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Modification of DNA by Alkylating Compounds

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The results of studies of the physicochemical properties of alkylating compounds—alkylnitrosoureas (ANU)—with a broad spectrum of biological activities (mutagenic, carcinogenic, and antitumour) as a result of the alkylation and carbamoylation of DNA as well as other cell components are presented. The identified chemical products of the interaction of ANU with DNA and its components are listed. The structural transformations of the DNA macromolecules as a consequence of their chemical modification are examined and the possible biological consequences of the modification of DNA by ANU are discussed. The bibliography includes 148 references.

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I. INTRODUCTION

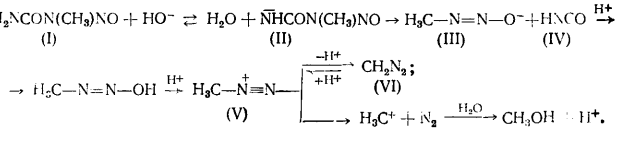
Alkylating compounds with a broad spectrum of biological activities have long attracted the attention of investigators. The knowledge of the mechanisms of the action of these compounds on the most important cell biopolymers will make possible their specific employment to achieve the desired effect. Among alkylating compounds, alkylnitrosoureas (ANU), which have been investigaged vigorously in recent years as chemical mutagens,[1-10] carcinogens,[11-17] and antitumour agents,[18-21] are of great interest.

Academician N.M.Emanuel' and his students have widely used for many years physicochemical and chemical kinetic methods in the study of many most important biological systems,[22-26] biochemical reactions,[27-29] and various pathological processes.[30-33] Their discovery of the antitumour properties of ANU and the introduction into clinical practice of *N*-methyl-*N*-nitrosourea (MNU) as a drug for the treatment of the undifferentiated cancer of the lung and lymphogranulomatosis[18] stimulated in recent years the wide-scale synthesis of new ANU derivatives and the study of the physicochemical properties and the molecular mechanism of the action of these compounds in order to discover ANU most effective in practical use.

One of the main (and perhaps most important) causes of the biological action of ANU may be reactions involving the interference with the functioning of the cell genome as a result of the modification of DNA due to the alkylation and carbamoylation of its macromolecule.

II. CHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF ALKYLNITROSOUREAS

Certain reactions of ANU can be analysed in relation to one of the representatives of compounds of this class which have been investigated, namely MNU. It has been established that the ANU are readily hydrolysed in aqueous solutions and that the rate of hydrolysis changes significantly as a function of the composition of the medium, pH, temperature, and other factors. A general scheme for the actions of MNU in aqueous solution can be represented in the following manner:[34-41]



Under the influence of hydroxide ions, the proton is abstracted from the nitrogen atom of the MNU molecule (I) with formation of a urea anion (II), which is converted into the diazotate anion (III) and isocyanic acid (IV).

The first stage of the decomposition of MNU is in this instance rate-limiting and is of pseudofirst order. This serves as the basis for the method involving the titration of MNU with alkali,[37] which is used together with other chemical methods for the investigation of the kinetics of this reaction.[38,39] The anion (III) formed is converted into the methanediazonium cation (V) under the influence of protons. In a highly alkaline medium, diazomethane (VI), which is an alkylating agent, is formed.[40]

In a neutral medium, diazoalkanes are usually not formed and the methylating properties are in this instance due to the methanediazonium cation,[41] whose addition to nucleophiles is accompanied by the evolution of nitrogen. In an aqueous medium hydrolysis takes place with formation of methanol:

$$H_3C\overset{+}{N}{\equiv}N + H_2O \rightarrow CH_3OH + N_2 + H^+.$$

The rate of reaction of MNU in aqueous solution depends particularly on the pH of the solution and it has been established that in a Britton–Robinson buffer the half-life of MNU diminishes with increasing pH. The composition of the medium influences the reactions of MNU in aqueous solutions. In the presence of buffer mixtures (phosphate and phosphate-citrate buffers) an increase in the rate of reaction of MNU owing to the action of nucleophiles is observed.[42]

In an acid medium one of the products of the reaction of MNU is nitrous acid,[38,39] which is known to be capable of functioning

as a deaminating agent:[43]

$$\underset{\mathrm{CH_3}}{\mathrm{H_2NCONNO}} + \mathrm{H_2O} \xrightarrow{\mathrm{H^+}} \mathrm{HNO_2} + \underset{\mathrm{CH_3}}{\mathrm{H_2NCONH}}.$$

Thus, depending on the conditions, the reactions of MNU in various media can give rise to products of several types: diazomethane (pH > 7), and methanediazonium cation and isocyanic acid (pH ~ 7), and nitrous acid (pH < 7). In the reactions with nucleic acids and their components, these products can exhibit their characteristic properties and can modify the biomacromolecules.

Analysis of the chemical nature and electronic structure of MNU established that this compound possesses donor–acceptor properties, which may be responsible for the varied manifestations of the biological activity of MNU.

It has been demonstrated by quantum-chemical and EPR methods that the NO group in the MNU molecule can behave as an electron acceptor or donor.[44–46] When an electron is captured by the NO group, the following reactions can take place:

$$\mathrm{H_2NCON(CH_3)NO} + \bar{e} \rightarrow \mathrm{H_2NCON(CH_3)N\dot{O}} \rightarrow$$
$$\rightarrow \mathrm{H_2NCONH\dot{C}H_2} + \bar{N}O \rightarrow \mathrm{CH_2NH} + \dot{C}ONH_2 + \bar{N}O.$$

The radical $\dot{C}ONH_2$ can behave as a carbamoylating agent.

The electron-donating properties of MNU are due to the abstraction of an electron from the nitrogen atom of the NO group with formation of a radical-cation whose decomposition can apparently give rise to the following products:

$$\mathrm{H_2NCON(CH_3)NO} \xrightarrow{-\bar{e}} \mathrm{CH_3{-}N{=}N{-}O^{\cdot}} + [\mathrm{CONH_2}]^+ \rightarrow$$
$$\rightarrow \mathrm{CH_3{-}N{=}N{-}O^{\cdot}} + \mathrm{H^+} + \mathrm{HNCO} \rightarrow \mathrm{CH_3^+} + \mathrm{N_2} + {}^{\cdot}\mathrm{OH} + \mathrm{HNCO}.$$

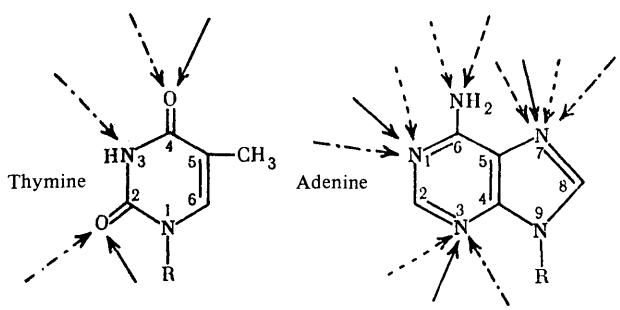
In this case the chemical reactivity of MNU is determined mainly by the radical $CH_3N = NO^{\cdot}$, whose interaction with biomacromolecules can lead to methylation. Carbamoylation takes place due to HNCO.

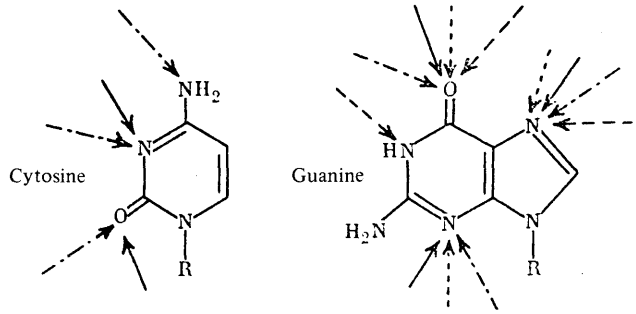
The radicals $\dot{C}ONH_2$, $CH_3{-}N = N\dot{O}$ and $H_2NCON(H)\dot{C}H_2$ have been detected by EPR in a frozen NMU solution under the conditions of the photosensitised electron transfer in the presence of an electron acceptor (donor).[45]

III. CHEMICAL PRODUCTS OF THE REACTIONS OF ALKYLNITROSOUREAS WITH DNA AND ITS COMPONENTS

In order to elucidate the molecular mechanism of the biological action of ANU, it is important to determine the principal sections of the biomacromolecules which may be modified by treatment with ANU. The first studies of the mechanism of the action of ANU derivatives were undertaken to investigate their alkylating properties.

The objects of study were nucleosides,[40,47] nucleotides,[48,49] polynucleotides,[50] and nucleic acids.[7] The observed sites where nucleic acid bases are alkylated can be represented schematically in the following manner (R represents the ribose or doxyribose residue):





The positions in which the bases are alkylated are indicated by arrows:
In nucleosides – – →
In nucleotides (polynucleotides) - - - →
In single-stranded DNA ——→
In double-stranded DNA –·–·→

The alkylation of triphosphate groups with formation of the monoethyl ATP ester (11% of the total amount of alkyl groups) has been demonstrated in model experiments with ATP and ethylnitrosourea (ENU).[49]

The possibility of alkylating DNA was first established in relation to the formation of 7-methylguanine in the reaction of DNA with MNU.[51] Later nine methylated derivatives were identified in an *in vitro* reaction of DNA with MNU: 1-methyladenosine, 3-methyladenine, 7-methyladenine, 3-methylguanine, 6-*O*-methyldeoxyguanosine, 7-methylguanine, 3-methyldeoxycytidine, 3-methylthymidine, and 4-*O*-methylthymidine. 3-Methyladenine (11.2% ^{14}C) and 7-methylguanine (75.7% ^{14}C) are formed in highest yields, while 4-*O*-methylthymidine (0.1% ^{14}C) is formed in the lowest yield. The amount of alkylated phosphate groups was 18% of the total radioactivity corresponding to DNA.[52,53]

It is noteworthy that, in those cases where MNU reacts with polynucleotides without a guanine residue, the contribution of the pathway involving the alkylation of phosphate groups increases sharply. For example, according to Shooter and Venitt,[54] the corresponding yield for the MNU-modified DNA is 18%, while according to other data, on treatment of polyadenylic acid with [$^{14}CH_3$]MNU, 23% of the yield is due to the alkylation of the bases and the remaining 77% is associated with the formation of phosphotriesters. The difference between the yields of the alkylated phosphate groups is frequently explained by the different experimental conditions and also by the instability of the products in alkaline media.[56]

The methyl and ethyl phosphotriesters are formed in DNA on treatment with the majority of alkylating carcinogens and are retained in it for a long time.[54,57] A dinucleotide containing alkylated phosphate groups has been isolated in the hydrolysis of DNA modified with MNU.[57]

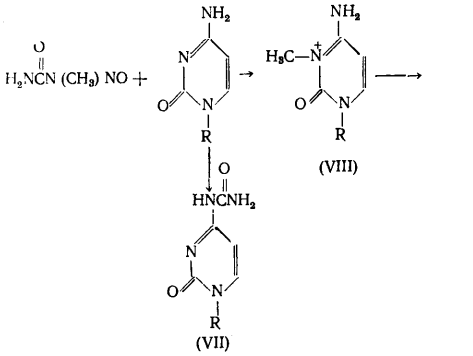
Comparative study of the reactivities of different alkylating compounds, namely dimethyl sulphate, diethyl sulphate, ENU, and MNU, in relation to the phosphodiester linkage in DNA established that the efficiency of the interaction was lowest for dimethyl sulphate and highest for MNU. This can apparently be explained by the fact that in the case of MNU and ENU the reaction intermediate is the alkanediazonium cation, which interacts electrostatically with DNA.[58]

The possibility of the carbamoylation of the DNA molecule in the reaction with MNU was first demonstrated[59] with the aid of tracer atoms. A DNA solution was treated for 72 h with MNU labelled at the carbonyl carbon atom with subsequent removal of the excess MNU (the ratio of the MNU and DNA phosphorus concentrations was 100 : 1).

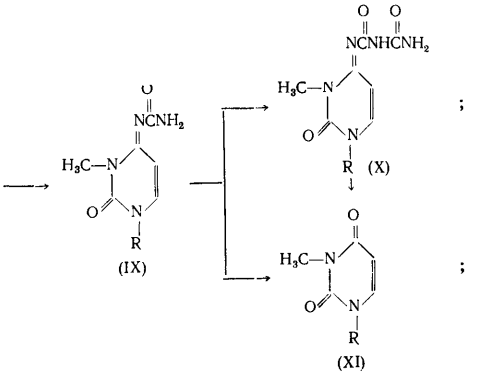
The hydrolysis of the modified [^{14}C]DNA to a depurinated acid with subsequent separation of the reaction products on ECTEOLA-cellulose demonstrated the presence of the label

in the depurinated fragment. It was suggested that the label is located in the phosphate fragment of DNA and (or) in cytosine.

Parallel experiments with nucleosides, polynucleotides, RNA, and DNA [60-67] confirmed the possibility of the carbamoylation of the NH_2 group of cytosine. The overall scheme for the reaction of MNU with cytidine[60,61] can be represented as follows:



(VII)

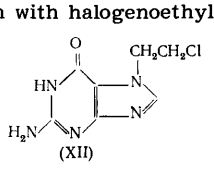


(IX) (X) (XI)

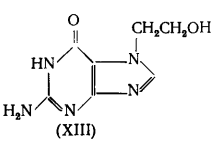
where R = ribose residue, (VII) = 4-*N*-carbamoylcytidine, (VIII) = 3-methylcytidine, (IX) = 3-methyl-4-*N*-carbamoylcytidine, (X) = 3-methyl-4-*N*-dicarbamoylcytidine, and (XI) = 3-methyluridine. Evidently the reaction of cytidine with MNU proceeds via two pathways—either via only carbamoylation to form compound (VII) or via alkylation with subsequent carbamoylation to form compounds (VIII), (IX), and (X). The carbamoylation products are readily hydrolysed with formation of compound (XI). On reaction with MNU, adenosine and guanosine give rise to carbamoylation products: 6-*N*-carbamoyladenosine, 1-methyl-6-*N*-carbamoyladenosine, and 2-*N*-carbamoylguanosine.[62]

The chloroalkyl derivatives of nitrosoureas have also been widely investigated.[68-71] Like MNU, these compounds decompose with formation of diazohydroxides, which alkylate the bases and phosphate groups of nucleic acid, and also isocyanates, which carbamoylate proteins, enzymes, and lipids.[72] Products of the interaction of ANU with nucleosides and DNA bases such as 7-chloroethylguanine (XII), 7-hydroxyethylguanine (XIII), 1,2-diguanylethane (XIV), 3-chloroethylcytosine (XV), and others have been identified.[73-76]

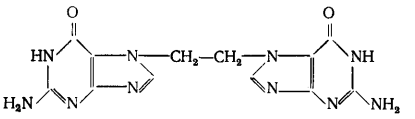
It has been established that the formation of DNA–protein and DNA–DNA cross-links, which have a significant influence on the cell metabolism, is possible in the reactions of chromatin with halogenoethylnitrosoureas:[71,76]



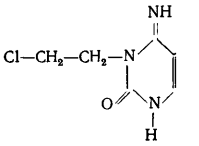
(XII)



(XIII)



(XIV)



(XV)

IV. STRUCTURAL CHANGES IN DNA MODIFIED WITH ALKYLNITROSOUREAS

The chemical modification of DNA (alkylation, carbamoylation, etc.) can lead to significant changes in the DNA structure. Some of the damage can be made good by the operation of enzymes in the repair system. The irreparable structural defects alter the normal functioning of the cell genome.

The structural changes in the DNA molecule (single- and double-strand breaks, etc.) have been observed *in vitro* and *in vivo* by many investigators when DNA was treated with the usual alkylating compounds.[77-79] Alkylation of the bases can lead to the degradation of the DNA chains as a result of the removal of the purine, which leads to the rupture of the sugar–phosphate linkages in the molecule as a result of consecutive transformations in the DNA chain.[80-82] The alkylated phosphate groups of DNA (phosphotriesters) are formed on treatment with the majority of alkylating compounds. They are unstable in an alkaline medium, but their thermal stability is fairly high.[83]

In order to detect the chemical modification of DNA, one may use infrared (IR) spectroscopy, the application of which to nucleic acids and their components has been widely discussed in reviews and monographs.[84-87] It has been established that the IR spectra of films of DNA modified with MNU reveal a change in the intensities of a number of bands, although, overall, the form of the spectrum is altered only slightly.[30,88] Fig.1 illustrates the change in the intensities of a number of IR bands of DNA as a function of the time of its interaction with MNU. Evidently, the light absorption by a number of groups and bonds in DNA (with amino-groups and conjugated double bonds of the bases as well as the sugar–phosphate linkages) diminishes during the reaction, which shows that, in the course of the first 3 h of the reaction of MNU with DNA, there is a possibility of deamination, and the rupture of the double bonds of the bases and of the DNA chains, most probably as a consequence of the methylation and carbamoylation of the bases.

Combination of various methods of physicochemical and biochemical analysis[88-95] made it possible to identify and estimate quantitatively the principal types of the structural defects in DNA occurring under the influence of MNU. The numerical parameters defining the overall damage to the macromolecular structure [determined by the viscometric method after the thermal denaturation of DNA (10.4 ± 1.0) and by the kinetic formaldehyde method (the KF method)[88] (11.0 ± 1.0)] agree well with one another. The application of the methods involving neutral and alkaline electrophoresis in agarose gels and the enzymic treatment of the modified DNA with endonuclease S_1,[92,96] which is specific to the single-stranded sections of DNA,[93,94] made it possible to determine the ratios of the numbers of double-strand breaks (DB), single-strand breaks (SB), the alkali-labile sections (ALS), and the thermolabile sections (TLS).[89]

Data obtained by pulse polarography are consistent with the above finding.[91] It has been shown that a much greater number of SB are formed in DNA treated with MNU after thermal denaturation than after brief treatment with alkali.

The thermolabile sections apparently contain alkylated nucleotides, whose glycosidic and phosphoester linkages are weakened on raising the temperature.[97]

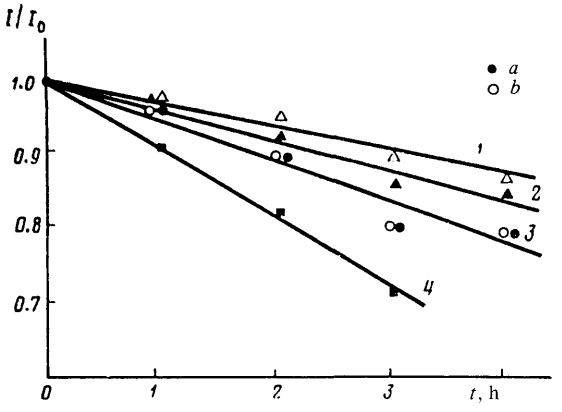


Figure 1. Dependence of the ratios of the intensities of a number of infrared bands of the modified DNA to the intensities of the infrared bands of native DNA on the duration of the interaction between DNA and MNU: 1) 960 cm^{-1} (vibrations of the sugar–phosphate linkages); 2) 1605 cm^{-1} (vibrations of the amino-groups of the bases); 3) 3350 cm^{-1} (*a*) and 3200 cm^{-1} (*b*) (vibrations of the amino-groups of the bases); 4) 1580 cm^{-1} (vibrations of the conjugated double bonds of the bases).[30]

The overall damage to the hydrogen bonds in the modified DNA can be estimated by two independent methods: from the decrease of the "melting" point on DNA T_m (the temperature of the transition of DNA from the helical to the disordered structure corresponding to the point of inflection on the curve relating the optical densities of the DNA solutions to temperature) and from the amount of the acid-soluble fragments (ASF) leached by nuclease S_1 from the modified DNA.[90]

Fig. 2*a* presents curves relating the optical densities of the DNA solutions to temperature for the control DNA (curve *1*) and after treatment with MNU for 4 h (curve *2*) and 16 h (curve 3). Evidently T_m decreases during the reaction of DNA with MNU, which indicates an increase of the number of damaged hydrogen bonds in the modified DNA. This is also indicated by the increase of the amount ASF (Fig. 2*b*, curve *1*) in the reaction of DNA with MNU. The parallel increase of the relative amount of ASF with increasing difference between the T_m for DNA before and after its interaction with MNU (δT_m) (Fig. 2*b*, curve *2*) indicates a correspondence between the overall amount of sections with damaged pairing of the bases (which are converted by nuclease S_1 into the acid-soluble fraction) and the decrease of T_m, the quantitative relation (a decrease of T_m by 1° corresponds on average to 1% of damaged hydrogen bonds) being consistent with the data of Pritchard and Eichinger.[98]

It is noteworthy that the number of damaged hydrogen bonds is always greater than the number of alkylated bases and breaks produced. Thus, after 1 h of the reaction between MNU and DNA, the total damage to hydrogen bonds amounts to 2.5%,[90] while according to other data[53,99] the amount of alkylated DNA bases produced during the same period under identical conditions is 0.2–0.5%. Taking into account these data, the occurrence of the observed damage can be represented as follows.[100] Alkylation of different bases in the DNA molecule leads to the rupture of hydrogen bonds. The subsequent formation of depurinated sections also damages the hydrogen bonds. This leads to the formation of SB, which, as a result of statistical accumulation, can give rise to DB when they are close to one another. This pathway to the formation of SB can be accelerated by treating the modified DNA with alkali or by heating. According to Verly at al.,[95] 90 min heating at 100 °C is sufficient for the virtually complete removal of alkylated purines.

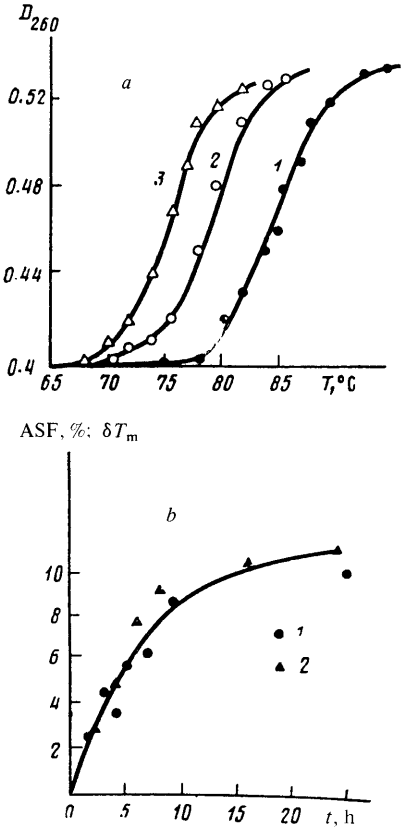


Figure 2. Melting curves relating the optical densities of the DNA solutions to temperature: 1) for native DNA; 2) after reaction with MNU for 4 h; 3) after reaction with MNU for 16 h; b) kinetic curves for the increase of the percentage content of the acid soluble fragments (ASF, curve *1*) and of the difference between the T_m of DNA before and after interaction with MNU (δT_m, curve *2*) in the reaction of DNA with MNU.[90]

The amount of sections where DNA has lost its helical structure as a result of alkylation (and other modifications) can be determined by the KF method,[101,102] which is based on the interaction of formaldehyde with the amino- and imino-groups of the DNA bases (not involved in the formation of hydrogen bonds) at temperatures below the "melting" point of DNA. This method has been used successfully to

observe the defects in the secondary DNA structure caused by the action of nucleases,[103] ionising radiation,[104] and thermal denaturation[105] and also to discover defects in the secondary structure of DNA in tumour cells.[106–108] The employment of the KF method for the analysis of the defects in the secondary structure of DNA produced in the reaction with MNU established that sections in which the DNA locally loses its helical structure are formed in the modified DNA and that their number agrees well with the number of SB determined by other methods.[89]

It has been established that the reaction of MNU with the tumour cell DNA is rapid[88,89] owing to the presence of sections in which the tumour cell DNA has locally lost its helical structure.[106–108]

V. BIOLOGICAL CONSEQUENCES OF THE CHEMICAL MODIFICATION OF DNA

The majority of the alkylated products detected in the reaction of DNA with MNU under *in vitro* conditions are also found under *in vivo* conditions.[109–111] As shown above, the main bulk of the alkylated products (up to 80% of the total amount) consists of 7-alkylguanine; adenine, guanine, cytosine, and thymine derivatives have also been detected. However, from the standpoint of mutagenesis and carcinogenesis, the greatest interest attaches to 6-*O*-alkylguanine and other oxygen-alkylated products.[110,112–114] In the course of the reactions of the alkylated products *in vitro* and *in vivo*, various intermediates can be produced, which alters significantly the mutagenic and carcinogenic activities of ANU and this must be taken into account when ANU are employed in experiments on living systems.[115,116] Mutations due to the alkylation of the DNA bases can arise as a result of erroneous base pairing. For example, when 6-*O*-alkylguanine is formed, the latter can pair with thymine (whereas under normal conditions thymine forms an A—T pair with adenine).[7,9,117–119]

As a result of the development of new methods for the identification of alkylated products, in recent years it has become possible to analyse the relation between the formation of these products and various manifestations of their biological properties.[120,121] A relation between the ability to alkylate guanine at the oxygen atom attached to the C(6) atom and the carcinogenic activity of the compounds has been established for many alkylating compounds;[122] however, the relation between the overall degree of alkylation and the carcinogenic activity was weak or almost altogether absent.[123] Alkylating agents [*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) and methyl methanesulphonate (MMS)] produce similar types of damage in DNA. When a combination of NG and MMS was used to investigate the different types of damage leading to mutations, transformation, and toxicity, it was shown that the frequency of mutations is correlated with the number of methylated adducts of 6-*O*-alkylguanines, but the cytotoxicity is not correlated either with the number of individual adducts or with the total level of alkylation of DNA (NG is more than 100 times more toxic than MMS).[124]

Comparison of the carcinogenic activities of the alkylating compounds (*N*-methyl-*N*-nitrosourethane, MNU, ENU, NG, MMS, etc.) in experiments on rodents with the mutagenic effects of these substances on bacteria established that the alkylating activity is fully correlated with the mutagenic effect but there is no correlation between each of these effects and the carcinogenic activity. A relation has been observed between the initial ratio of the 7-*N*- and 6-*O*-alkylguanines formed as a result of the reaction of the alkylating compound with DNA *in vitro* and its carcinogenic activity *in vivo*. This is evidence that the alkylation of guanine in the 6-*O*-position plays a key role in damaging DNA and is responsible for the experimentally observed carcinogenic activity of the alkylating compounds.[4,114]

The malignant transformation of cells depends on the duration of the existence of the modified base in DNA, which in the case of 6-*O*-methylguanine proved to be longest for brain cells, which are the most sensitive to MNU. On the other hand, its removal from the liver DNA is much faster and liver proved to be resistant to the malignancy-inducing activity of MNU.[125–127] Thus alkylation of DNA may be one of the principal reactions in carcinogenesis and in the mutagenic action of chemical compounds, which themselves (or their reaction products) can add to the nucleophilic centres of DNA.[128,129]

The carbamoylation reaction, which is characteristic of ANU, makes it possible in many instances to account for the high mutagenic and antitumour effects of such compounds by the formation of carbamoyl derivatives. Thus, mutations can occur due to the products of the carbamoylation of the bases by isocyanic acid, since it has been shown that when many plants are treated with its salt (potassium isocyanate), mutant forms are obtained.[130] However, one should note that a clear-cut correlation has not been observed between the degree of carbamoylation of nucleic acids and the antitumour activity of ANU, although such correlation has been traced between the alkylating activity (for the same degree of carbamoylation) and the antitumour activity.[131]

It has been shown that the carbamoylation reactions can significantly inhibit the cell repair processes. The enzymes involved in the repair and replication of DNA can be inactivated as a result of carbamoylation by the isocyanates arising during the reaction with ANU.[70,71,132]

The protein carbamoylation reaction has assumed a definite importance as one of the possible explanations of the antitumour properties of ANU.[133,134]

The manifestation of the biological activity of ANU can be influenced significantly by the reactions of the alkylated products involving damage to the structural integrity of DNA (SB, DB, ALS, and TLS) and of the carbamoylated products (modification of proteins) and also by the formation of DNA–protein and DNA–DNA cross-links which have been detected with mammalian cells were treated with chloro-derivatives of ANU.[135,136] Experiments with bacterial cells established a correlation between the number of DB induced in DNA by MNU and the survival rate of the cells.[137] The destruction of cells under the influence of ANU may be also caused by the formation of a considerable amount of ALS, as happens when cells are exposed to ionising radiation.[138]

In many instances a definite relation has been discovered *in vivo* between the induction of breaks in DNA and the carcinogenic activity for a specific class of substances. However, one should bear in mind that single-strand breaks in cell DNA can arise additionally owing to the action of excision reparation enzymes, which are capable, for example, of excising 3-alkyladenine and 6-*O*-methylguanine with formation of a depurinated section, which subsequently gives rise to SB.[131,139]

The alkylation and carbamoylation reactions, accompanied by a significant damage to the DNA structure, can alter the template properties of the latter which are responsible for the replication and transcription process.[140]

A very sensitive test of the template properties of the modified DNA may be provided by RNA polymerase systems of purified nuclei and with a soluble RNA polymerase.[141–143] The interference with the synthesis of RNA using as a template DNA modified with MNU has been investigated.[144,145] The template activity was determined from the amount of

[^{14}C]CMP in the RNA produced relative to the control (Fig.3, curve *1*). The control consisted of a system with untreated DNA. Evidently, even during the first hours of the reaction, the template activity diminishes exponentially. The extent of the disturbance of the template activity was greater the longer was the reaction between MNU and DNA. After 10 h, there is virtually no synthesis of RNA. Comparison of these results with the appearance of SB and DB in the DNA molecule under the influence of MNU (Fig.3, curves *2* and *3*) demonstrates a greater sensitivity of the RNA polymerase system to the damage sustained by the DNA structure than is shown by other methods. The cessation of template synthesis after 10 h indicates a significant damage to the DNA template, leading to the inhibition of transcription.

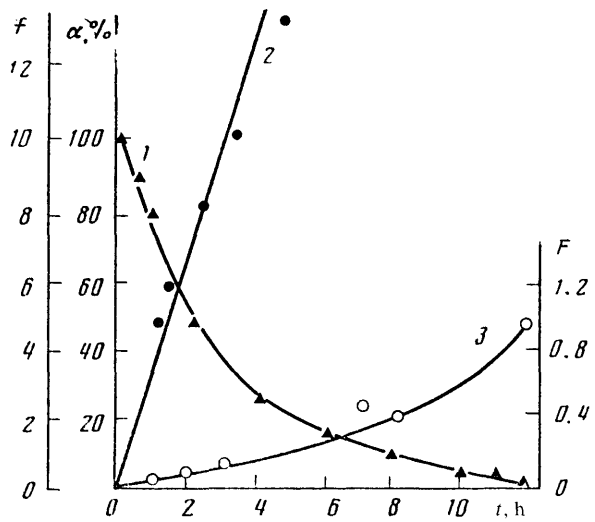


Figure 3. Variation of the template activity of DNA in the RNA polymerase system (curve *1*) {determined from the degree of incorporation α of [^{14}C]CMP in the RNA produced} and of the numbers of SB (*f*, curve *2*) and DB (*F*, curve *3*) as a function of the duration of the interaction of DNA with MNU.[143]

The change in the template properties of chromatin under the influence of MNU and the chloroethyl derivatives of nitrosoureas has been established[146] and in many instances the degree of inhibition of the template activity was found to be correlated with the antitumour effect of the ANU.

It has been established that ANU exhibit an antitumour activity with respect to malignant neoplasts of different origin and those induced by carcinogens in animals. However, the question of which of the DNA modification reactions is decisive in the manifestation of the antitumour activity of ANU is at present still obscure. It is assumed that the greater degree of inhibition of the DNA synthesis in tumour cells plays a significant role in this process owing to the decrease of the template activity of chromatin (as a result of alkylation and carbamoylation) and also the inhibition of the tumour cell reparation processes (resulting from the carbamoylation of the enzymes).[70] One cannot rule out the possibility that the selectivity of the action of ANU on tumour cells is based on their reactions with DNA whose structure differs from the DNA of the normal cells by the presence of sections in which the molecules have locally lost their helical structure,[106-108], which is responsible for the greater sensitivity of the tumour DNA to the action of chemical[100,147] and physical[148] agents.

Thus analysis of the possible ways of modifying DNA by alkylating compounds, particularly by ANU, makes it possible to relate the observed biological effects to different types of damage to the primary and secondary DNA structures and also to estimate the possibility of the specific synthesis and application of new biologically active preparations.

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