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# PHYSICAL, BIOCHEMICAL AND BIOPHYSICAL BASES FOR CREATION OF NEW EFFECTIVE ANTICANCER AGENTS

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**Abstract** - Main principles of screening of effective antitumor drugs grounded on the methods and concepts of biology, chemistry, physics, mathematics have been set up. Strictly quantitative criteria based on kinetic parameters of tumor growth were suggested for evaluation of therapeutic effect in experiment. A new classification of antitumor agents by their reactivity types have been suggested. Calculation of structures and selection of most effective agents have been concidered in terms of quantum chemistry method. Quantitative criteria were proposed for evaluation of effectiveness of cancer therapeutic treatment in clinics making use of data on patient survival. Principles of selection of antitumor agents were discussed with respect to certain biochemical tests:measurements of antioxidative activity of tissue lipids of inhibition of DNA synthesis and number of defects in DNA secondary structure. Nature of paramagnetic centers in normal and tumor cells, and variations of tissue paramagnetic properties in the course of tumor growth have been considered with respect to chemotherapeutic principles. Applicability of the EPR technique for studying the mechanism of antitumor drug interaction with cell components has been reported.

The methods and notions of main fundamental sciences such as biology, chemistry, physics and mathematics are widely employed in modern cancer chemotherapy. Therefore formulation of principles directing the research of various scientists is of great importance.

The principles must clarify the correlation between the structure, chemical reactivity and the antitumor properties of the agent. The methods of mathematical statistics and computing must become the basis of quantitative evaluation of drug effectiveness and optimization of chemotherapy.

Of important role is the kinetic study of tumor growth and drug pharmacokinetics since the chemotherapeutic effectiveness depends on the stage of process development and pharmacokinetic characteristics of the drugs.

## QUANTITATIVE CRITERIA OF EFFECTIVENESS

The kinetics of all experimental tumor processes such as transplanted, induced and spontaneous experimental leukemias, solid tumors and their ascites sublines can be described by exponential, s -shaped or power functions [1]. The differential equation for growth of tumor cell populations can be written as

$$\frac{1}{F} \cdot \frac{dF}{dt} = \phi(t) \qquad (1)$$

where F is any measurable property of the system, $\phi(t)$ is the specific growth rate.

Function

$$\phi(t) = \phi_1(t) - \phi_2(t) \qquad (2)$$

where $\phi_1(t) \geq 0$ characterizes the total rate of cell proliferation and $\phi_2(t) \geq 0$ - the total rate of their killing. Meanwhile $\phi(t)$ can be both positive and negative.

The types of kinetic curves for tumor growth observed in practice can be described by expressions F(t) obtained by integration of equation (1) for various functions $\phi(t)$. The effectiveness of antitumor therapeutic treatment manifests in greater or lesser inhibition of tumor growth, in complete or partial regression (Fig.1).

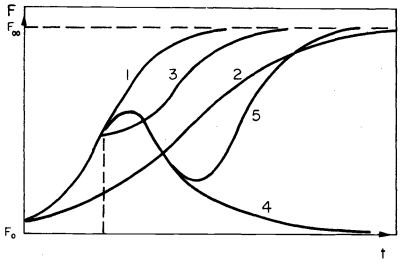



Fig.1. Types of kinetic curves for tumor growth. 1, control; 2, inhibition for early therapy; 3-5, treatment of the developing processes with inhibition effects (3), complete (4) and partial (5) regression; F is tumor size ($F_0$- initial, $F_\infty$- maximum achievable).

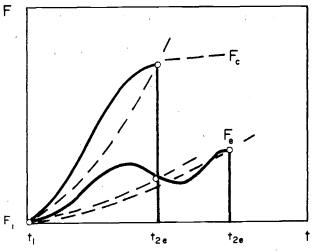



Fig.2. Plotting of equivalent exponents.

In many cases the kinetics of tumor growth satisfies the exponential dependences

$$F = F_o e^{\phi t} \tag{3}$$

which are obtained from integration of quation (1) at $\phi$ = const.

If the antitumor action results in inhibition of tumor growth only, the kinetic curve remaining exponential, it is convenient to use as a quantitative criterium of the treatment effectiveness the equation

$$x = \phi_c/\phi_e \tag{4}$$

where values $\phi_c$ and $\phi_e$ refer to control and experimental curves respectively. In case of complex shapes of control and especially experimental kinetic curves the treatment effectiveness can be evaluated by comparing the experiment and control taking for comparison two "equivalent exponents" (Fig.2).

The ratio of average specific tumor growth rates in control and experiment is taken as measure of the effect.

$$x = \frac{\bar{\phi}_c(t_1,t_2)}{\phi_e(t_1,t_2)} = \frac{t_{2e}-t_{1e}}{t_{2c}-t_{1c}} \cdot \frac{\ell nF_c(t_{2c})-\ell nF_c(t_{1c})}{\ell nF_e(t_{2e})-\ell nF_e(t_{1e})} \tag{5}$$

In the general case the value $\bar{x}$ depends on the two averaging intervals chosen ($t_{1c}$, $t_{2c}$) and ($t_{1e}$, $t_{2e}$). In practice it is more convenient to use a modified criterion

$$x^* = 1 - \frac{1}{x} \tag{6}$$

This criterion is a linear function of the tumor specific growth rate in experiment

$$x^* = 1 - \phi_e/\phi_c \tag{7}$$

Then the value $x^* = 0$ corresponds to absence of effect, $x^* \geqslant 0$ - to effective treatment (inhibition of tumor process) and the negative values - to actions stimulating tumor growth (Fig.3).

## CLASSIFICATION OF ANTITUMOR DRUGS BY THE PRINCIPLE OF CHEMICAL REACTIVITY

Usually research for new effective antitumor drugs is realized inside certain empirically established classes of chemical compounds such as alkylating agents, antimetabolites, antibiotics, hormones.

These compounds can be divided by the mechanisms of action into two main groups - compounds natural or similar to those involved in metabolic processes in normal or tumor cells (biochemically functionally active compounds) and the compounds mainly involved in pure chemical interactions with cell molecules, thus causing more or less considerable changes in metabolism (chemically functionally active compounds).

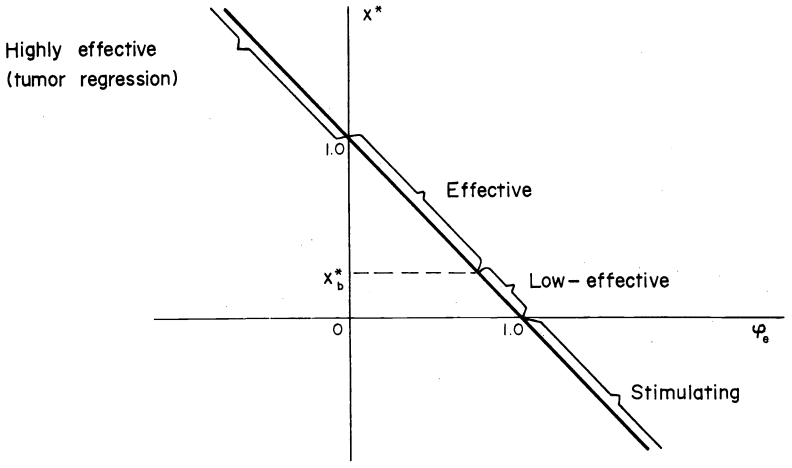
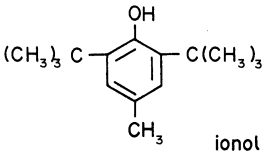


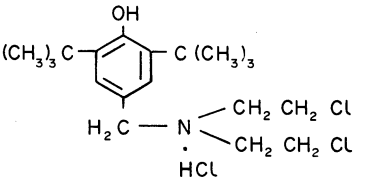

**Fig.3. Regions of effective therapeutic treatments. $x_b = 0.2$ is conventional value differentiated low-effective and effective treatment.**

The following compounds should be attributed to the biochemically functionally-active agents: enzymes (asparagenase, ribonuclease), hormones (cortisone, prednisolone, testosterone, diethylstilbestrol), some metabolites out of membrane-active regulators of metabolic processes including structural analogs of metabolites - the antimetabolites of nucleic metabolism (mercaptopurine, thioguanine, 5-fluorouracyl, phtorafur, 5-azacytidine, etc), the antimetabolites of folic metabolism (methotrexate, aminopterin).

Up to new possibilities to find new effective drugs among the above agents have been realized but slightly. Such a statement can be confirmed, in particular, by unexpectedly successful application of synthetic analogs of bioantioxidants such as ionol, ambunol, etc, in chemotherapy of tumors.




ionol




ambunol

The existent classification of antitumor agents is not sufficient to be the basis of rational planning of synthesis of new drug or for selection of potentially antitumor agents out of the great number of chemical compounds known to the present. Meanwhile, a more systematized classification based on the types of structures and chemical reactivities of these compounds can be proposed. Indeed, these can be divided in their specific reactions into 5 main groups:

1. Electrophilic reactions
2. Nucleophilic reactions
3. Free radical reactions
4. Complexing
5. Cycloaddition

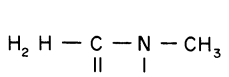
1. Electrophilic reactions

The main part of modern effective antitumor drugs consists of chemical compounds reacting by the mechanism of electrophilic interaction involving the formation of electrophilic species (carbonic cations) realizing the alkylation or acylation of nucleophilic centres in the main chemical components of the cell [2].

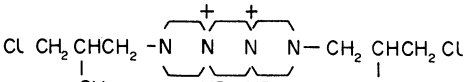
β-Chlorethylamines and β-chlorethylsulphides, epoxides and aziridines, sulpho- and phosphoesters, halogen methylketones and chlormethyl ethers, ammonium and sulphonic compounds, β-lactones, diazoketones, nitrosocarbamides act as alkylating agents in vivo.

Further progress in the synthesis of alkylating antitumor drugs is connected with finding new types of cytotoxic groupings and with modification of transport enzymes ensuring the selectivity of drug action. New effective antitumor drugs such as methylnitrosourea containing the nitrosocarbamide grouping as the functionally active agent [3], prospidine in which the spiroheterocycle with quaternary atoms of nitrogen contains the bis-β-chloralkylamine group [4] and 1,2-bis-diazoacetyl ethane(diazan) containing two diazoacetyl

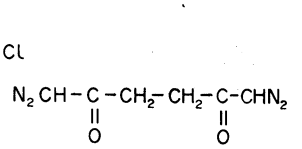
**fragments have been recently created [1].**



Methylnitrosourea

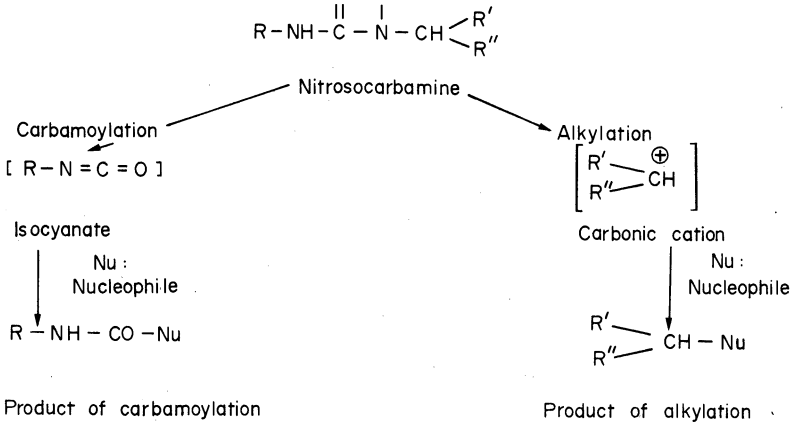


Prospidine

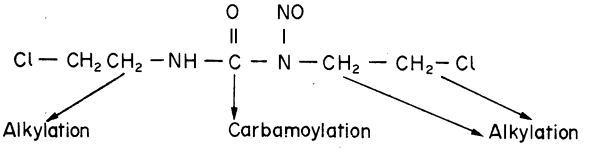


Diazan

**The methylnitrosourea proposed in 1964 as an antitumor drug, its homologs and analogs different from other alkylating agents exhibit a dual electrophilic reactivity, i.e. they exert both an alkylating and a carbamoylating action accounting for the peculiarities of the biological activity of the compounds of this class:**




**The nitrosocarbamide chlorethylamine derivatives contain several reaction centers, in particular, the well-known drug BCNU has one carbamoylating center and three alkylating centers:**



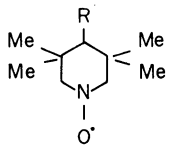

**Prospidine also contains two types of alkylating centers - ammonium and chloroalkyl groupings. The ionic centers of spiroheterocycles ensure specific binding of the drug with chemical components of the cell.**

**Methylnitrosourea is widely used for treatment of lung cancer, lymphogranulomatosis, and in combination with other drugs - for disseminated skin melanoma.**
**Prospidine was used for treatment of cancer of the larynx, of retinoblastomas, papillomatosis of the upper respiratory tract, Kaposi's antisarcoma [5]**

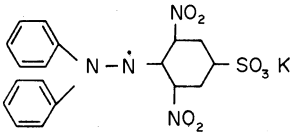
## 2. Free radical reactions.

**Free radical reactions represent a considerable part of chemical processes proceeding in living systems. Therefore compounds capable of being involved in these processes such as stable radicals and antiradical agents (inhibitors) are expected to possess a physiological activity.**

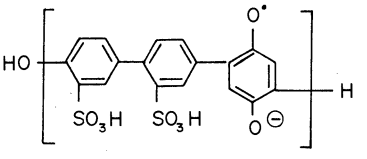
**The antitumor effect of free stable radicals was first found in 1964 for nitroxyl and triarylhydrazyl [6], and later for polysulphophenylene semiquinone [7].**



Nitroxyl



Triarylhydrazyl



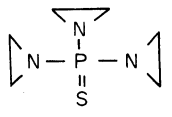
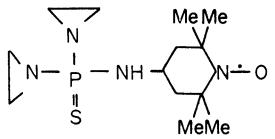
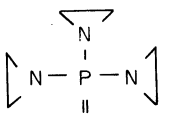
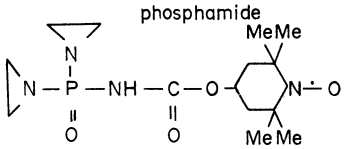
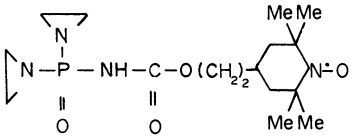
Polysulphophenylene semiquinone

A radiosensitizing effect of nitroxyl radicals was also reported.

The use of stable radicals for structural modification of several known antitumor drugs is rather promising [8]. The paramagnetic derivatives of thiophosphamide, phosphamide and triethylenemelamine synthesized and studied by now are characterized by a $10^1$ lower toxicity with retention or even increasing of the antitumor activity (Table 1).

Table 1

Antitumor Activity of Paramagnetic Analogs of Antitumor Agents.

| Formula | $LD_{50}$ mg/kg | Leukemia La, increase in mean life span, % | x* | Tumor growth inhibition,% WCS | SEM | EAT | S-180 |
|---|---|---|---|---|---|---|---|
|  thiophosphamide | 18 | 20 | 0.3 | 98 | 100 | 44 | - |
|  | 187 | 80 | 0.5 | 100 | 100 | 67 | 47 |
|  phosphamide | 15 | - | - | 65 | - | 50 | - |
|  | 150 | 171 | 1.0 | 100% of tumor inhibition | | | |
|  | 280 | 80 | 0.4 | 100% of tumor inhibition | | | |

*/ WCS - Walker carcinosarcoma, SEM - Svec erythromyelosis, EAT - Ehrlich ascites tumor, S-180 is for sarcoma 180

The synthesis of chemically polyfunctional antitumor drugs involving stable nitroxyl radicals as carriers of the alkylating group leads to a fundamentally new type of drugs with a combined mechanism of action and involving both the properties of the compounds with electrophilic reactivity and of the compounds involved in free radical reactions.

3. Complexing

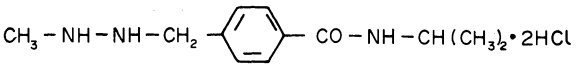
A large group of antitumor compounds dissimilar in structure consists of complexing agents capable for donor-acceptor interactions of various types (complexes with charge transfer, hydrogen bonds, hydrophobic interactions, metal complexes). Many antibiotics bind with nitrogen bases of DNA by the type of intercalating agents (antibiotics of the antracyclic series such as adreamycin, rubomycin, carminomycin, antibiotics from the aureolic acid group such as olivomycin, mitramycin, actinomycins) can be ascribed to this group.

The mechanism of intercalation can be suggested for many polycyclic compounds and ionic forms of polyheterocycles such as phenothiazines, phenoxazines, acridines. A specific group of antitumor agents consists of salts and complex metal compounds such as gallium nitrate, metal complexes of the platium group. The mechanism of their biological action is accounted for by

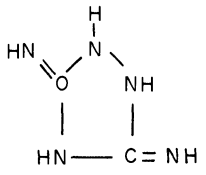
their capacity to bind with the informational biomacromolecules.

4. Nucleophilic reactions

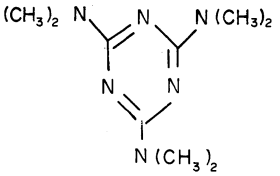
Nucleophilic reactions were not discussed in literature with respect to their antitumor effect. Meanwhile the electrophilic centers of metaloenzymes, carbonyl and isomethine groups can become targets for drugs with nucleophilic reactivity. Indeed, out of the antitumor drugs which can be classified as compounds with different (or even uncertain) mechanisms of action, the derivatives of hydrazine, carbamide and nitrogen bases which are strong nucleophilic reagents can be mentioned:



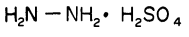
procarbazine



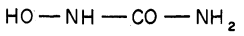
Guanasol



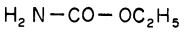
Hexamethylmelamine



Hydrazinesulphate



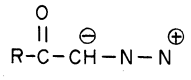
Hydroxiurea



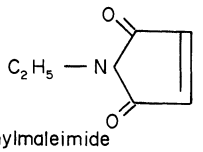
Urethane

5. Cyclo-addition

No direct data about the possibility of conducting cyclo-addition reactions in the living body, involving antitumor drugs, are available at present. However, the presence of activated double bonds in many metabolites does not exclude reactions of cycloaddition. N-Ethylmaleimide and diazoketones can be classified as antitumor agents capable of participating in these reactions.

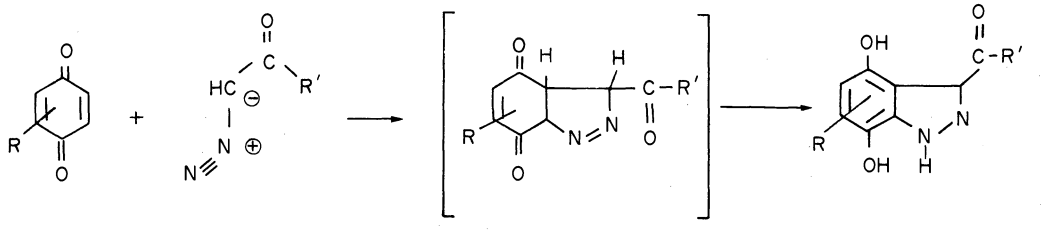


Diazoketone

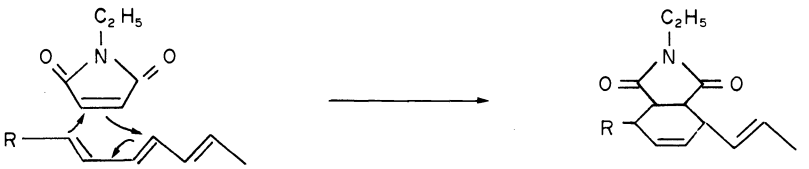


Ethylmaleimide

The possibility of 1,3-dipolar addition of diazoketones at the activated double bond (as for example in quinones) has been found recently in principle in model experiments:



N-Ethylmaleimide can act as dienophilic component in reactions of [1,4] --cyclo-addition to conjugate polyene systems:



Thus, along with known chemical mechanisms of antitumor action of the drugs (alkylation, complexing, free radical reactions) the possibility of cycloaddition or chemical interaction of the nucleophilic type is not excluded.

A new classification of agents by the reactivity types proposed by Soviet researchers is of a more general character than the traditional one, it permits interpreting in detail the chemical and biological mechanisms of antitumor drug actions and puts forth premises for rational planning of their synthesis.
The drugs with hybrid structures and correspondingly with hybrid reactivity readily fall into this classification.

## PHARMACOKINETICS OF ANTITUMOR DRUGS

Distribution of the compound in the living body corresponds to the simple kinetic model:

$$C_t \underset{k_1}{\overset{k_2}{\rightleftharpoons}} C_p(t) \xrightarrow{k_e}$$

where $C_t$ is drug concentration in the tissue, $C_p(t)$ is the function of concentration distribution in the blood plasma, and $k_1$ and $k_2$ are rate constants of direct and reverse transport. Excretion of many antitumor drugs of various classes from the blood plasma after single administration is described by the monoexponential dependence (Fig.4):

$$C_p(t) = \frac{D}{V} \cdot e^{-k_e t}$$

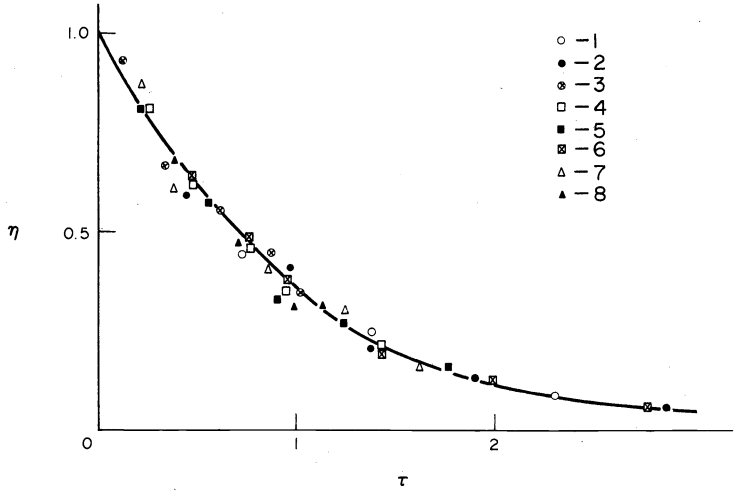



Fig.4. Generalized kinetic curve for removal of antitumor compounds of various classes from organisms of man and experimental animals: 1 - embiquin, 2 - ThioTEPA, 3 - TEPA, 4,5 - fluorouracil, 5 - methotrexate, 6 - cytosine arabinoside, 7 - colchicine, 8 - dawnomycin.

The changes in the drug concentration in the tissue are described by dependence:

$$C_t(t) = \frac{D}{V} \frac{k_1}{k_2 - k_e} e^{-k_e t} - e^{-k_2 t}$$

where D is the dose, V is the distribution volume, $k_e$ is the rate constant of excretion from plasma (Fig.5).

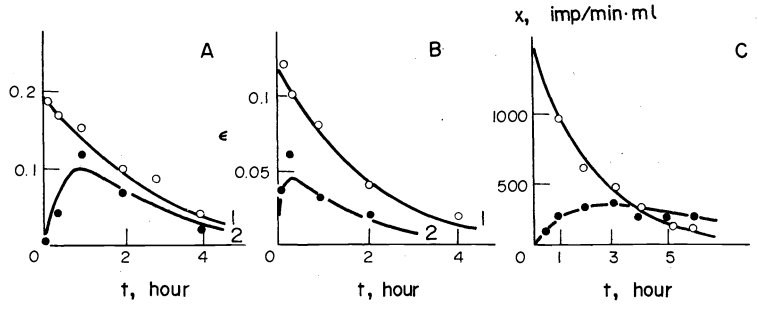



Fig.5. Changes in drug content in animal tumor and normal tissues: A,B, alkylating groupings in the blood (1) and in tumor (2), Guerin carcinoma) of rats after injections of cyclophosphamide(A) and ThioTEPA(B) C, label content in the blood plasma (1) and spinal fluid (2) of a dog after injection of cytozin-arabinoside-$^3$H.

## CORRELATION OF CHEMICAL STRUCTURE AND ANTITUMOR ACTIVITY

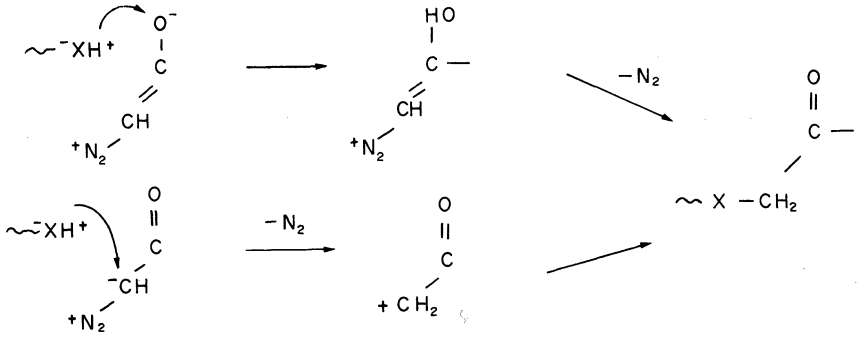
Prognostication of the drug properties based on generalized correlation between the structure and biological activity is an important trend in experimental and clinical chemotherapy. Among many others the Hansh and Free-Wulson methods of establishing quantitative correlation between compound structure and biological activity are most widely spread [9].

In Hansh general equation $\sigma$ characterizes the polar effects in a molecule, $E_s$ is for steric factors (for example, the Taft constants) and $\pi$ is for the drug distribution in the lipid-aqueous system.

$$\log A = \rho\sigma + \alpha\pi^2 + \gamma E_s + B$$

By applying the Hansh equation for series of chemical compounds close in structure, which steric factors and lipid-solubility characteristics changing insignificantly, one can obtain the correlation between the electron (polar) characteristics and biological effect.

For diazoketons the electrophilic attack of proton and alkylation of the acid anion is the first stage of diazoketon interaction with acid groups of biomacromolecules.




The changes in donor properties of the substituents in diazoacetophenons markedly affect the distribution of electronic density in diazoketon fragments (Table 2). It is seen that not only charges on atoms and the Wiberg characteristics correlate with the antitumor effect, but also Hammett constants, the energies of higher occupied orbitals ($E_{HOO}$) and lower free mole-

Table 2

Antitumor Activity of Para-substituted Diazoacetophenons and Values of Some Electronic Features of Molecules.

| Substituent | Inhibition of tumor growth P, % | | Atom negative charge value | | Free valency index | Hammett constant | $E_{LFO}$ | $E_{HOO}$ |
|---|---|---|---|---|---|---|---|---|
| R- | | S-180 | O | | | | | |
| $OCH_3$ | 79 | 70 | -0.375 | -0.186 | 0.122 | -0.27 | -10.959 | 1.916 |
| H | 64 | 63 | -0.372 | -0.172 | 0.114 | 0.00 | -11.013 | 1.896 |
| F | 30 | 44 | -0.343 | -0.162 | 0.066 | 0.06 | -11.144 | 1.441 |
| $NO_2$ | 25 | 32 | -0.324 | -0.158 | 0.050 | 0.78 | -11.697 | 0.959 |

cular orbitals ($E_{LFO}$) (quantum-chemical calculations were carried out by semiempyrical method CNDO/2).

For series of bifunctional diazoketons the quantum-chemical characteristics were computed for two most advantageous conformations of molecules. To find conformations the dihedral angles in diazoketone molecules were scanned in succession and rotation barriers were determined, which resulted in obtaining the energy surfaces. Two potential wells marked with an asterisk correspond to the most advantageous energy conformers of bis-diazoacetyl (Fig.6). The averaged values of charges on oxygen atoms and on methine atoms of carbon as well as their characteristics of free valency calculated by the Wiberg method are given in Table 2.

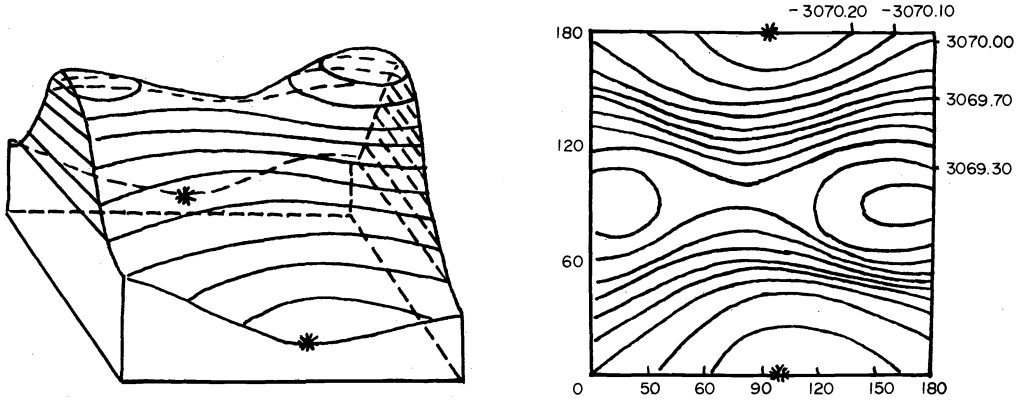



Fig.6. Energetic surfaces of bisdiazoacetyl - general outlook and contour diagram.

The values of electron densities on the above atoms as well as characteristics of free valency correlate with the value of antitumor activity (Fig. 7,8).

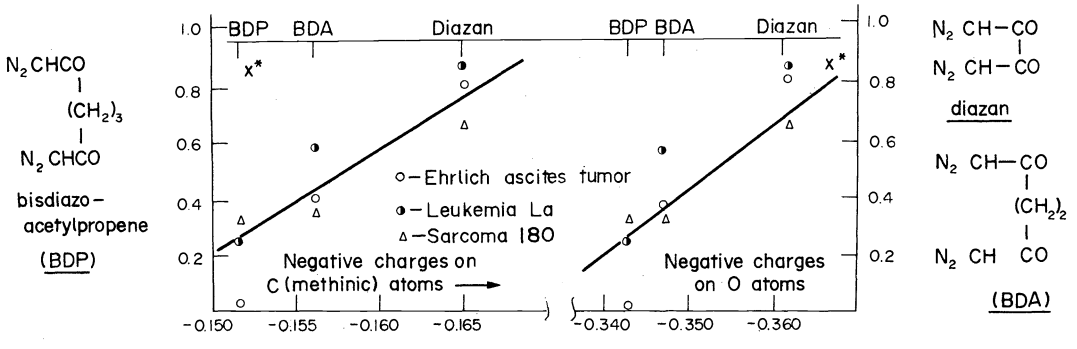



Fig.7. Correlation between negative charges of oxygen atom or carbon methine atom and antitumor activity of bisdiazoketons.

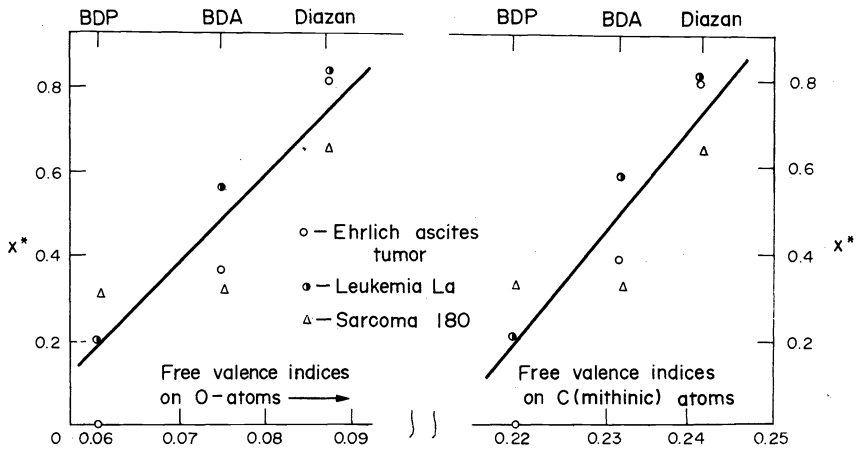



**Fig.8. Correlation between indices for free valency of oxygen atoms and carbon methine atom and antitumor activity of bisdiazoketons.**

## EFFECTIVENESS OF COMBINED CHEMOTHERAPY FACTOR ANALYSIS OF THE SYSTEM "TUMOR-DRUG-LIVING BODY"

The effectiveness of chemotherapeutic treatment of tumor is controlled by a great number of various factors and by essential intra-factor interaction. Certain functional groups of the drugs (or the drugs as such), the parameters of their spatial and electronic structures, the physico-chemical properties of compounds, the individual characteristics of the system "tumor-drug-living body" can be considered as such factors. The tumor type and animal strain, the extent of tumor process development and the kinetics of metastases, the cytokinetic parameters of tumor cells and drug resistance, the chemotherapeutic schedules, the toxic properties and pharmacokinetics of drugs, etc., belong to such characteristics. Factor analysis permits isolating the most important factors out of the great number of all possible characteristics of the system "tumor-drug-living body" under conditions of polychemotherapy.

At present the optimal schedules are chosen empirically. The number of possible combinations is extremely great and it is difficult to expect that an unbiassed optimal schedule can be found. In this connection of importance becomes the possibility of strict determination of the drug coaction as a basis for optimization of complex chemotherapy.

The effectiveness of combination of diazan and cyclophosphamide has been recently studied for leukemia P-388 and the method of factor analysis was first used for combined therapy of experimental leukemias [10]. This enabled the determination of the contribution of each drug (factor) to the total ultimate result of therapeutic treatment and the finding of value $\lambda$ characterizing the interaction of factors. The value $\lambda = 0$ denotes the additive effect, $\lambda < 0$ the subadditivity, $\lambda > 0$ stands for drug synergism.

The method of complete factor experiment with two factors taking two levels was used. The drug dose values were assumed to be the levels.

The experimental results programmed using the FORTRAN algorithm of the regression coefficient calculation by the Yates method [11].

The main effect (i.e. the contribution of each factors separately) and interaction of factors can be analytically expressed by the equation:

$$\lambda_{k_1 k_2} = \sum_{j=1}^{m} \left\{ (-1)^q \sum_{i=1}^{n} \tau_{ij} \right\}$$

where $k_1 = 1,2,\ldots \ell$; $k_2 = 1,2,\ldots \ell$; $q = 1$ or $2$, $\tau_{ij}$ is animal life times.

The experimental results are given in Table 3. 100% of animals were reported to be cured by combination of diasan at the doses of 150 mg/kg with cyclophosphamide at the doses of 100 and 200 mg/kg. Under these conditions the combined treatment effectiveness did not change with variations of cyclophos-

Table 3

**Effectiveness of the Combined Therapeutic Treatment of Leukemia P-388 by Diazan and Cyclophosphamide**

| Drugs | Single dose mg/kg | Increasing of mean life span in % to control | Number of exp. animals / Number of survived by 60 day | Coefficient of activity x | Main effects | Effect of coaction λ |
|---|---|---|---|---|---|---|
| Diazan | 100 | 45 | 6 / 0 | 1.4±0.04 | 17.4 | |
| Cyclophosphamide | 100 | 132 | 6 / 0 | 2.3±0.21 | 25.7 | |
| Diazan+Cyclophosphamide | 100+100 | 446 | 6 / 4 | 5.5±0.26 | | 13.60 |
| Diazan | 150 | 45 | 6 / 0 | 1.5±0.07 | 23.0 | |
| Cyclophosphamide | 100 | 95 | 6 / 0 | 1.9±0.14 | 27.7 | |
| Diazan+Cyclophosphamide | 150+100 | 544 | 6 / 6 | 6.4±0.11 | | 18.83 |
| Diazan | 150 | 45 | 6 / 0 | 1.5±0.07 | 14.5 | |
| Cyclophosphamide | 200 | 265 | 6 / 2 | 3.8±0.44 | 36.0 | |
| Diazan+Cyclophosphamide | 150+200 | 544 | 6 / 6 | 6.4±0.11 | | 10.42 |

$x = \frac{\tau_e}{\tau_c}$ ; $\tau_e$ is the mean life span of treated animals, $\tau_c$ is the mean life span of control animals,
The life span of control animals was 9.33-10.0 days.
The drugs were injected subcutaneously: diazan on the 1st and 6th day, cyclophosphamide - on the 2nd and 7th day.

phamide doses. The activity coefficient x was 6.4 in the both cases. However, the comparison of the values specific for the combined drug action showed that the extent of therapeutic synergism at cyclophosphamide dose of 100 mg/kg was 18.83 whereas with increase in cyclophosphamide dose the main effect contributed by the drug increases while the extent of the combined action becomes lower ( $\lambda_{1,2}$ = 10.42)

Factor analysis permits plotting equieffective curves for combined use two drugs (Fig.9). The experimental data are given in the same figure and show

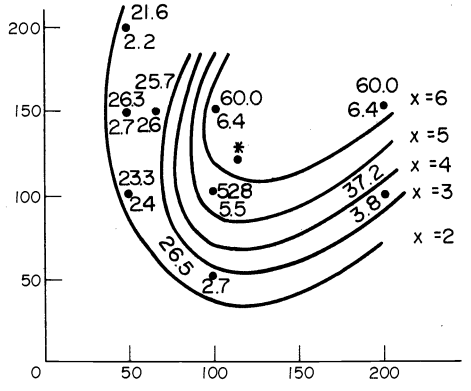



Fig.9. Equieffective curves for combined action of diazan and cyclophosphamide: X-axis is for cyclophosphamide doses, Y-axis is for diazan doses (mg/kg).

satisfactory agreement with the calculation (average values for animal life spans in days and values $x_{comb}$) are given near the points).

Optimal (maximal) value ($x_{comb}$)max and necessary minimal doses of each drug permitting to obtain value ($x_{comb}$)max. It was found out that ($x_{comb}$)opt = 6.4, while diazan and cyclophosphamide concentrations are 120 and 110 mg/kg respectively (asterisk in Fig.9). This point is seen to be located near the

equieffectiveness curve corresponding to $x_{comb} = 6.0$. The experiments carried out later showed that maximal effectiveness actually was observed for this point ( $x = 6.4$) (black point near the asterisk). Such an approach to analysis of polychemotherapy results opens up possibilities for strictly grounded clinical recomendations and permits planning ways for solving the optimization problem in complex therapy.

## SURVIVAL OF PATIENTS AND CHEMOTHERAPY

The working out of unbiassed quantitative criteria which can be of use in clinics is of great importance for the selection of new effective antitumor drugs [12]. The life span of patients is the most important indicator of effectiveness of malignant tumor treatment. The survival is described by certain functions for tumors of different localizations. The main types of such functions are given in Table 4.

Table 4

Main Functions Used for Describing Kinetic Curves for Survival of Patients with Malignant Tumors

| Tumor | Function | Equation of functional dependence |
|---|---|---|
| Stomach cancer (after radical operation) | Exponential | $P\,\% = 100^{-kt}$ |
| Cancer of the lung, mammary gland, rectum, kidney, bladder (after radical operation) | Hyperbolic | $P\,\% = \frac{100}{a - kt}$ |
| Cancer of the stomach, lung, mammary gland (inoperable patients) | Equation of log-normal distribution | $P\,\% = \frac{1}{\sqrt{2\pi}} \int_{\frac{1}{\sigma}\ln\frac{t}{T}}^{\infty} e^{-\frac{1}{2}x^2}\,dx$ |
| Chronic lympholeukemia Chronic myeloid leukemia | | |

The results became the scientific and methodological ground for an original kinetic method of comparative evaluation of the treatment effectiveness. The great amount of information accumulated in various clinics of the world is used as (so-called) "historical control". It permits to give up the simultaneous creating of special control groups of patients and to speed up the studies and assaying of new most effective drugs in clinical practice.

The data on survival of patients with inoperable lung cancer having received no special treatment ("historical control") in 33 clinics and those on survival of 168 patients with a similar disease but under chemotherapeutic treatment were generalized (Fig.10). It follows from the figure that the

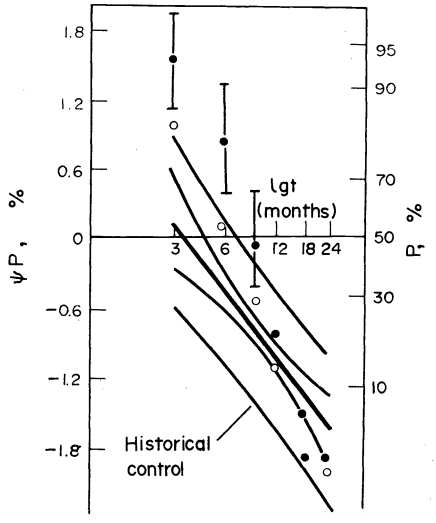



Fig.10. Anamorphosis of generalized curve for survival of patients with inoperable lung cancer (1) calculated by data from 33 clinics using the method of linear regression. Data on patients treated with chemotherapeutic drugs: o is for all patients received chemotherapeutic treatment, • is for patients with immediate improvement.

data on 3 and 6 months' survival were beyond 95% on the confidential level for patients with immediate improvement (most patients received methylnitrosourea - black circles). The survival of patients without improvement after treatment (white circles) was the same as for the control groups. Thus, the use of drugs causing immediate improvement in patients with lung cancer increases the time of their survival, but at present this time comes to a 6 months' survival only.

A number of tumor cells killed as a result of any treatment should be considered as an important complex characteristic of the antitumor drug effectiveness. Direct determination of this characteristic under clinical conditions is impossible. A model has been worked out correlating the survival kinetics with the tumor growth rate and the value of tumor cell mass in the body [13]. By means of this model it is possible to estimate the average multiplicity of tumor mass reduction due to effective treatment using the data on variations of survival of a group of patients after treatment.

The kinetic curves for patient survival radically operated for the lung cancer (data provided by the Hertzen Oncological Institute, Moscow) are given as an example in Fig.11. It is seen that combined treatment (surgery + cyclophosphamide) increased the time of survival. Using this model and data on

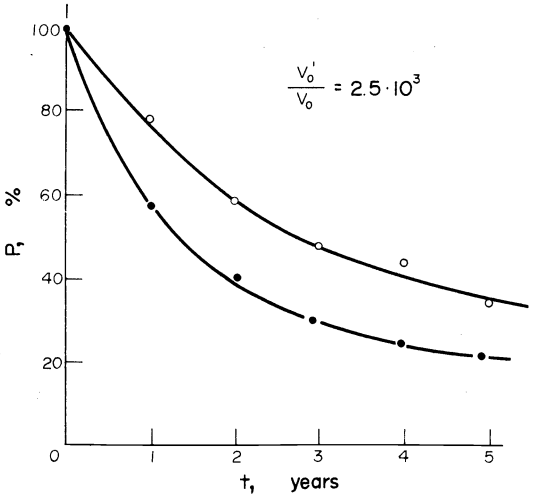



Fig.11. Kinetic curves for survival of patients radically operated for the lung cancer: ● is for patients received only surgical treatment, o is for patients received treatment with cyclophosphamide in addition to surgery.

changes in the survival after treatment it is possible to evaluate effective treatment that reduces the tumor mass on the average for a group of patients by a factor of 400. This value may be considered as a quantitative characteristic of the drug effectiveness and can be used for comparative evaluation of the drugs as well as the effect of combined therapy.

## ANTIOXIDATIVE ACTIVITY OF LIPIDS OF NORMAL AND TUMOR TISSUES AND CHEMOTHERAPY.

Around two decades ago the notion of antioxidative activity (AOA) of tissue lipids specific for participation of bioantioxidants in the metabolic processes appeared in biochemistry of tumors [14].

The value (AOA) was determined from the capacity of lipid fraction to inhibit the oxidation of model substrate as, for example, methyloleate which oxidizes already at physiological temperatures at a rate convenient for measuring

$$(AOA) = \frac{\tau - \tau_{stand}}{P} \quad hour.gr^{-1}ml^{-1}$$

where $\tau$ and $\tau_{stand}$ are the times of attaining a similar extent of substrate oxidation, P is weighted portion of lipid fraction. The AOA value is very sensitive to various normal physiological and pathological states of the living body and to the action of multiple physical and chemical factors.

The growth of transplanted tumors is accompanied by increase in AOA of lipid fractions of host tissues and organs and of developing tumor tissues compared to normal organ tissue and initial AOA value of the transplanted substance

(Fig.12) [14,15] .

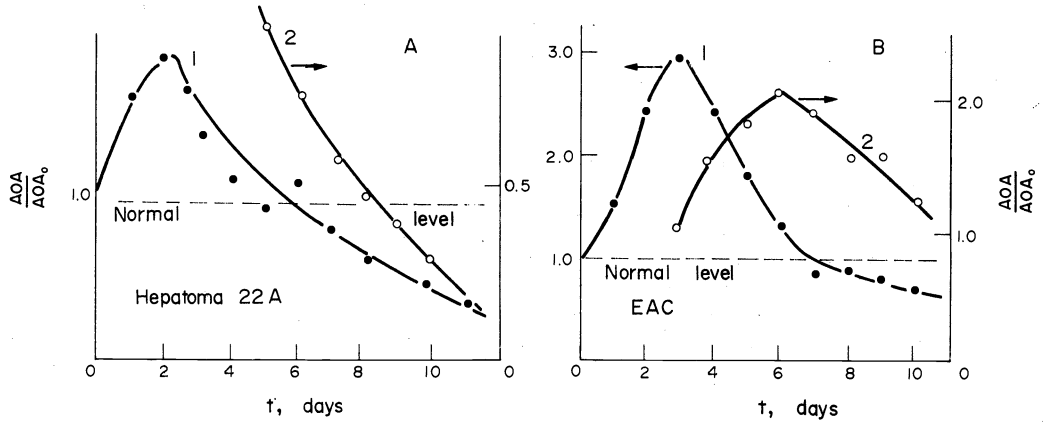



**Fig.12. A. Changes in AOA of liver lipids(1) and tumor(2) in the course of transplanted hepatome 22-a growth. B. Changes in AOA of liver lipids(1) and tumor cells(2) in the course of Ehrlich ascites carcinoma growth.**

**Changes in AOA can be used to control the course of chemotherapeutic treatment since increase in tumor size is accompanied by retarding the AOA growth (Fig.13).**

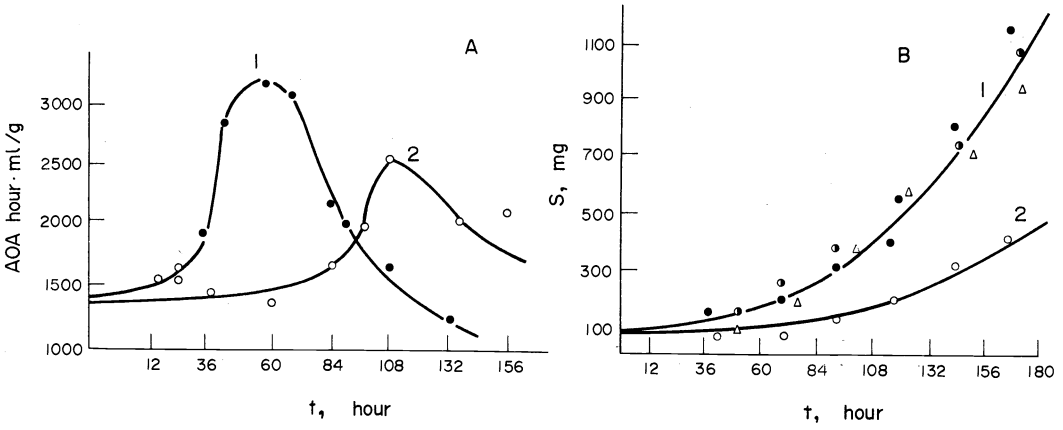



**Fig.13. Changes in the liver antioxidative activity (A) and the spleen weight (B) in the course of transplanted leukemia La development (curves 1) and for chronic injections of antitumor drug - 4-oxi-3,5-ditert.butyl -α- methylbenzylamine chlorhydrate (30 mg/kg x 6) beginning from the first day after transplantation of tumor (curves 2).**

**All effective antitumor agents injected to normal animals are capable of reducing AOA in comparison to the normal level [16,17] (Fig.14). Unspecific effects such as various doses of X-rays and stresses can also bring to fall in AOA and thus to appropriate antitumor effect.**

**Correlation between the antitumor action and AOA values was found for the model of transplanted leukemia La (Fig.15). The values $AOA_{least}$, i.e. the lowest of those which can be attained upon administering some drug at the given dose were used for deriving this relation.**

**A new type of antitumor drug screening using normal animals for evaluation of the drug effectiveness can be developed in terms of the above. Correlation**

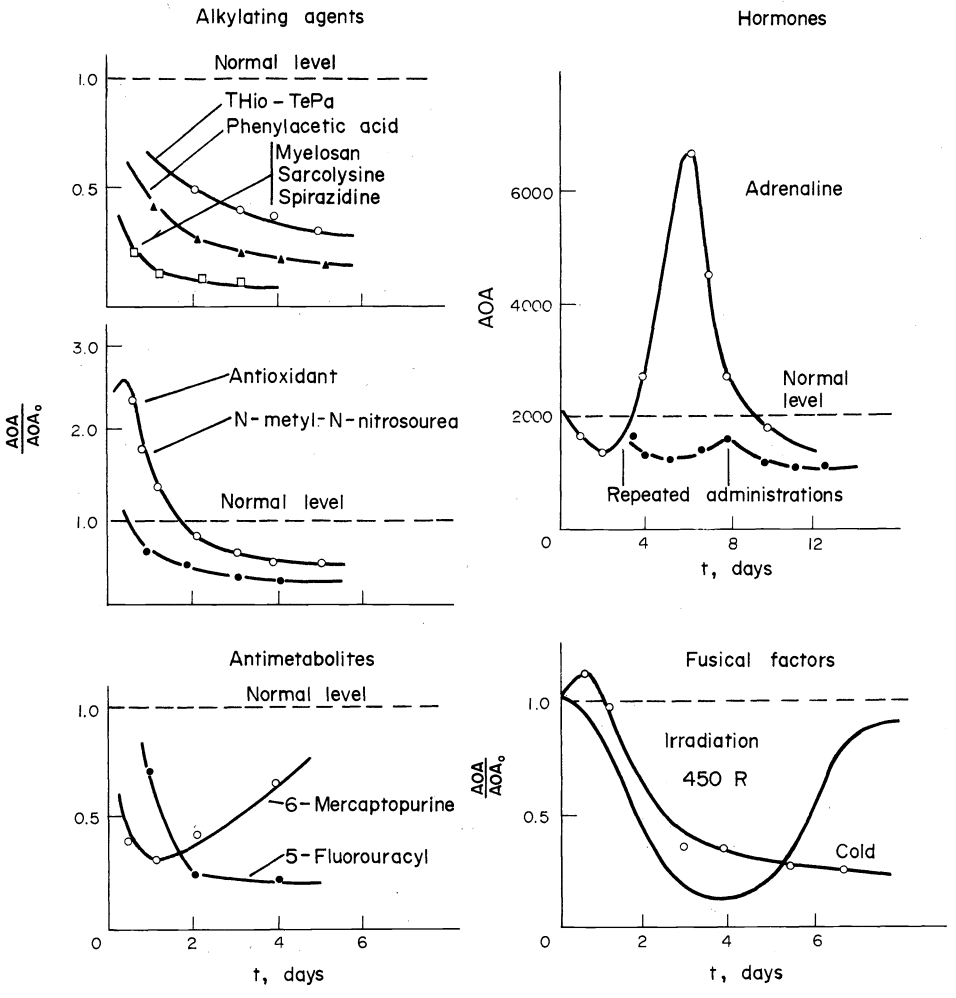



**Fig.14. Changes in lipid antioxidative activity of normal mice liver under the action of certain antitumor actions.**

of $x_s$ and $1/(AOA)_{least}$ as in Fig.15 was obtained in cases when the drugs are applied at early stages of tumor growth characterized by increase in AOA level. At the late stages when AOA of host tissues falls below the norm (Fig.12,13), the use of a strong chemotherapeutic drug may become ineffective as the toxic effect of the drugs on the living body can be much higher than on the tumor.

In such cases the use of these or those drugs increasing AOA and as if restoring normal metabolism can stimulate increase in chemotherapeutic effectiveness with strong antitumor agents. It is natural to expect that synthetic antioxidants would be the first to possess such properties.

The probability should not be excluded either that effective use of natural drugs such as anabol and some means of so called "people's medicine" is based on the above suggestion.

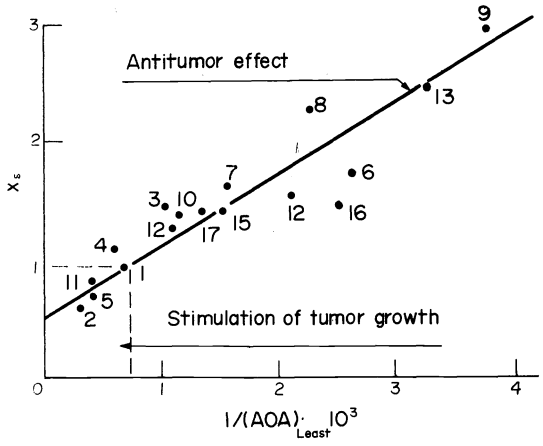



Fig.15. Inhibition coefficient (æ) of transplanted leukemia La development as a function of capacity of various compounds for decreasing the AOA level in liver lipids of normal animals: 1 - norm, 2 - 4-oxi-3,5-di-tert.butyl- -methylbenzylamine chlorhydrate (30 mg/kg), 3 - the same (6x30 mg/kg), 4- the same (4x30 mg/kg), 5 - 4-methyl-2,6-di-tert.butylphenol (30 mg/kg), 6 - the same (6x50 mg/kg), 7- 4-N-N-di(B-oxiethylaminomethyl)2,6-di-tert.butylphenol clorhydrate, 8 - n(B-B-bis-chlorethylamine)-phenylacetic acid (6 mg/kg), 9 - the same (x% mg/kg), 10 - n(B-chlorethylamine)-phenylacetic acid(6x8 mg/kg), 11 - 6-methyl-2-ethyl-oxipyridine, 12 - irradiation (300 r), 13 - irradiation (450 r), 14 - methyloleate, 15 - 4-methyl-2,6-di-tert.butylphenol, 16 - Thiophosphamide (ThioTEPA), 17 - cold stress.

In such cases the use of drugs increasing AOA and as if restoring normal metabolism can stimulate an increase in chemotherapeutic effectiveness. It is natural to expect that synthetic antioxidants would be the first to possess such properties. Thus, the probability should not be excluded that effective in several cases, use of natural drugs such as anabol - one of the means of so called "peoples medicine" - is based on the above suggestion.

Investigation of antioxidative properties of tissue can also help in the rational selection of drugs possessing anticarcinogenic properties. Changes in tissue AOA under carcinogenesis corresponds to morphological stages of cancerogenesis. A decrease in AOA was observed at the stage of the carcinogen toxicity, an increase in AOA to the initial value was observed at the stage of transition from diffusive to focal hyperplasia. A sharp increase in AOA as compared to the norm was reported in the period of tumor appearance (Fig.16) [18].

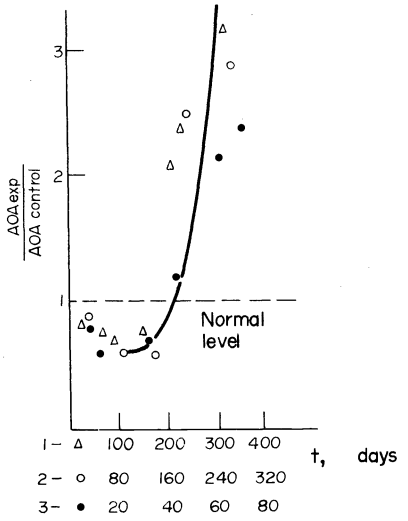



Fig.16. Changes in AOA of mice liver under carcinogenic effect or orthoamineazotiluene (1), γ -irradiation (2), 3,4-benzpyrene (3).

It is evident that at the stage of AOA decrease these drugs would possess anticarcinogenic activity increasing AOA to the values close to norm. At the later periods of carcinogenesis AOA has to be reduced [19]. Use of synthetic antioxidants at small doses at the early stages of carcinogenesis decreases the possibility of tumor appearance [19,20,21].

## INHIBITION OF DNA SYNTHESIS AS A BIOCHEMICAL CRITERION OF THE EFFECTIVENESS OF ANTITUMOR DRUG ACTION

Suppression of DNA synthesis by the antitumor agents is accounted for by their action on the DNA molecule as such and on the enzymes of synthesis and repair. Search for drugs mainly capable of suppressing DNA biosynthesis in tumors than in rapidly proliferating cells of tumor-bearing animal organs such as the bone marrow, intestines epithelium is needed for the purposes of antitumor therapeutic treatment. The use of drugs assisting to restore the DNA synthesis in normal cells earlier than in tumor ones can also exhibit certain effect.

From this point of view the nitrosourea derivatives have been recently studied as antitumor drugs effective for a number of malignant tumors in man [3,22]. DNA synthesis in the tumor cells of melanoma B16 in mice and also in normal dividing cells of the marrow and intestines epithelium was studied [23]. Curves for changes in $^{14}C$-thymidine incorporation in DNA cells of melanoma B-16, in the marrow and intestines epithelium as a function of time after nitrosourea injection to tumor-bearing animals are given in Fig.17.

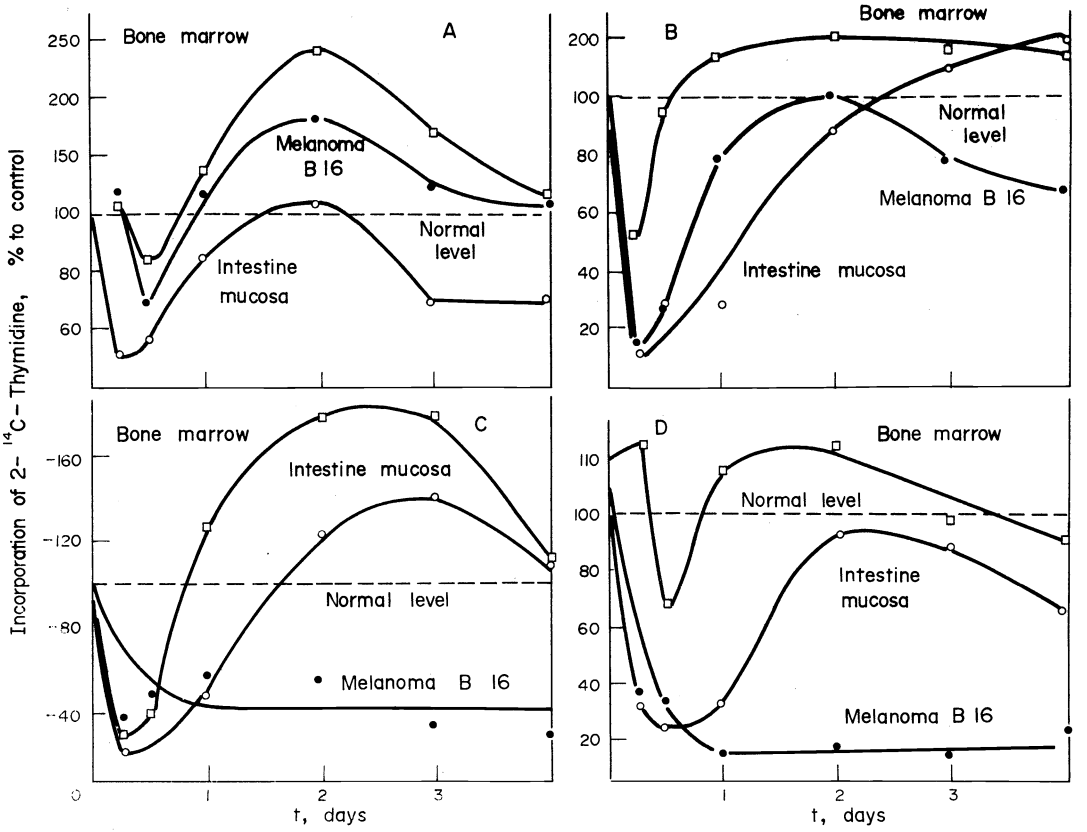



Fig.17. Inhibition of DNA synthesis in the cells of melanima B16, the bone marrow and intestine epithelium of tumor-bearing mice after a single injection of therapeutic dose: A - streptozotocin (200 mg/kg), B - N-methyl-N-nitrosourea (80 mg/kg), C - dimethylnitrosourea (80 mg/kg), D - chlorozotocin (15 mg/kg).

Streptozotocin is seen to only slightly inhibit the DNA synthesis in melanoma B-16 cells, with posterior undesirable stimulation of the synthesis (Fig. 17A). Short-term inhibition of DNA synthesis took place in the bone marrow, then it was followed by considerable stimulation which in all probability would be harmful on repeated injection of the drug. The processes of undesi-

rable suppression of DNA synthesis prevail in the intestines epithelium. Thus, streptozotocin possesses many undesirable properties, what seemingly coordinates with its low antitumor activity and limited application in chemotherapy.

In 6 hours after injection of N-methyl-N-nitrosourea the DNA synthesis is considerably inhibited in tumor cells and in the intestines epithelium ( by 90%) (Fig.17B). However later it is restored and attains control values in 24-48 hours (Fig.11). Inhibition of DNA synthesis in the marrow cells is less marked and after 12 hours does not practically manifest.

Of highest interest in terms of tumor therapy is the dimethylnitrosourea (Fig.17C): inhibition of DNA synthesis in tumor persists throughout the whole experiment (96 hours), whereas the DNA synthesis in marrow and intestines epithelium cells is already restored in 20 and 40 hours respectively. A similar result was obtained for chlorozotocin (Fig.17D).

The results obtained reveal the correlation of the antitumor activity with the extent and duration of inhibition of DNA synthesis in tumor cells of certain alkylnitrosoureas. It corresponds to known therapeutic effectiveness of these agents decreasing in the order: chlorozotocin --- dimethylnitrosourea --- methylnitrosourea --- streptozotocin 3 . It is expedient to make repeated injections of dimethylnitrosoureas and chlorozotocin only after complete restoration of DNA synthesis in normal actively-dividing cells.That would permit to reduce the undesirable toxic action of the agents used on the marrow and intestines epithelium.

An important direction of research on the mechanism of antitumor action of chemical substances is the study of direct interaction of drug molecules with DNA macromolecule. The differences in stability of secondary DNA structure of normal and tumor cells were determined in 24-26 . The kinetic-formaldehyde method (KF-method) used permitted detecting one defect per $10^4$ pairs of DNA bases. Kinetic curves for despiralization of normal and tumor cell DNA in the interaction with formaldehyde are given in Fig.18. The DNA

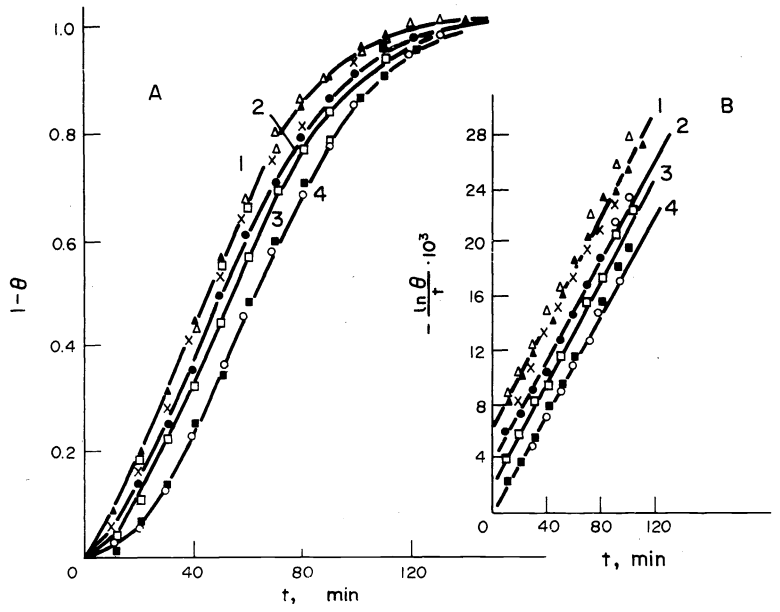



Fig.18. Kinetic curves for despiralization in the presence of formaldehyde (A) and their linear anamorphosis (B) for DNA of normal and tumor cells: ■ - spleen of intact mice, ○ - liver of intact mice, □ - spleen of mice with leukemia La (on the 7th day), ● - mice with hepatoma 22A (on the 8th day), × - Ehrlich ascites carcinoma (on the 7th day), ▲ - Svec erythromyelosis in rats, △ - Walker carcinosarcoma in rats.

of normal cells is seen to have no defects in the secondary structure (the respective semilogarithmic anamorphose falls to the origin of coordinates), whereas DNA of tumor cells has defects(from 1.5 to 6 defects per $10^4$ base pairs of different strains). The DNA of liver cells of tumor-bearers has up to 2.8 defects.

The existence of loci with disturbed secondary structure in tumor cell DNA was confirmed by independent experiments on the level of adenine and cytosine modification by dibromine ethylacetate with formation of fluorescent ethane derivatives [27,28].

It was found for reaction of DNA with methylnitrosourea (MNU) studied by KF-method (18) that the accumulation rate of secondary structure defects in tumor cell DNA (hepatoma 22A in mice) is two times higher than for the reaction with DNA of normal liver cells (Fig.19).

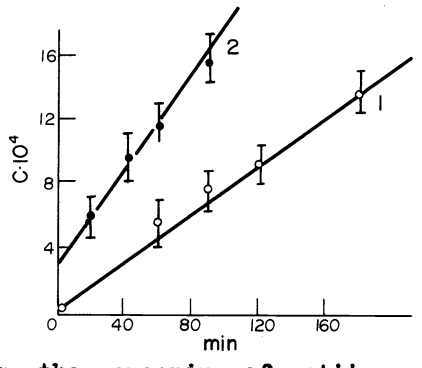



Fig.19. Kinetic curves for accumulation of DNA secondary structure defects determined by the KF-technique in reaction with MNU: 1 - mice liver DNA, 2 - DNA of hepatoma 22A. Ratio of MNU and DNA concentrations is 50:1 (mole).

Thus, the screening of antitumor agents interacting with DNA can be carried out by selecting drugs with higher rate of tumor cell DNA damaging. In this case a certain specificity of drug action can be provided.

## THE RESPIRATION CHAIN IS THE TARGET OF ANTITUMOR DRUG ACTION

Among the main targets of antitumor agent action the proteins - carriers of electrontransport chain of mitochondria, containing metal paramagnetic ions - occupy an important place. Therefore studies of the EPR spectra of tissues and their modifications under the agent action represent a route for controlling the functional state of the cell and its response to the agent action. At present much information has been accumulated on the nature of paramagnetic centers (PC) in different tissues, and evidence for the existance of quantitative correlation between PC content and intensity of redoxy reactions has been obtained.

Free radical centers in the tissues are practically completely localized in mitochondria. Two types of centers - semiquinones of flavoproteides localized in the inner membrane of mitochondria, and coenzyme Q semiquinones (ubisemiquinones) - are responsible for signal at g = 2.00 in frozen animal tissues. Signals of ferrous sulfide protein paramagnetic centers, cytochrome P-450, $Mn^{2+}$-, $Cu^{2+}$-, $Mo^{5+}$ paramagnetic complexes containing proteins are defected in normal tissues.

Two types of the EPR signals not observed before in normal tissues were found in malignant tissues of animal. This is the signal at g = 2.03 and a complex signal with triplet hyperfine structure with center at g = 2.007 and resolution 17 G. These signals were observed not only in tumor tissues but also in pathological nonmalignant animal tissues. For example, signal at g = 2.03 appeared under conditions of hypoxia and is related to inhibition of mitochondria respiration chains. The signal at g = 2.007 was found in preparations under various effects. Nitrosyl complexes of Fe-S- proteins are responsible for the signal at g = 2.03, nitrosyl complexes of ferrum ions of hemoproteins are responsible for the signal at g = 2.007.

It follows from the quantum-chemical calculations of MNU electronic structure parameters that breaking of N - N bond and detachment of NO can occur under conditions of restoration. The appearance of exogenous NO can result in forming nitrosyl complexes of cell metal enzymes. The appearance of exogenous NO in the cells owing to MNU molecule decomposition can be determined by formation of nitrosyl complexes.

The EPR spectra of liver homogenate with MNU and liver homogenate (control) exhibited at equal time intervals at room temperature are shown in Fig.20. The EPR signal characteristic of nitrosyl complexes of Fe-NO hemoproteins (signal with triplet hyperfine structure with a ratio of component intensities I:I:I, at g = 2.007 and a = 16G) was recorded for the samples of liver homogenate with MNU in an hour after the drug injection. The intensity of this signal increases with time, attains the maximum in 6-9 hours and then starts decreasing (Fig.21). A singlet signal at g = 2.004 and H = 7 G. The singlet signal is close in its parameters to the signal from ubisemiquinone, appears in the EPR spectra and increases with time of the drug action. The singlet signal is close in its parameters to the signal from ubisemiquinone.

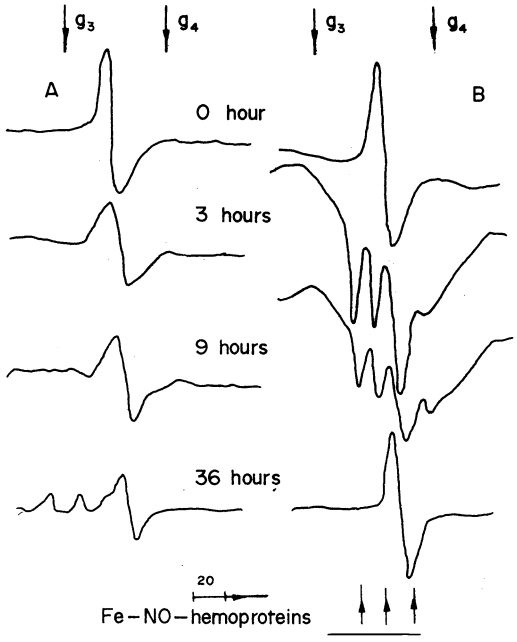



Fig.20. The EPR spectra of nitrosyl complexes of Fe-NO-hemoproteins for MNU decomposition in the course of its incubation in liver homogenate: A - homogenate, B - homogenate + MNU.

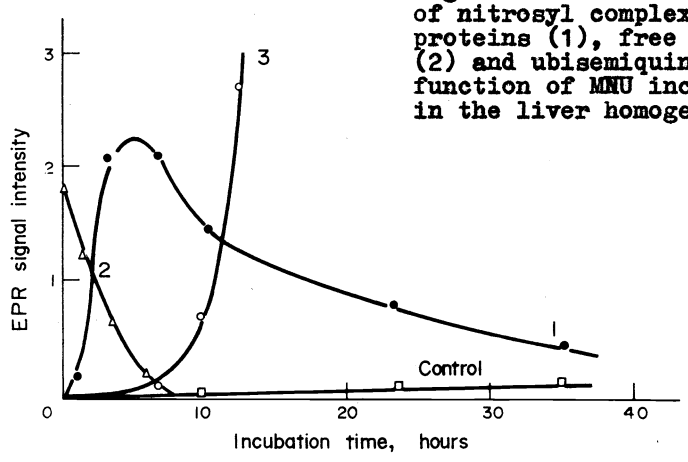



Fig.21. Variations in concentration of nitrosyl complexes of Fe-NO-hemoproteins (1), free radical centres (2) and ubisemiquinone (3) as a function of MNU incubation time in the liver homogenate.

Preferential binding of exogenous NO yielded in decomposition of MNU, with cytochrome b can be suggested to occurs. That results in inhibition of electron transport chains of mitochondria and accumulation of ubisemiquinones. The nature of this signal is being studied at present.

It is important to find out the possibility for MNU decomposition with detachment of NO directly in the blood of animals. The keeping of MNU with mice blood (1 mg of MNU per 1 ml of blood) or hemoglobin solution in vitro did not practically result in its decomposition with NO detachment (Fig.22). However, addition of ascorbic acid (Asc.A.) to the system brought to the formation of a considerable amount of Fe-NO complexes of hemoglobin. The first stage of such a process was the oxidation of Asc.A. and formation of its cation radical form. The result of MNU restoring is the NO detachment and binding of the latter with hemoglobin. The results obtained show that only under conditions of MNU restoring, there takes place its decomposition to form NO.

In the second (in vivo) series of the experiments MNU (200 mg/kg) was injected i.p. to noninbred mice: five minutes after injection the Fe-NO complexes of hemoproteins were recorded in the animal liver the concentration of which grew with time and attained the maximal value in the interval of 0.5±1 hour

and then decreased to zero 2 hours after MNU injection.

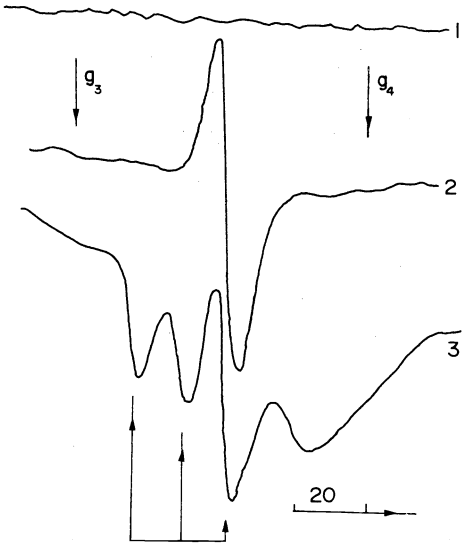



Fig.22. The EPR spectra of hemoglobin solutions: 1 - after MNU addition, 2 - after MNU and ascorbic acid (AA) addition ( in 5 minutes), 3 - after MNU and AA addition (in 3 hours of incubation at room temperature).

Thus, a new path of MNU decomposition with NO detachment under conditions of restoring has been found. It is possible that this mechanism underlies inhibition of electron transfer in the mitochondria respiration chain. Such inhibition occurs due to appearance of exogenous NO in the cell and as a result of it there occurs formation of nitrosyl complexes of metalenzymes.

Nevertheless it has to be borne in mind that this path of MNU decomposition can be responsible also for MNU inactivation since the agent deprived of the NO-group loses its biological activity. This can result in that only an insignificant part of unchanged MNU would reach the tumor.

Thus, investigation of paramagnetic centers of normal and tumor tissues affected by antitumor drugs in vitro and in vivo permits elucidating the action of antitumor drugs and evaluating their toxicity.

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