot{R}_A , \qquad (a)$$

$$\bar{e} + A \rightarrow R_A^{\cdot -} , \qquad (b)$$

$$O_2^{\cdot -} + A \rightarrow R_A^{\cdot -} + O_2 , \qquad (c)$$

$$\dot{R} + A \rightarrow \dot{R}_A + P_i , \qquad (d)$$

$$R\dot{O}_2 + A \rightarrow \dot{R}_A + \Pi , \qquad (e)$$

$$\Pi + A \rightarrow P_i . \qquad (f)$$

All these reactions inhibit to some extent the radiation-induced oxidation of proteins and nucleic acids. Naturally reactions (a) and (d) make the greatest contribution to the protection. A series of protectors of the type of cysteamine and cysteine act in accordance with this mechanism. The rate constants for their reactions with all types of active species are fairly large and these substances give rise to free radicals which are relatively inactive.[102]

The acceptor free radicals $\dot{R}_A$ react with biopolymers at sufficient concentrations of the latter to form macroradials:

$$\dot{R}_A + B \rightarrow P_A + \dot{R} , \qquad (g)$$

where P_A are the acceptor reaction products. There is a possibility of the competing reaction

$$\dot{R}_A + O_2 \rightarrow R_A\dot{O}_2 . \qquad (h)$$

Judging from the available data,[97,102,103] such protective effects with latent sensitisation have been observed for solutions of biopolymers and even for living cells when alcohols and other substances, which react effectively with hydroxy-radicals to form fairly active acceptor free radicals, are introduced. The rate constants for the reactions of alcohol radicals with proteins are close to the diffusion-dependent values,[103] the oxy-radicals being the most reactive; effects unusual for free radical reactions are observed: the more hydrophobic the alcohol radical, the more readily it penetrates the hydrophobic "gaps" in the protein macromolecules.

Effects of explicit radiosensitisation have been observed for proteins, nucleic acids, and whole cells.[5,6,11,12] As a rule substances, the rate constants for the reactions of which with hydrated electrons are high, exhibit a sensitising activity. Several sensitisation pathways have been proposed, the simplest of which involves the formation of an acceptor radical-anion via reaction (b) and the formation of a macroradical in the reactions with biopolymers:

$$R_A^{\cdot -} + B \rightarrow \dot{R} + P_A . \qquad (i)$$

There are many competing pathways leading to the destruction of acceptor radical-anions, namely the reactions with oxygen, disproportionation, etc.

VI. KINETIC SCHEME FOR SENSITISED PHOTOCHEMICAL OXIDATION

Studies on the degrative effect of "near" u.v.light and visible light on living organisms have recently become more vigorous and the problem of protection against such damage and its prophylaxis has arisen.[14,104] When light acts on solutions of sensitisers, numerous reactions of their electronically excited states and free radicals, radical-ions, and peroxides take place in the presence of oxygen and lead to the degradation of the sensitiser molecules. Naturally there are many sensitisation mechanisms. A triplet–triplet energy transfer from acetone, acetophenone, and benzophenone to the DNA bases has been observed.[105,106] It involved the formation of cyclobutane dimers and breaks in the DNA chain were also observed. In the presence of oxygen the amount of dimer diminished, but the number of breaks increased. In photochemical oxidation this sensitisation pathway is probably fairly rare. Here we shall consider a possible but extremely simplified mechanism of the photochemical oxidation of macromolecules. The probable kinetic scheme for the primary processes is indicated below:

Scheme 3

$$S \xrightarrow{h\nu} {}^3S \qquad (XVII)$$

$${}^3S + S \xrightarrow{k_5} S + S, \qquad (XVIII)$$

$${}^3S + O_2 \xrightarrow{k_6} S + O_2, \qquad (XIX)$$

$${}^3S + O_2 \xrightarrow{k_7} S + {}^1O_2, \qquad (XX)$$

$${}^3S + B \xrightarrow{k_8} S + B, \qquad (XXI)$$

$${}^3S + B \xrightarrow{k_9} \dot{R} + \dot{C}H, \qquad (XXII)$$

$$\dot{C}H + O_2 \rightarrow S + O_2^{\cdot -} + H^+, \qquad (XXIII)$$

$${}^1O_2 \xrightarrow{k_{10}} O_2 , \qquad (XXIV)$$

$${}^1O_2 + B \xrightarrow{k_{11}} \Pi , \qquad (XXV)$$

where S is a sensitiser molecule in the ground state and ^3S the triplet electronically excited state of the sensitiser. The triplet state of the sensitiser can be quenched physically in reactions (XVIII)–(XXI), reaction (XXII), involving the formation of singlet oxygen, being the most important. Reaction (XXII) involves formation of free radicals from the macromolecules via a dehydrogenation or charge transfer mechanism and reaction (XXIII) represents the regeneration of the sensitiser. Reaction (XXIV) is the deactivation of singlet oxygen and reaction (XXV) involves the addition of singlet oxygen to the macromolecule. On the basis of the kinetic Scheme 3, subject to the condition that the concentrations of all types of active species are quasi-stationary, an expression has been obtained for the rate of oxidation ω:

$$\omega = \frac{a_0\omega_T cp\,(a_3 + a_4 p + c + a_7)}{(a_1 + p)\,(a_3 + c)\,(a_5 p + c + a_6)} ,$$

where ω_T is the rate of formation of triplet excited states of sensitiser:

$$a_0 = \frac{k_9}{k_8 + k_9}, \quad a_3 = \frac{k_{10}}{k_{11}}, \quad a_4 = \frac{k_7 O_2^{air}}{k_9},$$

$$a_5 = \frac{(k_6 + k_7)\,O_2^{air}}{k_8 + k_9}, \quad a_6 = \frac{k_5}{k_8 + k_9}, \text{ and } \quad a_7 = \frac{k_1 k_7}{k_2 k_9}$$

In the presence of a fairly high oxygen content ($p > 0.1$), the term a_1 may be neglected and apparently so can the parameter a_7, which simplifies the formulae. We shall consider special cases. At a high oxygen concentration, the dependence of the relative rate of the concentration of macromolecules is given by:

$$z = \frac{c}{a_3 + c} \quad (3)$$

Relations of this type have been observed for many systems: glycyltryptophan, in the presence of xanthene dyes, psoralen, and other sensitisers, and trypsin with sensitisation by eosin and retinal.[107-112]

When there is no generation of singlet oxygen, the concentration dependence simplifies:

$$z = \frac{c}{a_5 p + a_6 + c} \quad (4)$$

It may be assumed that this type of relation obtains in the systems glycyltryptophan–ketones, human serum albumin–riboflavin, and bovine serum albumin–anilinonaphthalenesulphonate (ANS).[107,111]

The dependence of the relative rate of oxidation on the relative oxygen concentration has a by no means simple form:

$$y = \frac{a_6 + a_5 + c}{a_3 + a_5 p + c} \cdot \frac{a_3 + a_4 p + c}{a_3 + a_4 + c} \quad (5)$$

For an experimental confirmation of a relation of this type, it is necessary to have numerous data with wide-scale variation of the concentrations of the substrate oxidised and of oxygen. Such studies have been performed in two systems: glycyltryptophan–eosin and trypsin–eosin.[108,110] So far as we are aware, studies of this kind on DNA have not been performed. Finally, when the contribution of singlet oxygen to the photochemical oxidation is low, the dependence on the oxygen concentration assumes the following form:

$$y = \frac{a_6 + a_5 + c}{a_6 + a_5 p + c} \quad (6)$$

It is seen from Eqn.(6) that the relative rate of photochemical oxidation falls with increasing oxygen concentration. This type of dependence has been observed for the human serum albumin–riboflavin system.[107] The parameters a_3, a_4, and a_5 have been determined from the experimental data presented above, which made it possible to estimate certain rate constants and their ratios (Table 5). The rate constants for the reactions of singlet oxygen with a series of peptides and proteins are close to the diffusion-dependent values. The quenching of triplet electronically excited states by proteins is also extremely effective. The quanitity $(k_6 + k_7)/(k_8 + k_9)$ characterises the efficiency of the quenching of triplet excited states of sensitisers by oxygen relative to the quenching by proteins and peptides; for proteins, the above quantity is of the order of unity. The ratio k_7/k_9 characterises the relative efficiency of the formation of singlet oxygen and of its involvement in the oxidation in relation to the formation of protein free radicals; Table 5 shows that the pathway involving singlet oxygen predominates.

VII. PHOTOPHYSICAL AND PHOTOCHEMICAL REACTIONS OCCURRING WHEN MACROMOLECULES ARE ACTED UPON BY ULTRAVIOLET RADIATION

The photophysical processes in proteins have been studied in much greater detail than in DNA. This is associated with the high luminescence yields in proteins and the insignificant luminescence yield in DNA molecules. The photochemical reactions in these systems are also different. Whereas approximately the same products are formed in the case of proteins as on exposure to ionising radiation and the photochemical oxidation proceeds with formation of free radicals, the main product of the photochemical reactions of DNA is cyclobutane dimers, while the yields of the remaining products are low. The dimers are fairly readily destroyed by enzymic repair systems, which ultimately lead to a very high photostability of the DNA molecule in living cells. The appearance of photobiological effects requires u.v. radiation doses, in terms of the energy absorbed by the cell nuclei, higher by 2–3 orders of magnitude than on exposure to ionising radiation.[14,17,100] A simplified scheme for the photochemical oxidation of proteins is given below:

Scheme 4

$$B \xrightarrow{h\nu} {}^1B, \quad (XXVI)$$

$${}^1B \rightarrow B, \quad (XXVII)$$

$${}^1B \rightarrow {}^3B, \quad (XXVIII)$$

$${}^3B \rightarrow B, \quad (XXIX)$$

$${}^3B + O_2 \rightarrow B + O_2, \quad (XXX)$$

$${}^3B + O_2 \rightarrow B + {}^1O_2, \quad XXXI)$$

$${}^1B \rightarrow P_i, \quad (XXXII)$$

$${}^3B \rightarrow P_i, \quad (XXXIII)$$

$${}^1B \rightarrow [{}^1B^{\cdot+} \ldots \bar{e}] \rightarrow \dot{R}^{\cdot} + \bar{e}, \quad (XXXIV)$$

$${}^3B + B \rightarrow \dot{R}H + \dot{R}, \quad (XXXV)$$

$${}^3B \rightarrow \dot{R} + \dot{H}(\bar{e} + H^+), \quad (XXXVI)$$

$${}^3B + O_2 \rightarrow \dot{R}^+ + O_2^{\cdot-}, \quad (XXXVII)$$

$$[{}^1B^{\cdot+} \ldots \bar{e}] + O_2 \rightarrow \Pi, \quad (XXXVIII)$$

$$\Pi \xrightarrow{h\nu} P_i, \quad (XXXIX)$$

where reactions (XXVI)–(XXXI) are the usual photophysical reactions.

Table 5. The rate constants and their ratios for the sensitised photochemical oxidation reactions of a number of substances and the constants calculated within the framework of thekinetic Schemes 1 and 3 from the literature.[98,107-112]

Substance	Sensitiser	$10^{-9} k_1$, litre mol^{-1} s^{-1}	$10^{-9}(k_8 + k_9)$, litre mol^{-1} s^{-1}	$\frac{k_6 + k_7}{k_8 + k_9}$	k_7/k_9
Glycyltryptophan	Eosin	1.1	1	27	6.2
Ditto	Uranin	1.2	—	—	—
»	Psoralen	1.1	—	—	—
»	Nicotinic acid	1	—	—	—
»	Acetone	—	1	8.6	—
Trypsin	Eosin	8.6	3	1	3.8
Ditto	Uranin	8	—	—	—
»	Retinal	6.4	—	—	—
Human serum albumin	Eosin	21	—	—	—
Ditto	Riboflavin	—	4.8	0.54	—
Bovine serum albumin	ANS	—	4	0.75	—

The principal chromophores in proteins are tryptophan, tyrosine, and phenylalanine; the fluorescence yields of these molecules in neutral aqueous solutions are 23, 21, and 3.8% and the lifetimes of the singlet electronically excited states are 3.1, 3.3, and 5.3 ns respectively.[113-115] The

fluorescence yields for tryptophan-containing proteins are 1–30%, the lifetime of the singlet electronically excited state is 1–6 ns, and the yield of the triplet electronically excited states is up to 10%.[113,114,116–118] In photochemical oxidation oxygen is absorbed with a quantum yield of 0.5–2%, the tryptophan residues decompose with a yield of 1–2%, and enzymes are inactivated with yields of 0.1–3%.[113–120]

The fluorescence quantum yield in DNA is extremely low (10^{-3}–10^{-4}%) and the lifetimes of the singlet electronically excited states are 1–5 ps.[121] The yield of the dimers reaches 4% and on exposure to irradiation by a laser the number of single breaks and other defects increases.[32,122,123] It has been suggested that dimers may be formed with participation of both singlet and triplet electronically excited states—reactions (XXXII) and (XXXIII). The main bulk of these defects are probably formed with participation of singlet excited states, because oxygen does not interfere with dimerisation. There are insufficient experimental data to examine the possible DNA photochemical oxidation processes.

It has come to be assumed that the main reaction occurring in the irradiation of proteins with light at wavelengths in the range 260–300 nm is photoionisation with participation of singlet electronically excited states in the aromatic groups of the protein—reaction (XXXIV), which includes the formation of a complex with partial charge transfer. This reaction proceeds via a one-quantum mechanism in liquid aqueous solutions and via a two-quantum mechanism in frozen solutions.[34,113,116–119,124,125]

Reaction (XXXIV) results in the formation of a radical-cation and a hydrated electron. Other possible reactions leading to the formation of free radicals are also considered in the literature quoted.

Reaction (XXXV) represents the abstraction of hydrogen atoms in the reactions of triplet excited states of the aromatic groups of the protein with neighbouring groups in the macromolecule to form a pair of free radicals and, finally, reaction (XXXVI), which represents the formation of hydrogen atoms or photoionisation with participation of triplet electronically excited states of aromatic groups, is relatively unlikely, being energetically unfavourable.

It has usually been assumed that reaction (XXXI), involving the formation of singlet oxygen, is relatively ineffective in the case of proteins, although there is no evidence for this. We believe that this contribution to photochemical oxidation may prove to be significant.

In constructing a photochemical oxidation mechanism, account is taken of reaction (XXXVII)—the formation of the radical-cation derived from the aromatic groups and of the superoxide radical-anion. This reaction probably occurs in the photochemical oxidation of free tryptophan and peptides containing tryptophan. In order to determine the contribution of the superoxide, experiments have been performed[93,94] in which superoxide dismutase was added during the photochemical oxidation of glycyltryptophan. The enzyme inhibits sharply the formation of unstable peroxides, which indicates a significant contribution of the superoxide to the oxidation. In the case of proteins the addition of the enzyme reduces the yield of peroxide by only 20–30%, i.e. O_2^- is only partly released into space and a "latent radical" mechanism involving the formation of radicals, possibly via reaction (XXXVIII), operates. Oxygen reacts either with the complex in the excited state or with groups in the protein in the triplet excited state, forming peroxides directly. In dilute solutions, at a low optical density, unstable peroxides undergo photochemical decomposition [reaction (XXXIX)].

Thus examination of questions associated with the action of u.v. light on proteins and DNA leads to the conclusion that their reactions follow different pathways: in the case of proteins a free-radical mechanism of the formation of damage-induced defects operates, while in the case of DNA the reactions of free radicals are not important.

VIII. THE POSSIBLE REDISTRIBUTION PROCESSES INVOLVING THE INITIAL DAMAGE-INDUCED DEFECTS IN MULTICOMPONENT SYSTEMS CONTAINING DNA, PROTEINS, AND OTHER SUBSTANCES

The main target when living cells are acted upon by ionising radiation is the cell nuclei or more precisely the DNA molecules. The biochemical composition of the cell nuclei is extremely varied and the principal component consists of a wide variety of proteins. The protein : DNA ratio in different cells varies within broad limits,[126–128] such data being presented in Table 6. Proteins can perform a wide variety of biochemical functions and are effective acceptors of all types of active species. Tables 3 and 4 present the estimated rate constants for different reactions. Proteins and DNA interact effectively with the active water radiolysis products and the free radicals derived from DNA can react with proteins. In qualitative terms, it is understandable that the higher the protein concentration, the smaller the damage to DNA. Here we shall make an attempt to estimate the contribution of proteins to the radiation stability of DNA.

We shall consider a simplified kinetic scheme for the processes occurring in the protein–DNA system on irradiation in aqueous solution. We shall take into account only the reactions of hydroxy-radicals with the macromolecules and DNA:

$$H_2O \xrightarrow{h\nu} \dot{O}H,\ \bar{e},\ \dot{H}\ ,$$

$$\dot{O}H + B \xrightarrow{k_1'} \dot{R}_1\ ,$$

$$\dot{O}H + D \xrightarrow{k_2'} \dot{R}_2\ ,$$

where $\dot{R}_1$ and $\dot{R}_2$ are protein and DNA free radicals respectively. The macroradicals could enter into numerous reactions, including the following:

$$\dot{R}_1 + D \xrightarrow{k_3'} B + \dot{R}_2,$$

$$\dot{R}_2 + B \xrightarrow{k_4'} D + \dot{R}_1.$$

Finally we have the destruction of radicals leading to chemical modification:

$$\dot{R}_1 \xrightarrow{k_5'} P_1\ ,$$

$$\dot{R}_2 \xrightarrow{k_6'} P_2\ ,$$

where P_1 and P_2 are the modification products.

The contribution of the reaction with k_3' to the degradation of DNA can be hardly large, because the protein free radicals are not very active and the absolute DNA concentration in the nucleus is low. Subject to the condition that the concentrations of all the active species are quasi-stationary, the rate of degradation of DNA ω_D is given by

$$\omega_D = \frac{k_2'k_6'[D]\,\omega}{(k_6' + k_4'[B])\,(k_1'[B] + k_2'[D])},$$

where ω is the rate of formation of hydroxy-radicals. We introduce the variable $x' = [B]/[D]$ and obtain the following equation for the relative radiation stability of DNA (y'):

$$y' = \frac{\omega}{\omega_D} = (a_1'x' + 1)(a_2'x' + 1)\ , \qquad (7)$$

where

$$a_1' = \frac{k_1'}{k_2'} \quad a_2' = \frac{k_4'[D]}{k_6'}\ .$$

The parameters were estimated from the rate constants (Tables 3 and 4) and the DNA concentration (approximately 4×10^{-4} M). The quantity a'_1 varies in the range 0.1–1 and $a'_2 = 0.05$–0.2. If it is assumed that $a'_1 = 0.5$ and $a'_2 = 0.1$, we obtain from Eqn.(7)

$$y' = (0.5\,x' + 1)\,(0.1\,x' + 1) \qquad (8)$$

Table 6 presents the results of the calculation by Eqn.(8) and it is seen that the stability of DNA increases sharply with increase of the protein : DNA ratio. According to radiobiological data, the stability of cells increases in the same sequence.[4,18,21,25]

Table 6. The radiation stability (y') of DNA as a function of the "protein : DNA" ratio in the cell nuclei of a number of organs and tissues.[126–128]

Object	Protein : DNA	y'
Lymphocytes	2.8	3.1
Thymus	3	3.3
Bone marrow	3.3	3.5
Spleen	6.3	7
Small intestine	8.7	10
Lungs	11.2	14
Kidney	16.7	24.8
Liver	23.4	42.5
Brain	24.6	46
Heart	55.6	188

Remarks: The quantity y' was calculated by Eqn.(8) for a homogeneous solution of the protein and DNA at concentrations comparable to those within the cell nuclei, the stability of DNA in the absence of protein being adopted as unity.

Table 7. The average concentrations (c) and the effective first-order rate constants (k) for the reaction of the components of cell nuclei and membranes with singlet oxygen.

Substance	c, M	k, s^{-1}
Nuclear proteins	$3 \cdot 10^{-3}$	$3 \cdot 10^{6}$—$3 \cdot 10^{7}$
Nuclear DNA	$4 \cdot 10^{-4}$	4
Membrane proteins	10^{-2}	10^{7}—10^{9}
Membrane lipids	1	10^{3}—10^{5}
Inhibitors of the type of tocopherol	10^{-3}	10^{2}—10^{4}

Remarks: k is the product of the rate constant for the reaction of a given substance with singlet oxygen and the concentration of the latter (Table 3).

When the known protector cysteamine is introduced into the system (Tables 3 and 4), its competing reactions with hydroxy-radicals and the DNA free radicals are included in the system. Fairly effective protection can be achieved at protector concentrations of the order of 10^{-3} M, which is comparable to the concentrations giving rise to an effect in a radiobiological experiment.[129] Naturally, the formal kinetic results make it possible to estimate the degree of primary protection of DNA by the protein in solution and cannot claim to be able to account for the complex final biological results of the action of radiation.

Table 3 presents certain data for the reactions of singlet oxygen, which permits a rough estimation of the redistribution of damage-induced defects in multicomponent systems in sensitised photochemical oxidation involving such oxygen. Table 7 also presents estimates for the components of cell nuclei and membranes. It shows that the effective rate constants for the first-order reactions of cell components with $^1O_2$ are higher by several orders of magnitude for proteins than for the remaining components, i.e. proteins are the main target.

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Data concerning the mechanisms of the action of radiation on proteins and nucleic acids are of significant interest from the standpoint of the control of the biological consequences of such influences in the primary physicochemical stages of the initial damage. It is seen from the results presented that numerous intermediates participate in the photochemical reactions of biopolymers—electronically excited states, radical-ions, free radicals, peroxy-radicals, and peroxides, as suggested by N.M.Emanuel' in the 1950's. In a number of cases the experimental data can be described within the framework of simplified kinetic mechanisms with participation of a small number of active species. Kinetic equations have also shown that the damage to the "main" DNA molecule can be greatly reduced in the presence of protein—the principal component of cell nuclei.

The complexity of the composition and structure of proteins and nucleic acids preclude the detection of all the reactions leading to the chemical modification of the primary structure of the biomacromolecules and the majority of the mechanisms proposed are tentative. All this indicates the necessity of further studies of these systems using modern methods for the determination of intermediate and final reaction products.

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Institute of Chemical Physics,
USSR Academy of Sciences,
Moscow