Translated from *Uspekhi Khimii*, **44**, 1887-1913 (1975) U.D.C. 577.3.08 : 547.963.32

Physicochemical Mechanism of the Damage Sustained by Deoxyribonucleic Acid under the Influence of Various Agents

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Data obtained in studies on the damage suffered by DNA molecules under the influence of ionising ultraviolet, or ultrasonic radiation, and chemical mutagens are reviewed. The application of chemical kinetic and other physicochemical methods (ESR, chemiluminescence, spin labels, electron microscopy, etc.) to the study of the mechanism of the damage to DNA molecules acted upon in different ways is considered and mechanisms whereby chemical and structural changes occur in DNA are analysed. The hypothesis of the free-radical mechanism of the damage sustained by DNA under the influence of radiation and of the possible use of radical-reaction inhibitors as agents offering protection from radiation is discussed and evidence for its validity is presented.
The bibliography includes 181 references.

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I. INTRODUCTION

In 1957 Emanuel[1] suggested that physicochemical and chemical kinetic methods be used to investigate many pathological processes such as radiation sickness, tumour growth, ageing, and others[1-5]. These processes were studied at various levels of organisation—molecular[6,7], cellular[8-10], and at the level of the whole organism[11-14].

Studies at the molecular level included analysis of changes in important biomacromolecules such as proteins[15-17], nucleic acids[18-23], lipids[24-26], and enzymes[27-29], slight changes in the structure of which can lead to the breakdown of the normal vital processes in the cell. Much attention has been devoted to the analysis of the damage suffered by nucleic acids, particularly deoxyribonucleic acid (DNA)[30,31].

It is known that the change in the structure of DNA acted upon in different ways can easily lead to the breakdown of hereditary information, to a change in the synthesis of specific proteins, and in many instances to cell death. The use of physicochemical methods for quantitative investigation of the damage sustained by the DNA structure under the influence of various factors in model experiments yields data about the primary molecular "events" leading to damage to the macromolecule. This information is important for the solution of the general problem of nucleic acids, associated with the processes involved in their functioning in the cell (in particular for the understanding of the mechanism of the occurrence of mutations or of the death of the cell).

II. THE CHANGE IN THE PHYSICOCHEMICAL PROPERTIES OF DNA UNDER THE INFLUENCE OF IONISING RADIATION. THE MECHANISM OF THE PROTECTIVE ACTION OF RADICAL-REACTION INHIBITORS

Only the first steps have been taken so far in many attempts to elucidate the physicochemical nature of the processes occurring in the irradiated cell[32-34]. To determine the characteristics of the development of reactions leading to the death of the cell, it is important to discover which cell structures are damaged and in what sequence. Numerous radiobiological studies have shown that the nucleus is damaged by ionising radiation[35,36], cell division is affected[37], and chromosomal aberrations occur[38]. It has been shown that the replacement of thymine in DNA by its halogenated analogues intensifies the sensitivity of the cell to irradiation[39,40]. Analysis of reduction processes in the irradiated cell established a relation between the chemical changes in the DNA macromolecule and the observed radiation-induced effect[41]. These data showed that DNA is one of the principal targets for radiation.

The first studies on the radiation-induced damage to DNA following the application of high radiation doses led to the detection of both chemical changes in the macromolecule (the formation of peroxides, evolution of ammonia)[42,43] and of structural damage in DNA (a change in the viscosity of the irradiated solutions)[44]. Later investigations demonstrated that the action of ionising radiation on DNA may lead to the single and double breaks in the macromolecular chains[45-48], to a chemical modification

of the bases, and to cross-linking between the complementary DNA chains[49-51].

As new physical, biochemical, and physicochemical methods for the investigation of biomacromolecules were developed, it was possible to differentiate the action of different radiation doses on DNA, which must be taken into account in studies on the mechanism of the radiation-induced damage to the cell and the organism as a whole. Thus the employment of the method of spin labels[52] for the analysis of the damage in DNA revealed conformational changes in the macromolecules under the influence of low radiation doses (0.5–8 krad)[53]. The spin label correlation time τ_c (the average time necessary for the rotation of the stable radical by 45°) and the associated inverse variation of the radical rotation frequency ν were calculated from the ESR spectra of spin-labelled DNA. These values yield information about the kinetics of the changes in the microenvironment of the label during irradiation. The observed sharp decrease of τ_c with increasing dose indicates a change in the DNA structure near the site of addition of the label (Fig. 1).

Analysis of the lg τ_c–$1/T$ curves made it possible to determine the temperature of the structural transitions (T_{st}), which shifts on irradiation towards lower values. This indicates conformational changes in the macromolecule. It was established that the values of T_{st} for DNA isolated from various sources differ significantly. Thus T_{st} for DNA isolated from tumour cells is lower than for DNA isolated from normal cells[53,54]. The observed difference may be caused by the fact that in tumour cell DNA there are more defects in the secondary structure[55]. The validity of this hypothesis was demonstrated by analysing the defects in the secondary structure of DNA, isolated from normal and tumour cells, by the kinetic formaldehyde method[56]. The applicability of this method to the analysis of structural damage in DNA for low γ-radiation doses (up to 5 krad) has been demonstrated[57]. The authors observed a single structural defect for 2600 pairs of nucleotides.

The conformational changes in DNA at low doses were also detected by pulse polarography[58]. Some investigators point out the possibility of damage to DNA bases at low doses (2–10 krad)[59].

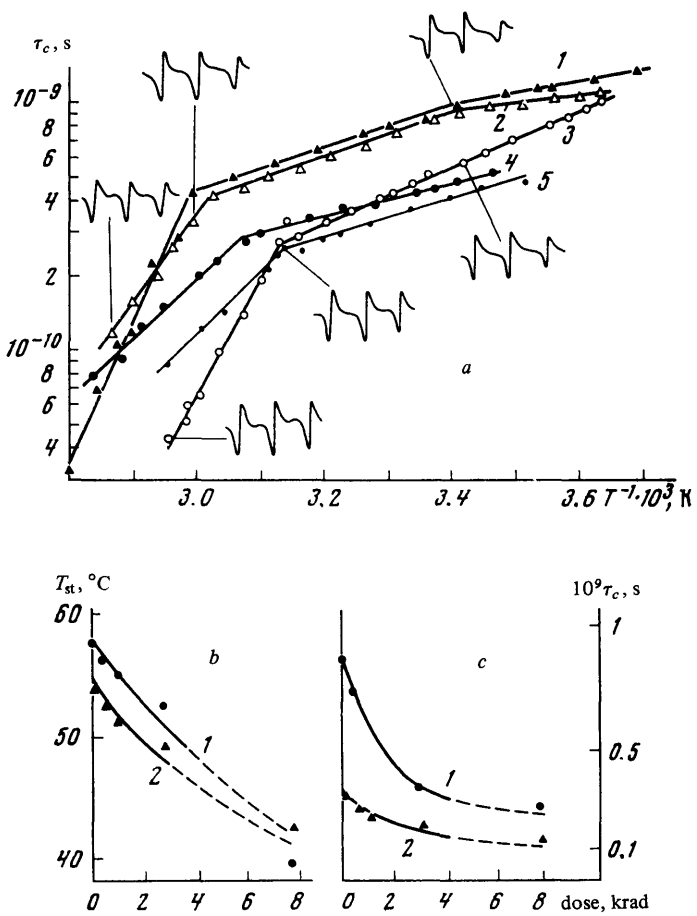


Figure 1. ESR spectra and temperature variation of the correlation time of spin-labelled DNA: *a*) [1) and 2) DNA isolated from the blood lymphocytes of two healthy cows; 3)–5) DNA isolated from the blood lymphocytes of three cows suffering from leukemia]; *b*) and *c*) variation with the dose of T_{st} and τ_c for DNA isolated from normal (curve *1*) and leukemic (curve *2*) cells.

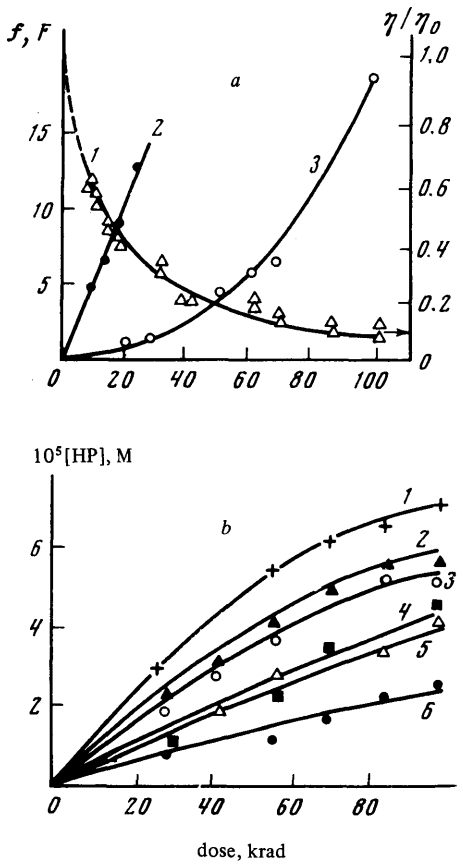


Figure 2. Variation of the physicochemical (*a*) and chemical (*b*) properties of DNA on irradiation with different doses: *a*) [1) intrinsic viscosity in relative units; 2) and 3) number of single (line *2*) and double (line *3*) breaks in DNA]; *b*) amount of DNA hydroperoxides formed on irradiation [DNA concentration (μg ml^{-1}): 1) 1000; 2) 700; 3) 500; 4) 400; 5) 100; 6) 50].

High doses (20–100 krad) lead to significant structural changes in the macromolecule (single and double breaks occur) and to chemical changes (the formation of hydroperoxides)[60–63] (Fig. 2). Infrared spectroscopy[64,65] made it possible to observe a modification of the bases at doses of 20–100 krad from the change in the intensity of the bands in the spectra of irradiated DNA solutions: deamination (3350, 3200, and 1600 cm^{-1} bands); saturation of double bonds (1580 and 1650 cm^{-1} bands); damage to the secondary DNA structure—rupture of hydrogen bonds (1700 cm^{-1} band) and of phosphate ester linkages (953 cm^{-1} band) (Fig. 3). The radiation-induced damage to DNA may be observed in electron micrographs of the macromolecule[66,67]. Long linear molecules are converted into short segments and coils after irradiation with doses in the range 60–300 krad.

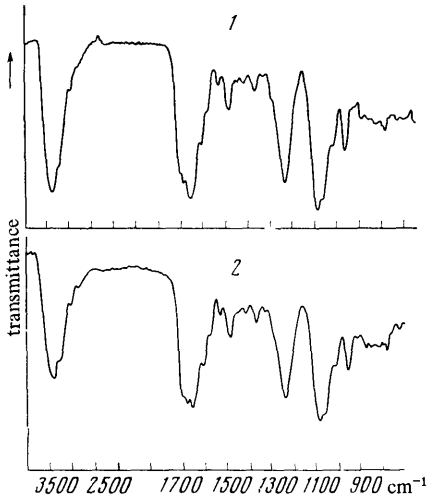


Figure 3. Infrared spectra of a calf thymus DNA film for 32% relative humidity: 1) native DNA; 2) γ-irradiated DNA (dose 55 krad).

The question arises of the causes of these significant changes in the DNA molecule and of the stages in the multistage mechanism of the damage sustained by the macromolecule where external action is necessary in order to liquidate or arrest the process. Several years ago Emanuel' put forward the following considerations on the basis of general concepts in chemical kinetics, radiation chemistry, and biochemistry[68]:

"1. The action of ionising radiation leads to the formation of free-radical states of the chemical components of the cell which may give rise to biochemical processes which are not characteristic of the living organism under normal conditions.

2. The development of free-radical reactions in the organism must lead to a decrease of the amount of inhibitors in the tissues, as happens in chemical radical processes induced by radiation in the presence of inhibitors. The decrease of the amount of tissue inhibitors impairs the ability of the organism to regulate correctly biochemical processes, which may be one of the causes of the changes characteristic of radiation sickness".

The above ideas made it necessary to investigate the free-radical states in biochemical systems, particularly in biomacromolecules—proteins, lipids, and nucleic acids. It has been shown by ESR[69,70] and chemiluminescence[71] methods that both the primary radicals formed in the course of irradiation of DNA and the secondary radicals generated as a result of the decomposition of hydroperoxy-compounds derived from DNA are important for the structural damage in the macromolecule.

The formation and reactions of DNA radicals were detected in the course of irradiation of aqueous DNA solutions at low temperatures (77 K). All the DNA radiolysis products are stable under these conditions[72]. It has been shown that the ESR spectrum of the irradiated DNA solutions contains lines due to paramagnetic species formed from water (ȮH radicals and stabilised electrons e_{st}) and DNA macroradicals. When the specimen is heated, the ȮH radicals and the electrons e_{st} vanish and the spectral lines due to the DNA radical appear. The doublet for the DNA radicals is gradually "converted" into a more complex quartet and at high temperatures (193 K) an eight-component spectrum is recorded, similar to that of the thymine radical[72,73].

With increase of DNA concentration in solution and of irradiation temperature, the yield of radicals rises. Kinetic curves for the accumulation of DNA radicals satisfy the equation $c = c_{st}(1-e^{-kD})$, where c_{st} is the steady-state concentration of radicals at high irradiation doses, k the rate constant for the destruction of radicals during irradiation, and D the radiation dose in Mrad.

To determine the nature of the radicals formed from DNA and the location of the free valence in the macromolecule, a study was made of the effect of radiation on compounds simulating individual fragments of the biopolymer—nitrogenous bases[74,75], carbohydrates[76–80], and phosphoric acid[81]. The formation of free radicals from nitrogenous bases was investigated under various conditions. The most detailed study was made of the formation and reactions of thymine radicals. It was established that thymine radicals are formed as a result of the interaction of an electron with a thymine molecule and the formation of a radical-anion, to which a hyperfine doublet structure corresponds in the ESR spectrum. On reacting with water, the radical-anion is converted into the thymine radical (of the type ṪH) (eight-component ESR spectrum). The observed hyperfine structure of the spectrum is due to the localisation of the unpaired electron at the $C_{(5)}$ atom of thymine[75].

The primary reaction in the formation of radicals in the sugar fragment of DNA is the abstraction of a hydrogen atom by OH radicals and free hydrogen atoms. Analysis of the ESR spectra of irradiated preparations of 2-deoxyribose and other carbohydrates[76,80,82] permitted an interpretation of the ESR spectrum of 2-deoxyribose as a result of the superposition of lines due to radicals in which the free valence is mainly located at the $C_{(1')}$, $C_{(3')}$, and $C_{(5')}$ atoms.

Irradiated frozen solutions of phosphate salts and phosphoric acid itself, DNA, and compounds simulating its sugar fragment were investigated by ESR. The ESR spectra of DNA did not contain lines characteristic of phosphoric acid radicals. This led to the conclusion that, even if the phosphate fragment in DNA is damaged, ultimately the free valence is located at the sugar fragment[81].

A comparative study of the ESR spectra of DNA, 2-deoxyribose, and nitrogenous bases led to the conclusion that at least two types of free radicals may be formed in DNA: with the free valence localised in the sugar fragment

and in the nitrogenous bases. The formation of radicals in DNA is possible as a result of both direct and indirect action of radiation.

The DNA macroradicals ($\dot{R}_{DNA}$) formed on irradiation are converted in the presence of oxygen into peroxy-radicals $R\dot{O}_2$ [72,73] and further into peroxide compounds (hydroperoxides):

$$\dot{R} + O_2 \rightarrow R\dot{O}_2 \rightarrow RO_2H.$$

Hydroperoxides are known to be radiotoxins and can in turn decompose with formation of radicals, initiating undesirable processes both in DNA solutions (a decrease of the molecular weight of the biopolymer, the occurrence of single breaks) and in the cell.

The formation of DNA hydroperoxides on irradiation has been established in a number of studies [84,85]. Kinetic analysis of the DNA hydroperoxide formation and decomposition processes showed [63,86,87] that hydroperoxides of two types are formed—relatively unstable and more stable. In a general form the rate constants for their decomposition can be represented as follows:

$$k_1 = 3.6 \cdot 10^5 \exp(-13000/RT),\ s^{-1};$$
$$k_2 = 1.3 \cdot 10^{11} \exp(-23000/RT),\ s^{-1}.$$

These expressions agree satisfactorily with the rate constants for the decomposition of hydroperoxides (HP) derived from thymine (k_t) and cytosine (k_c):

$$k_t = 4.5 \cdot 10^{11} \exp(-23000/RT),\ s^{-1};$$
$$k_u = 6.4 \cdot 10^5 \exp(-11500/RT),\ s^{-1}.$$

This suggested that, among the DNA hydroperoxides, those derived from cytosine and thymine are respectively less and more stable. The addition of inhibitors [88] demonstrated that the decomposition of cytosine hydroperoxide and hence of the less stable DNA hydroperoxide has a radical mechanism [87]. The decomposition of cytosine hydroperoxide (4.0×10^{-5} M) was retarded in the presence of α-naphthol, an effective inhibitor of free-radical reactions. The α-naphthol itself was consumed at a rate proportional to the rate of decomposition of the hydroperoxide, which indicated the possibility of the formation of radicals in the decomposition of cytosine hydroperoxide. In a study of the nature of the chemiluminescence of irradiated DNA solutions it was shown that the glow is due to the radical decomposition of DNA hydroperoxides and probably cytosine hydroperoxide [89-92]. This followed from a number of experimental findings (Fig. 4): a linear relation was observed between the chemiluminescence intensity and the concentration of hydroperoxides; the kinetic curve for the decomposition of the less stable DNA hydroperoxide had a shape closely similar to that of the kinetic luminescence curve; the activation energies for the chemiluminescence of the irradiated DNA solutions (E_{chl}) and for the decomposition of the less stable hydroperoxide (E_1) and cytosine hydroperoxide (E_c) had the following values:

$$E_{chl} = 13 \pm 1 \text{ kcal mole}^{-1};$$
$$E_1 = 13 \pm 1 \text{ kcal mole}^{-1};$$
$$E_c = 11 \pm 1 \text{ kcal mole}^{-1}.$$

The overall hydroperoxide decomposition mechanism can be represented as follows [22,71]:

$$ROOH \xrightarrow{k_0} R\dot{O} + \dot{O}H;$$
$$\dot{O}H + ROOH \xrightarrow{k_1} R\dot{O}_2 + H_2O;$$
$$R\dot{O} + R\dot{O} \xrightarrow{k_8} h\nu + \text{products};$$
$$R\dot{O} + R\dot{O}_2 \xrightarrow{k_{12}} h\nu + \text{products};$$
$$R\dot{O}_2 + R\dot{O}_2 \xrightarrow{k_{22}} h\nu + \text{products}.$$

Assuming a steady-state concentration of radicals in the system, it is possible to formulate equations for the rate of reaction of $\dot{O}H$, $R\dot{O}$ and $R\dot{O}_2$, the solution of which on the assumption that $k_8 = \sqrt{k_6 k_9}$ yields an expression for the concentration of these radicals. The destruction of $\dot{O}H$ radicals may be neglected since in the presence of ROOH the rate of their recombination is lower by several orders of magnitude than the rate of their reaction with ROOH. [93] The steady-state concentrations of the radicals are given by

$$[R\dot{O}] = \sqrt[6]{\frac{k_{22}}{k_6}}\,[R\dot{O}_2];$$
$$[R\dot{O}_2]^2 = \frac{k_0\,[ROOH]}{k_{22} + k_{12}\sqrt{k_{22}/k_6}}.$$

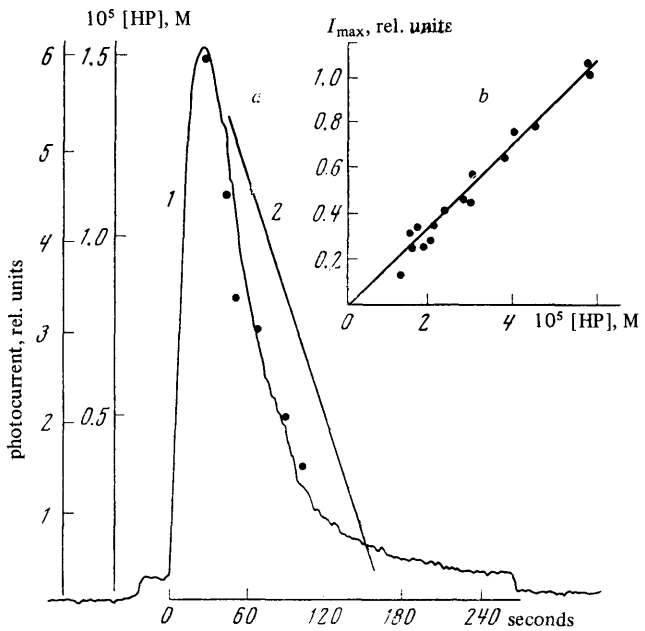


Figure 4. Chemiluminescence in the decomposition of DNA hydroperoxides: *a*) time variation of the chemiluminescence intensity (curve *1*) and of the concentration of the less stable hydroperoxide (circles on the curve) in irradiated DNA solution at 72°C; curve *2* is the semilogarithmic form of kinetic curve *1*; *b*) variation of the maximum intensity of the chemiluminescence of an irradiated DNA solution (700 μg ml^{-1}) with the initial concentration of hydroperoxides.

Since the chemiluminescence intensity I_{chl} is proportional to the rate of recombination of free radicals, one can write on the basis of the mechanism adopted

$$I_{chl} = Ak_6[R\dot{O}]^2 + Bk_{12}[R\dot{O}] \cdot [R\dot{O}_2] + Ck_{22}[R\dot{O}_2]^2.$$

Taking into account the expressions for $[R\dot{O}]$ and $[R\dot{O}_2]$, we obtain

$$I_{chl} = \alpha k_0\,[ROOH] = \alpha k_0\,[ROOH]_0 e^{-kt},$$

where

$$\alpha = \frac{Ak_{22} + Bk_{12}\sqrt{k_{22}/k_6} + Ck_{22}}{k_{22} + k_{12}\sqrt{k_{22}/k_6}}.$$

The expression obtained for I_{chl} agrees satisfactorily with experimental data[22].

Thus irradiation of aqueous DNA solutions may result in the formation of radicals both in the primary irradiation stages and as a result of the transformation of chemical products in secondary processes. Both reactions can be the primary cause of undesirable processes in the cell and can lead to breaks in the DNA chain and impairment of transcription. It was reasonable to suppose that the most effective means of preventing undesirable reactions involving free radicals might be inhibitors of free-radical processes (IRP), which have been used successfully in chemistry and have been proposed by Emanuel'[68] as agents offering protection from radiation (radioprotective agents or radioprotectors).

Studies on the radioprotective properties of IRP in model experiments with DNA[94,95] established that the most effective radioprotectors are derivatives of gallic acid, 3-hydroxypyridine, and phenylethylamine. The radioprotective effect of all the compounds under the conditions of appreciable damage to the molecule (60 krad) amounts to 80–90% when they are added before irradiation; derivatives of gallic acid and 3-hydroxypyridine exhibit a definite protective effect (20–48%) when they are used after irradiation[95]. Radical-reaction inhibitors greatly reduce the inactivation of the transforming activity of DNA under the influence of ionising radiation[96].

The observed linear relation between the protective effect and the antiradical effectiveness of the test compounds confirms the hypothesis of a significant role of free radicals in the damage to the DNA molecule and the analogous relation found in experiments on animals[97,98] shows that these compounds are promising radioprotective agents.

The protective effect of IRP added before irradiation can involve two mechanisms:

1. Via the interaction of the inhibitors with radical products of the radiolysis of water. This follows from the finding that the yield of DNA radicals on irradiation in the presence of propyl gallate (PG) is significantly reduced (G is 0.1 without PG and 0.02 with PG).

2. Via the reduction of the DNA radical by the inhibitor before the radical undergoes further reactions:

$$\dot{R}_{DNA} + InH \rightarrow RH + In\cdot.$$

This follows from experiments designed to study the changes in the DNA and propyl gallate radicals, irradiated at low temperatures, when the specimens are heated[99]. It has been shown that, when aqueous DNA solutions (5%) are irradiated in the presence of propyl gallate (0.03 M) at 77 K, $\dot{O}H$, $\dot{R}_{DNA}$, and $\dot{R}_{PG}$ radicals are formed. It has been established that, with increase of specimen temperature from 77 to 123 K, the $\dot{O}H$ radicals disappear, but the sum of the $\dot{R}_{DNA}$ and $\dot{R}_{PG}$ radicals remains unchanged. Subsequently, with increase in temperature from 173 to 193 K, the overall concentration of radicals does not alter, but $[\dot{R}_{DNA}]$ falls, while $[\dot{R}_{PG}]$ increases simultaneously. The operation of this mechanism of the protection of the macromolecule may be of interest in connection with the possible reduction reactions in the cell during irradiation.

As regards the protective action of inhibitors added after irradiation, presumably under these conditions they can interact with the radicals formed on decomposition of secondary products, for example hydroperoxides, and thus inhibit the reactions involving them. The intensity of the observed chemiluminescence of irradiated DNA solution decreased in the presence of various inhibitors (Fig. 5), the decrease being more marked the more efficient the inhibitor[90].

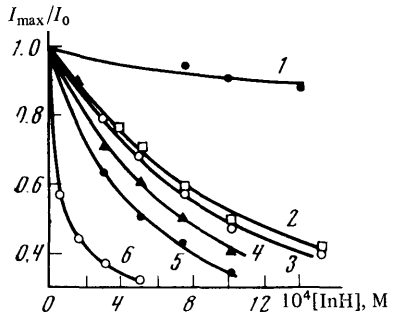


Figure 5. Variation of the intensity of the chemiluminescence of DNA solutions with the concentration of inhibitors at 72°C; inhibitor: 1) 4-ethylphenol; 2) hydroquinone; 3) catechol; 4) propyl gallate; 5) pyrogallol; 6) cysteine.

The mechanism of the possible reactions in this instance can be represented as follows[22,71]:

$$ROOH \xrightarrow{k_0} R\dot{O} + \dot{O}H;$$
$$R\dot{O} + InH \xrightarrow{k_7} ROH + In\cdot;$$
$$\dot{O}H + InH \xrightarrow{k_7} HOH + In\cdot;$$
$$R\dot{O} + R\dot{O} \xrightarrow{k_8} h\nu + \text{products};$$
$$\left.\begin{aligned} R\dot{O} + In\cdot &\xrightarrow{k_8} \\ In\cdot + In\cdot &\xrightarrow{k_8} \end{aligned}\right\} \text{without excitation of luminescence.}$$

This mechanism makes it possible to derive a relation between the intensity of the luminescence in the inhibited (I) and uninhibited (I_0) processes and the inhibitor concentration in the system:

$$\sqrt{\frac{I_0}{I}} = 1 + \frac{k_7}{\sqrt{2k_0k_8[ROOH]}}[InH].$$

A linear relation between $\sqrt{I_0/I}$ and the concentration of the inhibitor employed was indeed observed experimentally[91].

However, one should bear in mind that the reaction of the inhibitor involving the formation of free radicals may prove important in many cases as regards damage to cell components. Experiments on tumour cells by Lipchina, Pelevina, and coworkers[100] established that propyl gallate, the most effective radical-reaction inhibitor, can act in two ways depending on the experimental conditions. When PG was added to cells a short time before irradiation (15 min), a radioprotective effect was observed; on the other hand, when it was added to cells after 18 h, the latter were sensitised to radiation. The author suggested that the observed effect may be due to the possible damage of cell DNA by the PG reaction products.

To discover whether the PG reaction products can indeed react with the DNA molecule and how this affects the protective effect of PG, experiments were performed on isolated DNA where PG was added to irradiated DNA solutions at different times before irradiation and for

different degrees of oxidation of PG. [101,102] It was found that the addition of PG to the DNA solution immediately before irradiation results in a protective effect, which is enhanced with increasing PG concentration. The degree of protection falls when PG is added in an oxidised form or several hours before irradiation. The extent of the reduction of the protective effect increases with increasing contact time between PG and DNA before irradiation (Fig. 6).

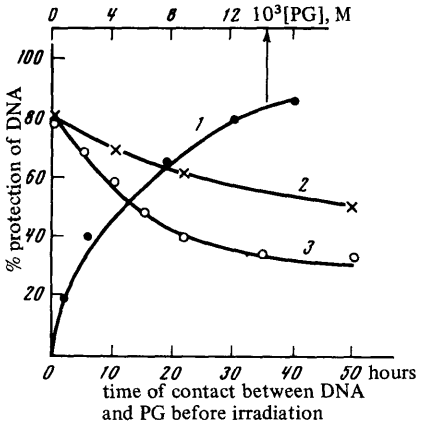


Figure 6. Effect of propyl gallate on the degradation of DNA during irradiation: 1) increase of the protective action in relation to DNA with increase of the initial propyl gallate concentration; 2) and 3) decrease of the protective action of propyl gallate in relation to DNA as a function of the duration of its contact with DNA before irradiation and of the degree of its oxidation [2) unoxidised propyl gallate; 3) propyl gallate oxidised to the extent of 30%].

One of the possible causes of the observed effect may be damage to the DNA structure by active PG oxidation products long before irradiation, which make the molecule more sensitive to subsequent irradiation.

Propyl gallate is known to be oxidised in an aqueous medium in the presence of oxygen, forming various biologically active radical and non-radical products: semiquinone radical-ions, quinones, etc. [103]. These intermediates can interact with the SH groups of proteins [104], can suppress the activity of many enzymes [105], can exhibit radiomimetic activity, and in particular can cause the rupture of chromosomes [106]. The formation of active radical intermediates is indicated by the chemiluminescence in the oxidation of PG. [102] The addition of DNA to the system both initially and at the instant corresponding to the maximum chemiluminescence intensity (Fig. 7) sharply reduces the yield of the oxidation products and the chemiluminescence. Free-radical oxidation products of the oxidation of PG were detected by ESR, their amount decreasing in the presence of DNA (Fig. 8). These findings show that the PG reaction products interact with DNA.

Experiments where PG oxidised to different extents had been added to DNA solutions showed that the longer the oxidation time of the added PG the more severe the damage to the DNA structure—single and double breaks appear in the molecule.

Thus, if one of the causes of the radiation-induced injury to cells is indeed damage of the DNA structure, then model experiments with isolated DNA will undoubtedly prove useful for the understanding of the mechanism of the damage of macromolecules and in the search for chemicals capable of causing specific changes in the sensitivity of the cells to radiation. In this sense radical reaction inhibitors are the most promising.

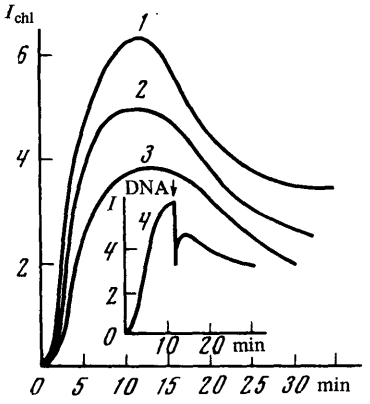


Figure 7. Variation of the intensity of chemiluminescence in the oxidation of propyl gallate: 1) at 70°C; 2) and 3) in the presence of added DNA at various concentrations [2) 80 μg ml^{-1}; 3) 160 μg ml^{-1}]; 4) variation of the chemiluminescence after the addition of DNA (80 μg ml^{-1}) at the instant corresponding to its maximum intensity.

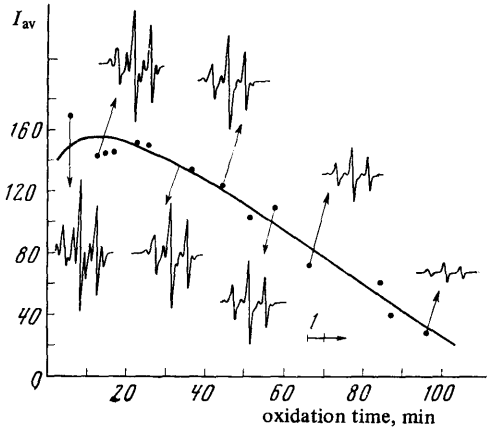


Figure 8. Variation of the intensity of the middle line in the ESR spectrum and of the shape of the propyl gallate spectra during oxidation in an aqueous alcoholic medium at 20°C and pH 11.5.

III. KINETICS OF THE DENATURATION OF DNA BY ULTRAVIOLET LIGHT

Ultraviolet light is known to be selectively absorbed by nucleic acids. DNA has been for many years the main object of studies on the effect of ultraviolet light at the

molecular level[107-109], since the nature of the observed changes in the cell[110] subjected to ultraviolet irradiation—mutations, chromosomal aberrations, and inhibition of cell respiration and DNA synthesis—indicate significant damage to the DNA.

The structural changes in DNA have been analysed by various physicochemical methods[111-116]. It has been shown that the exponential decrease of viscosity is associated with a parallel increase of optical density (hyperchromic effect), the kinetic constants for these processes having the same absolute magnitude (7.6×10^{-2} min^{-1}) [111]. Since it is well known that the hyperchromic effect is based on the impairment of the plane-parallel stacking of the bases due to hydrogen bonding and hydrophobic interaction between neighbouring bases are characteristic of native DNA, this agreement between the constants can be accounted for by the fact that the change in viscosity is due to the impairment of precisely these bonds. Thus the action of ultraviolet light reduces mainly to the denaturation of the molecule. This conclusion has been confirmed by the sedimentation analysis of DNA solutions after ultraviolet irradiation. It was shown that the sedimentation constant hardly changed in the course of the irradiation of DNA solutions (40 μg ml^{-1}) with ultraviolet light, which may indicate the absence of a sharp change in the molecular weight of the irradiated preparation[114].

The denaturation of the DNA molecule induced by ultraviolet light has been clearly illustrated by photomicrographs[112] recorded at specified time intervals. In the course of the first 5 min the photomicrographs show individual condensed sections which become clearly visible after irradiation for 20 min. On further irradiation (after 30 and 50 min) coils, the number and size of which increase, are visible in the field of view together with elongated molecules (Fig. 9). Evidently the denaturation process proceeds gradually with accumulation of denatured sections along the molecular chain.

These local conformational changes have been studied by ESR using 4-(β-aziridinopropionyloxy)-2,2,6,6-tetramethylpiperidin-1-yloxy-radicals as the spin labels[117]. It was shown that the denaturation of DNA is accompanied by an appreciable increase of the intensity of the ESR signal due to the label bound to DNA [115], which constitutes evidence that the label is joined in the first place to partly unwound or denatured sections of the DNA molecule.

The denaturation of the macromolecule can be followed by the change in the intensity of the signal of the label bound to DNA. Comparison of the changes in the ESR signal intensity I of the label and in the intrinsic viscosity of the solution η during the initial stage of irradiation showed that, as I increases η decreases. The change in the signal intensity of the label and the decrease of viscosity characterise to an equal extent the macroeffect of the ultraviolet irradiation on DNA solutions, i.e. the process in which local denaturation sites accumulate. The correlation times τ_c, which pass through an extremum (a maximum) as a function of the irradiation dose, were calculated. For low irradiation doses (up to 1.4×10^6 erg mm^{-2} for c_{DNA} = 600 μg ml^{-1}), τ_c increases, i.e. the mobility of the label diminishes; in the dose range ($1.4-3.5) \times 10^6$ erg mm^{-2} τ_c remains constant, falling sharply at still higher doses (Fig. 10).

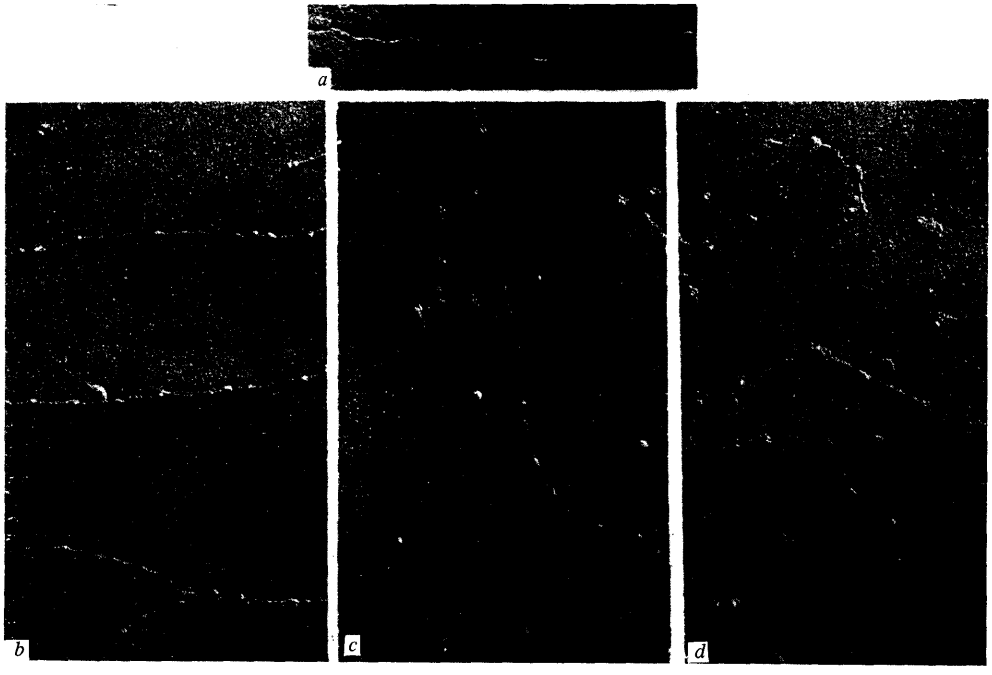


Figure 9. Photomicrographs of DNA preparations irradiated with ultraviolet light: *a*) fragment of the molecule of a native DNA; *b*) dose 3.6×10^5 erg mm^{-2}; *c*) dose 7.2×10^5 erg mm^{-2}; *d*) dose 1.2×10^6 erg mm^{-2}. The dimensions indicated in the photomicrographs correspond to 0.5 μm.

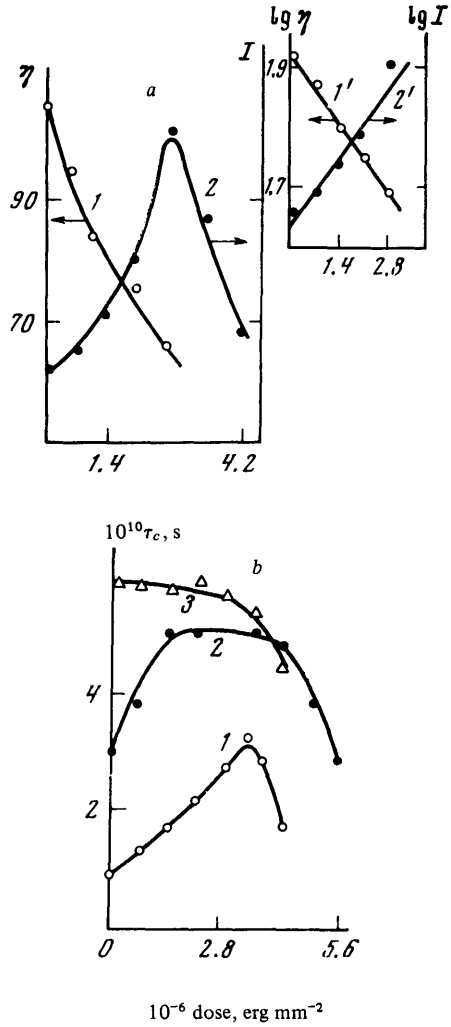


Figure 10. Variation of the viscosity and of the parameters of the ESR spectra of spin-labelled DNA as a function of the dose of ultraviolet irradiation: *a*) variation of the viscosity of DNA solutions (curve *1*) and of the intensity of the ESR signal (curve *2*) with the dose; lines *1'* and *2'* are semilogarithmic plots corresponding to curves *1* and *2*; *b*) variation with the dose of the correlation time for the spin label at different DNA concentrations [1) 400 μg ml^{-1}; 2) 600 μg ml^{-1}; 3) 900 μg ml^{-1}].

The decrease of the mobility of the radical may be caused by the increase of microviscosity within the local denaturation region. Two processes are known to occur in such regions: the accumulation of single breaks and the formation intramolecular cross-links as a result of the dimerisation of thymine bases. It has been suggested that thymine dimers accumulate in a single locally denatured region. The increase of τ_c can probably be a consequence of an increase in the yield of thymine dimers (for a single break in the polynucleotide chain). The constancy of τ_c observed in the dose range (1.4–3.5) × 10^6 erg mm^{-2} probably shows that the ratio of the thymine dimers and the breaks in the polynucleotide chain reaches a constant value. For fairly high doses (in excess of 3.5 × 10^6 erg mm^{-2}), the energy absorbed is consumed mainly in the formation of coils and globules. Under these conditions, the attached label is most probably distributed on the surface of the globule, which leads to a sharp increase of the mobility of the label and to a decrease of τ_c.

The structural changes in ultraviolet-irradiated DNA are indicated by infrared spectroscopic data. It has been observed[118] that ultraviolet irradiation of a DNA film with a 32% relative moisture content using a dose of 4.2 × 10^6 erg mm^{-2} decreases the absorption by the amino-groups of the bases by about 10%; the intensity of the C=N band at 1655 cm^{-1} falls by 10%, that of the C=C band at 1580 cm^{-1} falls by 25%, etc. The observed changes can be explained by the occurrence of photochemical reactions such as the dimerisation of thymine and the deamination of the cytosine dimer with its conversion into the uracil dimer[119]. The dimerisation reaction can be clearly followed in the infrared spectra of ultraviolet irradiated single-stranded DNA.[116]

A study of the denaturation of DNA by ultraviolet radiation led to the discovery of the phenomenon of the stepwise alteration of the rate constant for the decrease of viscosity as the concentration of the irradiated solution is altered[113]. It was suggested that the observed stepwise effect is due to the aggregation of DNA molecules in solution. The aggregation can be caused to some extent by the "cross-linking" of the macromolecules, including the dimerisation of thymine by ultraviolet light. Factors influencing aggregation (ionic strength, temperature) facilitated the manifestation of the "stepwise effect"; a decrease of ionic strength led to its complete disappearance. This is consistent with the data of Eigner and Doty[120] for the aggregation of molecules in solutions at different ionic strengths. The possibility of this behaviour follows also from the data of Doty and Glisin[121] concerning the dimerisation of the thymine in the DNA molecule in solutions at moderate ionic strengths and the decrease of the degree of dimerisation to a minimum at low ionic strengths. The stepwise effect was also shown in solutions at low ionic strengths to which metal salts (Ca^{2+} and Fe^{2+}), stabilising the DNA molecule, had been added.

Comparison of these results with data for the variation of τ_c of spin-labelled DNA solutions at different concentrations as a function of the irradiation dose shows that the minimum damage to DNA preparations occurs when the concentration is highest (900 μg ml^{-1}). This is probably a consequence of the maximum extent of aggregation of the macromolecules. Presumably the aggregation (it can also occur in a cage) "protects" the molecule from ultraviolet irradiation[115].

Since the changes in the DNA molecule observed under ultraviolet irradiation lead to a significant impairment of the vital processes in the cell, the question of seeking effective protective measures arises. Chemicals of various classes—phenols, pyrimidines, and mercapto-compounds—have been investigated[112] as protective agents. Propyl gallate, 3-pyridinol, hydroquinone, and thymine afforded 40–60% protection, while cysteine and glutathione afforded 15–17% protection. Estimation of the degree of absorption of ultraviolet light by the test compounds established that the main mechanism of the protective effect involves the screening of the DNA molecule. A similar protective mechanism was observed when glutathione, cysteine, and tryptophan were used in ultraviolet irradiation of cells[122].

The finding that the protective effect of radical-reaction inhibitors involves screening in such cases constitutes at the present time indirect evidence for the absence from

the system of appreciable concentrations of free-radical states. This is consistent with data obtained in studies[123,124], which demonstrated instantaneous recombination of the radicals formed on ultraviolet irradiation of aqueous DNA solutions at room temperature. The absence of stable radicals from ultraviolet irradiated DNA solutions was demonstrated by ESR[125]. The dominant role of the denaturation effects in DNA under the conditions of ultraviolet inactivation of bacteriophages has also been established[126].

Thus the use of physicochemical methods led to the discovery of structural and chemical changes in ultraviolet-irradiated DNA, which may be diminished by the introduction of various chemicals absorbing ultraviolet light.

IV. SPECIFIC FEATURES OF THE EFFECT OF ULTRASOUND ON DNA

Ultrasonic energy is used in making DNA preparations with various molecular weights for the investigation of the physicochemical and biological properties of DNA[127-130]. For this reason, the problem arises of the mechanisms of the depolymerisation of the DNA molecule and of the elucidation of the relative susceptibility to damage of its structural components.

It has been shown[131-133] that ultrasonic degradation of DNA, like that of other polymers, is mechanical. The existing ideas about the action of ultrasound on macromolecules indicate a dominant role of mechanical forces in the depolymerisation effect (shockwaves, hydrodynamic shear). The chemical changes in DNA are caused by the specific interactions of the molecule with activated dissociation products of water molecules (free radicals and ions) under cavitation condition[131]. Since the damage to the DNA structure by ultrasound can have consequences at the cellular level, the degradative changes in DNA were studied. The types of mechanical degradation of its strands and the initial chemical reactions of the carbohydrate–phosphate groups and the nitrogenous bases were investigated[134-136].

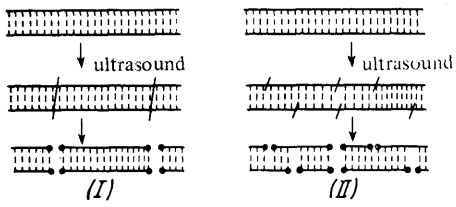


Figure 11. Schematic representation of various ways in which DNA is degraded by ultrasound.

Analysis of the physicochemical characteristics of single-stranded DNA[137,138] (sDNA) obtained from a native preparation (nDNA) subjected to ultrasound (UZG-250 generator, vibration frequency 750 kHz, intensity on the emitting surface 8–10 W cm^{-2}, duration of treatment 10–50 s) established that the DNA can be degraded either by the simultaneous rupture of both its strands (Fig. 11, scheme I) or by the independent accumulation of breaks in single strands (Fig. 9, scheme II)[131,136].

Ratios of the expected weight-average molecular weights of single-stranded DNA[139] and its intrinsic viscosities $[\eta]$ in 0.01 M Na^+ solution were calculated as a function of the postulated type of degradation of native NDA[135] from experimental values of F (the number of symmetrical breaks on depolymerisation of DNA) for s = 2 (where s is the maximum number of nucleotides between the breaks in the two DNA strands). The values of F were determined from the formula

$$F = \frac{M_0^{nDNA}}{M_t^{nDNA}} - 1 \quad .$$

The Table lists the experimental parameters of single-stranded DNA isolated from native DNA preparations exposed to ultrasound for given values of F and quantities calculated according to scheme I or scheme II. Evidently the experimental values agree with the calculated values for the degradation of DNA according to scheme I, i.e. as a result of the simultaneous rupture of both strands. There is no accumulation of random breaks in the DNA strands.

The molecular weights M and intrinsic viscosities of single-stranded DNA $\{[\eta]$ in 0.01 M $Na^+\}$ isolated from native DNA exposed to ultrasound and comparison with experiments of the values calculated in accordance with Schemes I and II.

Γ_{nDNA}	Scheme I		Scheme II		Experiment	
	$[\eta]$	$M\cdot10^{-6}$	$[\eta]$	$M\cdot10^{-6}$	$[\eta]$	$M\cdot10^{-6}$
0.28	57.3	2.29	5.6	0.22	58	2.32
0.45	53	2.12	4.46	0.18	52	2.08
0.77	46	1.85	3.48	0.14	46	1.6
2.03	31.6	1.27	2.2	0.09	23.5	0.94
3.75	22	0.88	1.6	0.06	13.7	0.55

Evidently DNA behaves as a single-stranded polymer in relation to the depolymerising action of ultrasound. The equation relating the rate of degradation of the polymer to the number of its strands is

$$\lg \frac{1-B}{B} = n \lg t + \text{const},$$

where

$$B = \frac{M_t^{nDNA}}{M_0^{nDNA}},$$

n is the number of strands in the polymer, and t the duration of action of the depolymerising factor. Fig. 12 shows that degradation by ultrasound does indeed obey the law characteristic of single-stranded polymers (with $n = 1$). Under these conditions, DNA having a molecular weight (M) of 1×10^7 is seen to decompose into fairly large molecules with M ranging from 0.3×10^6 to 1.2×10^6 (according to sedimentation analysis[129]). Ultrasound can thus be used to obtain native DNA fragments, which extends the scope of the application of ultrasound in biological experiments.

It is noteworthy that the observed depolymerisation of the macromolecules occurred under conditions of exposure to ultrasound where chemical processes associated with cavitation had not yet appeared. For longer exposures to ultrasound, leading to an increase of F, there is a slight discrepancy between the experimental data and the values calculated according to scheme I (see Table), which is probably due to the onset of chemoacoustic degradation of DNA. With increase of the duration of exposure to ultrasound from 1 to 15 min in the absence of protective substances, definite damage to individual chemical groups and bonds in the DNA molecule is observed. In particular, the intensities of a number of infrared absorption bands of DNA exposed to ultrasound change [136].

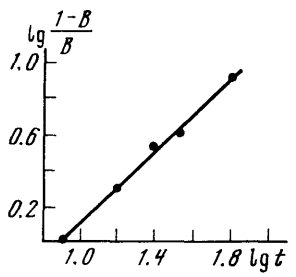


Figure 12. Kinetics of the depolymerisation of DNA by cavitation ultrasound.

In the region corresponding to the vibrations of the double bonds in the nitrogenous bases of DNA the intensity of the 1650 and 1590 cm^{-1} bands (due to the C=C and C=N bonds) diminishes, while in the region corresponding to the vibrations of the carbohydrate–phosphate chain the intensity of the 1010 cm^{-1} band [ν(COC) for deoxyribose] falls. Deoxyribose is then damaged to a greater extent than the nitrogenous bases: the decrease of the intensity of the 1010 cm^{-1} band is 17%, while that of the bands corresponding to the vibrations of the chromophoric groups of the nitrogenous bases is on average 10% (after exposure to ultrasound for 10 min). Deamination is observed only after more prolonged treatment with ultrasound.

The observed results show that the nature of the damage suffered by DNA on exposure to ultrasound differs from the effect of ionising radiation. As stated above, in the latter case the nitrogenous bases are deaminated together with the dissociation of their conjugated bonds; the bases are damaged to a greater extent. This difference is caused by the partial retention of the native DNA structure during ultrasonic degradation (caused by the simultaneous mechanical rupture of its two strands) and hence by the smaller accessibility to free radicals of the bases located within the helix. On the other hand, depolymerisation of DNA by ionising radiation entails independent accumulation of single breaks in each DNA strand [137,140], which must be accompanied by an appreciable damage to its secondary structure with a simultaneous damage to the rings of the bases.

In the literature on the infrared spectroscopy of DNA there is no unambiguous assignment of the 1060 and 960 cm^{-1} (970 cm^{-1} for a 92% relative humidity) bands to the vibrations of the individual C–O or P–O bonds of the carbohydrate–phosphate chains of DNA. A linear correlation has been observed between the relative decrease of the intensity of the 970 cm^{-1} band and the number of double breaks in DNA measured viscometrically. The correlation permits a tentative assignment of these bands to the vibrations of the C–O bond, since ultrasonic depolymerisation of DNA entails the preferential rupture of the C–O (90%) rather than P–O (10%) bonds [141]. The assignment of the 1060 cm^{-1} band to the vibrations of the C–O bonds appears to be less justified, since the intensity of this band does not decrease with increase of the duration of exposure to ultrasound.

A new band appears at 1190 cm^{-1} in the infrared spectra of DNA in the same wavenumber range. When DNA is ruptured, the number of terminal phosphate groups increases. The 1190 cm^{-1} band can be tentatively assigned to their vibrations, since absorption in the range 1190–1140 cm^{-1} is characteristic of alkyl phosphates. [64]

More far reaching changes in DNA under the influence of cavitation ultrasound led to successive decomposition of the pyrimidine and purine bases. Chromatographic analysis of the DNA hydrolysis mixtures exposed to ultrasound for periods ranging from 2 to 3.5 h demonstrated a greater resistance of the purine bases (adenine and guanine) to ultrasound compared with the pyrimidine bases (cytosine and thymine) [134]. This behaviour persists also in the susceptibility of the free bases and purine and pyrimidine derivatives to damage by ultrasound, which may have a definite biological significance.

The results obtained for the influence of ultrasound on the DNA molecule permit the employment of ultrasonic vibrations for specific and selective alterations in the DNA structure. The observed damage in DNA can have consequences at the cellular level, which has been confirmed, for example, by the formation on exposure of DNA to ultrasound of fragments with transforming activity [142] and by the results of studies on the influence of ultrasound on the chromosomal apparatus of cells and on genetic processes [143,144].

V. KINETICS AND MECHANISM OF THE REACTIONS OF AZIRIDINE DERIVATIVES AND NITROSOALKYLUREAS WITH DNA

More than 200 chemical mutagens have now been discovered. The great interest in compounds of this class is primarily due to the fact that chemical mutagens make it possible to obtain useful mutations in plants and microorganisms and have therefore found practical applications in the selection of agricultural crops and in the manufacture of antibiotics.

Since it has been shown that hereditary changes are mainly associated with nucleic acids (particularly with DNA), studies were begun to determine the possible mechanism of the action of chemical mutagens on DNA. A number of these established that mutations can arise both as a result of interaction with DNA (both in the state of replication and at rest) and as a result of reaction with its precursors. Under these conditions, alkylation of the bases and phosphate groups, replacement of one base by another, elimination of purines, etc. are possible.

The most effective mutagens proved to be substances with alkylating properties (derivatives of aziridine, *N*-nitroso-compounds, derivatives of mustard gas, epoxides, etc.). Studies *in vitro* and *in vivo* established a number of physicochemical characteristics of their action on nucleic acids. Some of the mutagens (aziridine derivatives,

mustard gas, etc.) cause changes in the macromolecule resembling the effect of radiation: a change in molecular weight, impairment of molecular shape, the formation of intramolecular and intermolecular "cross-links", etc.[145,146].

Since one of the principal problems in modern genetics concerns the possibility of achieving specific mutations, kinetic studies on the mechanism of the action of active mutagens assume particular importance. The effect of aziridine derivatives [2-aziridinylethyl methyl ketone (2-AEMK) and *N*-acetylaziridine (*N*-AA)] on DNA has been studied[147-152]. It has been shown that the effectiveness of these derivatives in terms of the intrinsic viscosity of DNA solutions increases in the sequence aziridine < 2-AEMK < *N*-AA.

The kinetics of the reaction and the physicochemical mechanism of the effect on DNA were studied in relation to *N*-AA. The kinetic curves for the variation of the viscosity of DNA solutions in the presence of *N*-AA are *S*-shaped and can be described by the equation for first-order autocatalytic reactions[151,147]:

$$\lg\frac{\eta_0-\eta_t}{\eta_t}=\lg\eta'+0.434\,\varphi t$$

$$\left(\text{when } \eta'\ll\eta_0,\ t>\frac{1}{0.434\,\varphi}\right),$$

where η_0 and η_t are the viscosities of the DNA solutions at the initial instant and at time t, lg η' characterises the size of the "seed" ensuring autocatalysis, and φ is the acceleration factor of the autocatalytic reaction. The changes in the kinetic curve show that the DNA molecule acted upon by *N*-AA undergoes not only conformational changes but also more far-reaching alterations with a change in the molecular weight.

The kinetic curve for the variation of the molecular weight of DNA can also be described in terms of the law governing first-order autocatalytic reactions, the value of φ being close to that for the variation of the intrinsic viscosity of the solution:

$\varphi = 0.31 \pm 0.02$ h^{-1} (from sedimentation data);
$\varphi = 0.28 \pm 0.02$ h^{-1} (from intrinsic viscosity data).

The changes in the DNA structure[147] discovered by electron microscopy are consistent with these observations. Only 2 h after the interaction of *N*-AA with DNA, the photomicrographs show modified DNA molecules (Fig. 13). The thickened regions formed throughout the extent of the molecule and at its ends lead to the conclusion that *N*-AA acting for a short period probably causes partial denaturation of DNA. At this instant the changes in the viscosity and molecular weight are very insignificant. After 12 h, low-molecular-weight fragments appear. By this time the molecular weight of DNA has fallen sharply. After 25 h, the photomicrographs show a large number of broken segments and coils resembling those observed after exposure to large radiation doses.

The action of 2-AEMK resembles that of *N*-AA. In this case too there is denaturation and depolymerisation of the molecules, but the reaction is slower.

The effect of aziridine (0.023 M) on DNA (1000 μg ml^{-1}) is somewhat different. After exposure to this substance for 2 min, thickened regions appear along the DNA molecule and at its ends, indicating partial denaturation. However, after 5 and 22 h the photomicrographs show, together with individual low-molecular-weight fragments, also large aggregates. A detailed study of the effect of brief exposure of DNA (50 μg ml^{-1}) to aziridine (10^{-4} M) was made using tungstophosphoric acid (TPA) as an electron-dense label which readily forms a salt-like linkage with the basic group of aziridine[150]. It was found that, after brief treatment with the mutagen (10 min), the density of the DNA filaments is uniform throughout the length of the molecule. Such uniformity of density is probably due to the fact that aziridine interacts with the phosphate groups of DNA. This is consistent with the literature data, showing that many alkylating agents can esterify the phosphate groups after acting on DNA for a short time.

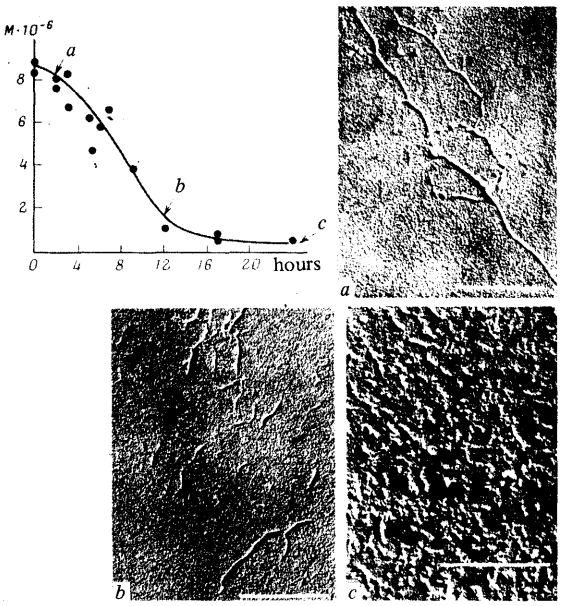


Figure 13. Photomicrographs of DNA and the kinetic curve for the variation of the molecular weight of DNA on interaction with *N*-AA; time (hours): *a*) 2.5; *b*) 12; *c*) 25.

A study of the effects of various concentrations of *N*-AA (0.012 M) on DNA[147] established that the change in the viscosity of DNA solutions remains autocatalytic in all cases. For the *N*-AA concentrations investigated, lg η' = 1.84 and $\varphi = 4.8\times10^{-2} - 2.3\times10^{-1}$ h^{-1}. The agreement of the values of lg η' for all the mutagen concentrations suggests that even at the initial instant the DNA–*N*-AA system contains the same amount of seed in all cases. Since the same DNA preparation was used in all the experiments, denatured DNA molecules, always present at the initial instant of the reaction, can evidently play the role of the seed. This is particularly likely, since denatured molecules are known to be more sensitive to various types of influences than the native molecules. The validity of this hypothesis was demonstrated in separate experiments using denatured DNA molecules[151].

DNA molecules denatured to different extents by ultraviolet light were added to the DNA-*N*-AA reaction mixture and the reaction rate was measured. It was shown that, with increase of the denaturation of DNA molecules, the induction period of the reaction fell, i.e. lg η' increased. Consequently the role of the denatured DNA molecules does indeed reduce to an increase of the amount of seed required to ensure the reaction between DNA and the mutagen.

Under the conditions of our experiments, the reaction of *N*-AA with DNA proceeds for a long time, in the course of which *N*-AA itself undergoes significant changes. It has been shown that the mutagen reaction products also play a definite role in the reaction. This is indicated by the "after effect" observed after removing the mutagen (Fig. 14). Thermochemical analysis of the transformation of *N*-AA made it possible to observe the conditions required for the formation of the maximum amount of the intermediate, the addition of which accelerated the process [149].

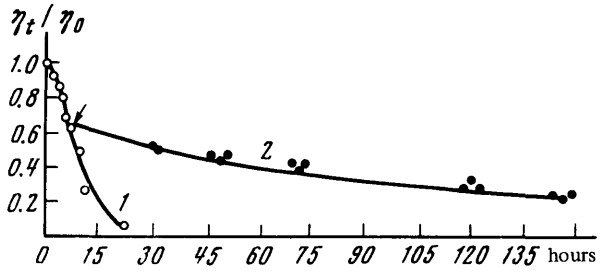


Figure 14. Variation of the intrinsic viscosity of DNA solutions acted upon by *N*-AA (curve *1*) and after the removal of *N*-AA (curve *2*) ("aftereffect"). The arrow indicates the time at which DNA was precipitated with alcohol.

The reaction of DNA with *N*-AA is accompanied by chemiluminescence [153], which appears 40 min after the start of the reaction and reaches a maximum in the course of 3 h with a subsequent slight decrease of intensity. The chemiluminescence spectrum consists of a broad band in the range 400–630 nm with a maximum at 490 nm. Kinetic chemiluminescence curves are *S*-shaped but are satisfactorily transformed into straight lines when lg $[I/(I_{max} - I)]$ is plotted. The chemiluminescence is due to oxidative processes, because its intensity falls sharply in the presence of O_2 and decreases in a stream of argon (Fig. 15). Since the change in the viscosity of DNA solutions containing the mutagen also occurs only in the presence of oxygen, the structural changes in DNA as well as chemiluminescence are probably a consequence of oxidative processes in the DNA–mutagen system.

In the reactions of the individual bases with *N*-AA there is no chemiluminescence, while in the reactions of nucleosides, nucleotides, and deoxyribose there is an appreciable chemiluminescence [154]. This shows that the reactions of 2-deoxy-D-ribose are responsible for the chemiluminescence. The latter probably arises as a result of the action of *N*-AA or its reaction products on the glycosidic carbon atom of ribose with subsequent oxidation at this atom. The stability of the glycosidic linkage is known to be influenced by the esterification of the hydroxy-groups and also by the nature of the base to which the carbon is bound: purine nucleotides are less stable than pyrimidine nucleotides and for this reason the chemiluminescence intensity in the reactions with purine nucleotides and nucleosides is higher than in the reactions with pyrimidine nucleotides. When deoxyribose is oxidised, the carbohydrate–phosphate linkages become labile and hydrolyse with rupture of the main macromolecular chain of DNA.

Radical-reaction inhibitors (propyl gallate, hydroquinone and 3-hydroxypyridine derivatives) reduce the chemiluminescence intensity to a greater extent the higher the antioxidant efficiency of the inhibitor [153].

Smotryaeva, Serebryanyi, and coworkers [155–158] investigated the mechanism of the reaction of DNA with *N*-alkyl-*N*-nitrosoureas (ANU). The interest in compounds of this class is not fortuitous, since, while behaving as supermutagens, they simultaneously exhibit both antitumour [159]

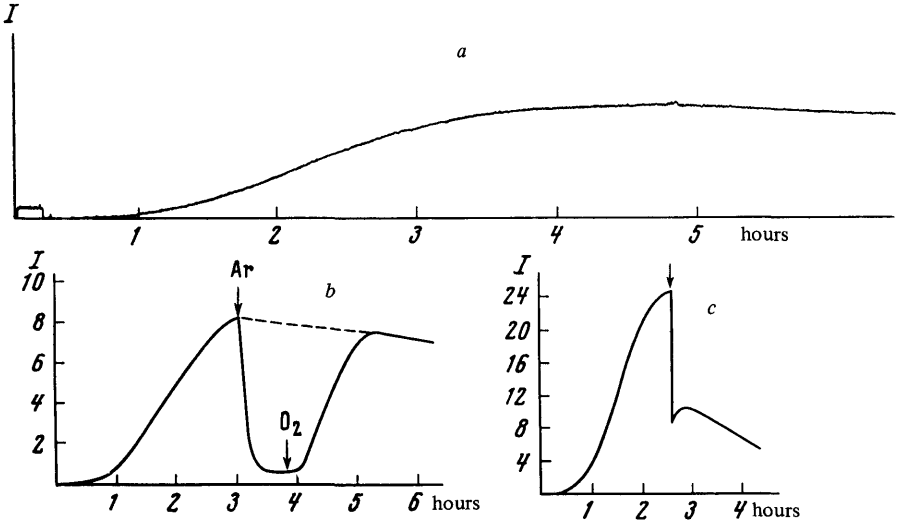


Figure 15. Chemiluminescence in the reaction of DNA with *N*-AA: *a*) kinetic chemiluminescence curve; *b*) variation of chemiluminescence intensity after the addition of argon and oxygen to the reaction mixture; *c*) variation of chemiluminescence intensity after the addition of propyl gallate at the instant corresponding to its maximum.

and carcinogenic[160] and mutagenic properties[161]. The reactions of *N*-alkyl-*N*-nitrosoureas with the genetic apparatus of the cell (in particular with the DNA molecule and the deoxynucleoprotein complex) can evidently play a significant role in such biochemical activity of the mutagens. One of the main physicochemical effects arising in the DNA structure as a result of its reaction with ANU is expressed by a sharp decrease of the viscosity of the solution and the occurrence of single and double breaks[156].

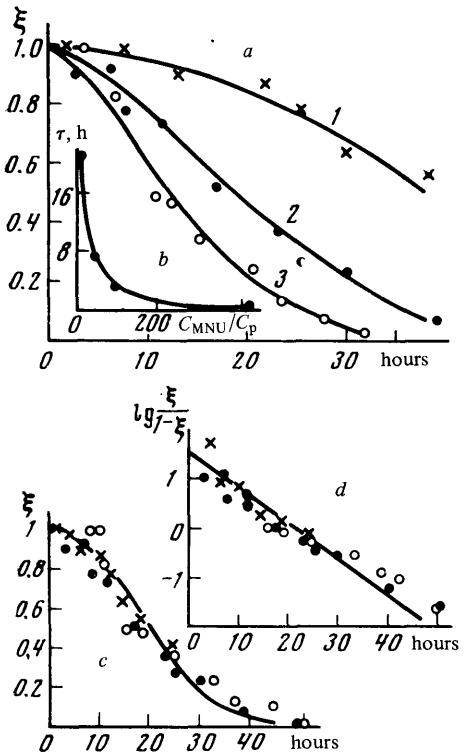


Figure 16. Kinetic curves for the variation of the intrinsic viscosities of DNA solutions (624 μg ml^{-1}) acted upon by MNU at different concentrations ($\xi = \eta_t/\eta_0$): *a*) [1) 0.02 M; 2) 0.1 M; 3) 0.2 M]; *b*) variation of the reaction induction period τ for different ratios of the amounts of MNU and DNA employed; *c*) transformation of the kinetic curves in *a* into curve *2*; *d*) semilogarithmic form of curve *c*.

The kinetic curves for the variation of the viscosity of DNA solutions in the course of reaction with *N*-methyl-*N*-nitrosourea (MNU) are *S*-shaped (Fig. 16), but give rise to straight lines when lg $[\xi/(1-\xi)]$ is plotted against time, in accordance with the equation for first-order autocatalytic reactions:

$$\lg\frac{\xi}{1-\xi} = \lg\xi' + 0.434kt, \quad t \neq 0,$$

where $\xi = \eta_t/\eta_0$ is a dimensionless coordinate characterising the viscosity of the DNA solution and lg ξ' and k are parameters of the kinetic curves. The kinetic curves for different concentrations can be transformed into a single curve with $k = 0.13$ h^{-1}.

The induction period of the reaction increases with decrease of the [MNU]:[DNA] ratio. In the course of the induction period chemical changes probably occur in DNA, which accumulate and are then manifested by single and double breaks in the DNA chains and by a decrease of the viscosity of the solutions (Fig. 17). This is also indicated by the experimentally observed aftereffect following the removal of the mutagen[155].

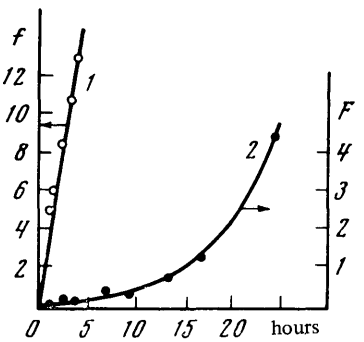


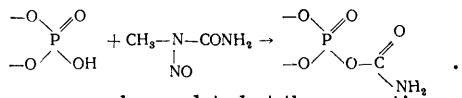
Figure 17. Damage to the DNA structure in the reaction with MNU: 1) accumulation of single breaks (*f*) in DNA molecules; 2) accumulation of double breaks (*F*).

A detailed study of the inactivating effect of MNU on different phages (T7, ΦX-174) showed that the reaction with DNA, in the first place alkylation of the DNA bases with subsequent elimination of purines from the alkylated fragments of DNA and degradation, may be responsible for the inactivation. A kinetic model has been proposed for the inactivation of phages, whereby it is possible to estimate quantitatively the influence of MNU on various phages[162].

More complex effects were observed in a study of the effect of MNU on deoxynucleoprotein[156]. It was shown that the strength of the DNA–protein linkage changes in the course of the reaction together with an integral property of the deoxynucleoprotein, namely its insolubility in a medium with the physiological ionic strength. The principal cause of the observed effect is chemical modification of the DNA and protein groups involved in the DNA–protein interaction and ensuring the integrity of the deoxynucleoprotein.

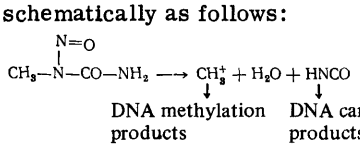
Analysis of the chemical changes in DNA and deoxynucleoprotein showed that the main chemical reaction giving rise to the degradation of DNA is again the alkylation reaction[157,163–165]. However, many biological data indicate a significant difference between the mechanism of the action of ANU and that of the action of other alkylating agents. To account for these differences, Serebryanyi[166] suggested that the ANU are agents with a complex action and their biological effect is due to the overall influence of two chemical reactions: methylation and carbamoylation. The use of MNU labelled with ^{14}CO in the reaction with DNA and deoxynucleoprotein established that there is indeed a transfer of the label from MNU to DNA and the protein, i.e. the carbamoylation reaction takes place[156,158]. The carbamoylation pathways were shown to occur in a study of the reaction of MNU with nucleotides, homopolynucleotides, and RNA.[167–169]. It was established that

mainly the phosphate groups are carbamoylated in polynucleotides:



The bases are carbamoylated at the same time, the main product of this reaction being N^4-carbamoylcytidine. Nucleotides can be arranged in the following sequence in terms of their reactivity in the carbamoylation reaction: cytidine ≫ adenosine ≃ guanosine, while in the alkylation reaction guanosine is the most reactive[168]. When MNU acts on the nucleotide complex, not only DNA but also the protein is methylated and carbamoylated[156]. The overall yields of both reactions are approximately the same; whereas both components of deoxynucleoprotein are methylated to an equal extent, mainly the protein is carbamoylated. Carbamoylation makes a significant contribution to the overall mutagenic activity of MNU, since carbamoylating agents such as isocyanic acid and its salts have been shown to possess mutagenic activity[170-172]. Later isocyanates were also found to have carcinolytic properties[173].

Thus the mechanism of action of MNU on DNA can be represented schematically as follows:



In connection with the antitumour properties of ANU, a study was made of the biochemical shifts arising after their administration *in vivo*. It was established that the synthesis of DNA, RNA, and protein is inhibited after the administration of ANU.[174-177]. The synthesis of DNA proved to be the most sensitive, that of RNA being inhibited to a lesser extent. In a study of the causes of the inhibition of RNA synthesis in a cell-free system it was shown that it involves both damage to the DNA matrix and a change in the properties of RNA polymerase due to the chemical modification of the enzyme. It is interesting that potassium cyanate, a product of the decomposition MNU, is also able to inactivate RNA polymerase[178].

The primary damage caused by MNU *in vivo* as well as *in vitro* arises as a result of methylation and carbamoylation reactions[179-181].

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