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Polymeric Chemotherapeutic Agents with Carcinolytic Activity

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The review deals with advances in research on the applications of polymeric compounds in active cancer chemotherapy and prospects for its development. The principal aims to which efforts are at present directed are indicated and arguments are adduced in support of the necessity for a further and comprehensive development of this problem. Attention is concentrated on the employment of synthetic polyelectrolytes as transporting agents for active chemotherapeutic preparations. The bibliography includes 52 references.

The treatment of different types of cancer is one of the most important problems being solved at present in medicine. The widespread occurrence of these affections in all parts of the world, their wide pathological variety, and the absence in certain cases of means for a complete cure — all these necessitate an active search for chemotherapeutic antitumour agents.

There exist several hypotheses at present concerning the nature of cancer and the mechanism of its occurrence and development. One can now state with complete certainty that cancer constitutes the formation of new tissue (tumour), which arises in different parts of the human organism as a result of continuous uncontrolled division of cancer cells. Usually this is a result of the response of the organism to the action of various external and internal irritants, toxic substances, physical (ionising radiation and others) and biological agents (certain types of viruses and hormones), etc.[1].

The concept of "cancer" is collective[2] (carcinomas, sarcomas, lymphomas, etc.). The wide variety of forms of cancer and their morphological, aetiological, and clinical differences make it necessary to select specific agents to treat the disease. The principal modern methods for the treatment of a cancer are surgery, radiotherapy, hormonotherapy, chemotherapy, and combined procedures[3]. When chemotherapy is used in the fight against cancer, it is necessary to overcome a specific difficulty — the identical response of normal and cancer cells to chemotherapeutic agents.

Many authors believe[3-5] that chemotherapeutic preparations used in modern practice can be divided into four principal groups: alkylating agents, antimetabolites, antibiotics, and preparations of plant origin. Larionov[4] includes hormonal preparations among them. Boesen and Davies[5] distinguish yet another group, combining all preparations with anticancer activity which cannot be included in any of the four groups stated above in terms of the principle of their action.

Antimetabolites are largely structural analogues of compounds important to the organism, such as vitamins, hormones, and aminoacids.

Antibiotics are not as effective against cancer cells as against bacterial infections. However, their capacity to influence the metabolism of tumours and also the virus theory of certain forms of cancer[6] resulted in the use of many antibiotics in the fight against cancer. Actinomycins have been most widely used.

Among preparations of plant origin, the alkaloids colchamine and vinblastine show the highest antitumour activity.

The main group of preparations includes alkylating compounds with anticancer activity. It has been most thoroughly investigated and a large number of carcinolytic preparations employed in clinical practice belong to it. The different types of biological alkylating agents, the mechanism of their action, the kinds of tumours which they effectively inhibit, the methods for the enhancement of their selectivity, and the prospects of finding new preparations have been considered in detail in reviews[4, 7-9].

A common property of biological alkylating agents is their capacity to alkylate the nucleophilic centres of biological systems under very mild conditions (aqueous solution at pH 7 – 7.5, temperature 37°C). The following nucleophilic centres are encountered in biological systems: anions of organic and inorganic acids and ionised sulphydryl groups and thioethers. The main alkylating compounds employed are β-chloroethylamines $R_2NCH_2CH_2Cl$, epoxy-compounds $R_2CH-CH-CH_2$ (O), aziridine derivatives



and alkanesulphonic acid esters.

It has been established that many alkylating agents react *in vitro* directly with the functional groups of proteins and nucleic acids. They attack the carboxy- or hydroxy-groups of aminoacids and phosphoric acids.

In order to account for the biological activity of alkylating substances, two hypotheses have been put forward[7]: (1) the formation of cross-links between two points in the same biomacromolecule or between two different biomicromolecules; (2) local polymerisation. In the latter case compounds capable of being alkylated and containing reactive groups are polymerised in the organism with formation of macromolecules. The majority of investigators tend to give preference to the first hypothesis. On the other hand, the second hypothesis makes it possible to detect the carcinolytic activity of certain alkylating polymers[10].

The principal requirement which must be met by alkylating agents (as well as all other anticancer chemotherapeutic agents) is selectivity of their action. The selectivity is measured by the so called chemotherapeutic index, which is defined as the ratio of the therapeutic and toxic doses of the given preparation[9].

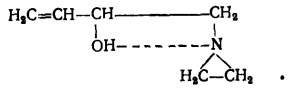
The high reactivity and low specificity of alkylating compounds introduced into the organism frequently preclude their employment as chemotherapeutic agents. Thus the idea arose that an inert carrier, which as a rule exhibits a specific affinity for the particular system toward which the therapeutic activity is directed, be employed.

An important principle of the preparation of chemotherapeutic compounds with high selectivity and low toxicity involves the employment of latent activity[8]. The principle is based on the possibility of introducing into the organism inactive preparations which are gradually converted into their active form under the influence of enzymes. Such preparations may be high-molecular-weight compounds.

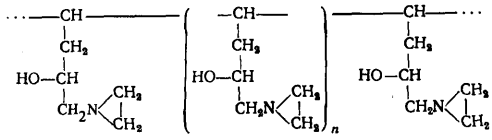
The successful application of polymers in all branches of medicine stimulated the synthesis of polymeric medicinal preparations with carcinolytic activity. Krentsel'et al.[11]

state that interest in polymeric materials increased particularly after the development of methods for stereospecific polymerisation whereby it is possible to synthesise rigorously ordered macromolecules, which were previously known only among natural compounds. The same workers believe that there are three promising trends in the synthesis of polymeric antitumour preparations.

The first important trend involves the synthesis of physiologically active polymers from physiologically active monomers. Alkylating aromatic preparations derived from di-(2-chloroethyl)amines, derivatives of aziridine, and ethylene derivatives in which the double bond is activated by groups such as NO_2, SO_2R, COR, etc. are believed to be the most promising in this respect. In the synthesis of such preparations the main factor is the endeavour to increase the selectivity by controlling the length of the macromolecule and the content of functional groups. In most cases polymerisation of monomeric anticancer alkylating substances of such types is difficult and advances in this field are so far insignificant. The first positive result was achieved by Lidaks and Hillers[12], who polymerised the familiar antitumour preparation ethoxen (1-azirindino-2-hydroxybutene):

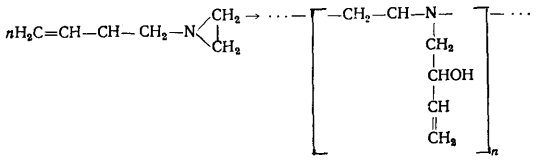


The main task in the synthesis was to achieve polymerisation via the double bonds and to prevent the opening of the aziridine ring. This was done by employing hydrogen peroxide at 100°C as the initiator. According to the authors, the resulting physiologically active polymer has the following structure:



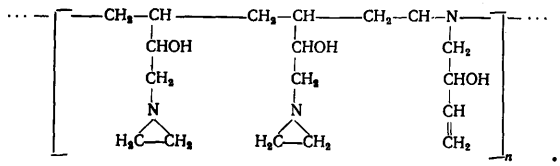
This structure presupposes the complete retention of the aziridine ring, which is unlikely. The polymeric preparation obtained was used in the treatment of the carcinoma of the alimentary canal and the stomach and was administered *per os*, which ensured good contact with the surface of the tumour and prevented a premature reaction of the reactive alkylating aziridino-group. The polymer exhibits almost the same anticancer activity against sarcoma S-45 in mice as the initial monomer, but is less toxic.

Subsequently it was established[11] that the polymerisation of ethoxen proceeds not only via the double bonds but also via the opening of the aziridine ring:



When synthesised by thermal polymerisation, the polymer includes up to 37% of units containing the aziridine ring, 58.8% of such units are retained on radical polymerisation under the influence of benzoyl peroxide and 67 to 68% on radiation-induced polymerisation (using γ-rays from a cobalt source). Thus the maximal antitumour effect is to be expected for the polymer obtained by γ-irradiation.

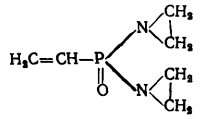
This polymer probably has a structure of the type[11]



i.e. of each three monomeric molecules two enter the growing chain as a result of the dissociation of the C = C double bond and one as a result of the opening of the ring.

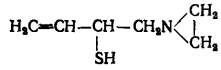
Shimanskaya[13] has listed the following other promising monomeric aziridine derivatives which are of interest for the synthesis of anticancer polymeric preparations:

bis(1-aziridinyl)vinylphosphine oxide:

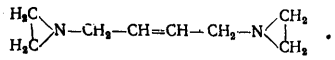


(the presence of the phosphoryl group might have increased the latent activity of the polymer);

1-aziridino-2-mercaptobut-3-ene (mercapto-analogue of ethoxen):

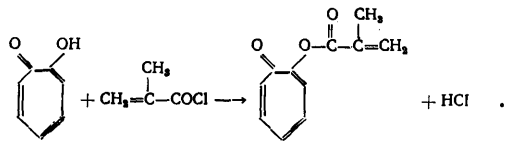


1,4-diaziridinobut-2-ene (product of the addition of aziridine to butadiene):

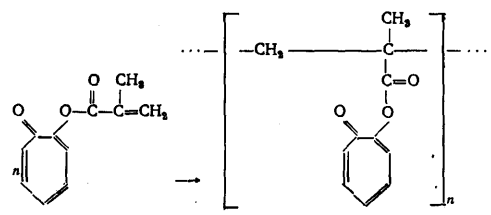


Cornell and Donaruma[14, 15] used 2-methacryloxytropone as the monomer. They assumed that the corresponding polymer would exhibit a moderate physiological activity, since at the biological pH it should be insoluble and resistant to biological decomposition. Indeed, the polymer obtained from 2-methacryloxynitrile[11, 12] does show a weak physiological effect. On the other hand, the homopolymer of methacryloxytropone exhibits a higher carcinolytic activity in relation to experimental strains.

Methacryloyloxytropone is a product of the reaction between tropolone and methacryloyl chloride:



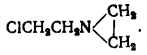
The polymerisation can be represented by the following scheme:



The authors do not explain the mechanism of the anticancer activity of the polymer which they synthesised. The carcinolytic activity of the monomer is probably due to the structural similarity between tropolone, colchicine, and other troponoids of this kind, the physiological activity of

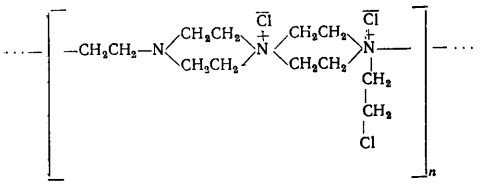
which is known. Colchamine is an analogue of this group substances among preparations of plant origin.

Galstyan[16] reported studies on the anticancer activities of the polymeric preparation Kh-1. This chloroalkylating preparation, obtained by Avetyan and Matsoyan[17], has been investigated clinically and introduced into chemotherapeutic practice under the name "polychlorimine". 2-Chloroethylaziridine, which constitutes a combination of two groups with a high cytostatic activity,

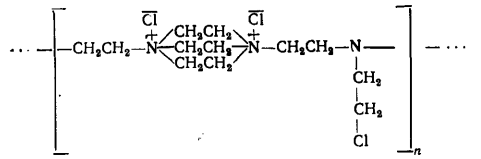


was used as the monomer for its synthesis.

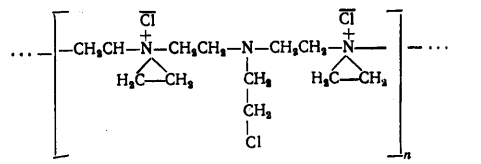
The monomer is unstable and polymerises readily in the presence of water. Controlled polymerisation was achieved in aqueous dimethylformamide solution at 30 – 40°C for monomer : water : dimethylformamide ratios of 1 : 5 : 1. The analytical data lead to the conclusion that the fundamental unit of the resulting linear polymers contains two ammonium ions formed by cyclisation. Three probable versions (A – C) of the structure of the polymer chain have been put forward:



(A)

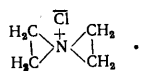


(B)



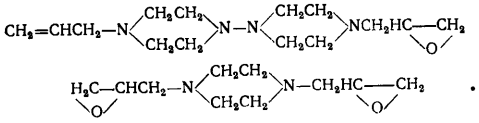
(C)

The authors believe that polymerisation proceeds via a cationic mechanism involving self-initiation with participation of a quaternary ammonium salt produced from the monomer:



The mechanism of the anticancer activity of the preparation has not been established. Its effectiveness can hardly be accounted for solely by the presence in the chain of a large number of active alkylating groups. This could lead to an extremely high toxicity and would reduce the selectivity of the polymeric chloroalkylating agent. The preparation is probably a polyelectrolyte. Thus, bearing in mind certain characteristics of cancer cells, which will be discussed below, one can assume that the effectiveness of the preparation is based on its combined action as a polyelectrolyte and as an active alkylating agent.

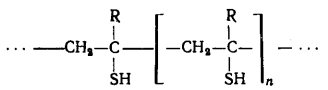
It would probably be interesting to obtain and test polymeric analogous of a number of compounds containing the epoxy-group, for example:



The copolymerisation of methacrylic acid with alkylating derivatives based on it is also interesting.

According to Krentsel' et al.[11], a second important trend in the synthesis of polymers with carcinolytic activity involves the formation of simple macromolecules containing physiologically active groups. Such studies are also important because they make it possible to explain the influence which a given polymer structure can exert on the reactivity of physiologically active functional groups. Polymers containing sulphydryl and imidazole groups are particularly interesting in this respect[18]. These polymers can inhibit the influence of ionising radiation, which is responsible for the carcinogenic degeneracy of cells.

Polyvinylmercaptans with various substituents in a side chain may be quoted as a possible example:



The presence of phenolic rings increases the resistance of polyvinylmercaptans to oxidation.

Certain reports concerning the use of polymeric molecules as carriers of active anticancer low-molecular-weight substances can also be referred to studies of this kind. The inclusion of an active sarcolysine preparation in the molecules of a number of polymers and copolymers (the copolymers are conventionally designated by K_2, K_7, K_{10}, K_{12}, etc.) led to the synthesis of effective agents with a comparatively low toxicity and high selectivity[19].

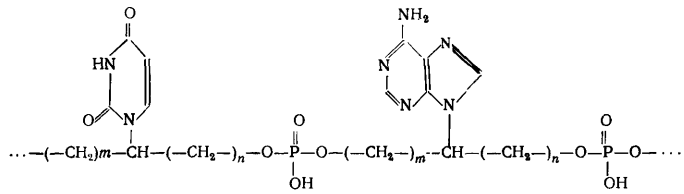
Vernick and Hoppe[20] discovered that the polyvinylpyrrolidine – iodine complex has an inhibiting effect on Walker carcinosarcoma 256. The possibility of using polyvinylpyrrolidine as a polymer forming complexes with various cytostatic substances and increasing their selectivity has also been reported by other workers[21].

A third, exceptionally promising but unfortunately as yet uninvestigated type of synthesis of polymeric carcinolytic substances is asymmetric synthesis[22]. The synthesis may be based on the relation demonstrated by many investigators between the stereoisomerism of chemical substances, in particular sarcolysin derivatives[23, 24], and their carcinolytic activity. The method of polymerisation on polymeric matrices might find an application here[25].

The study of the use of polymeric analogous of natural macromolecules to suppress the selective action of the enzyme RNA-polymerase[26] showed that polymers with a specific configuration may inhibit the synthesis of protein and arrest the growth of malignant tumours. It has been suggested[26] that it might be possible to synthesise polymers whose structure would resemble various enzymes with a rigorous alternation of a large number of aminoacids in the monomeric unit with an appropriate chain length and appropriate distances between individual units. Such "depressant polymers" can "recognise", via one of their units much more readily than low-molecular-weight chemotherapeutic substances, the appropriate enzyme centre susceptible to

attack and block its activity. Merrifield's method – solid-phase polymerisation – is most suitable for the synthesis of such polymers[27].

The synthesis of a polymer of this kind was achieved by Hillers[28], who obtained oligomeric polynucleotides complementary to natural aminoacids and having the following structure:

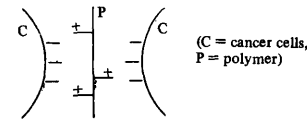


In all the instances mentioned, the activity was explained on the basis of the already established mechanisms of the action of alkylating substances on biological centres or the mechanisms of the suppression of the activity of enzymes responsible for cell division. Polymeric preparations exhibit almost identical activities in relation to both cancer and normal cells. Therefore, even a slight qualitative difference between such cells has been the subject of numerous wide-scale investigations in order to find new ways for the inhibition of the growth of tumours.

Another interesting type of study, which gives rise to the possibility of creating various combinations and modifications of polymeric substances, involves the use of polyelectrolytes. Both polycations and polyanions, i.e. polybases and polyacids, can probably be used with equal success.

The majority of natural and biological polymers are polyelectrolytes. They include, for example, peptides and enzymes[29, 30]. The surface of normal and cancer cells has a definite electrical charge. The possibility of the formation of electrostatic bonds between natural or synthetic polyelectrolytes and a biopolymer of a living organism constitutes yet another basis for the inhibition of tumour growth. For example, by binding a suitable polyelectrolyte to enzymes e.g. deoxyribonuclease responsible for cell division, one might retard or arrest such division. It is then necessary to protect the organism from the toxic action of the polyelectrolytes, since they act with equal force on both cancer and normal cells[29]. Cationic polyelectroyltes proved to be the most selective. This may be attributed to the negative surface charge of the cells, arising mainly from ionised phosphate or carboxy-groups.

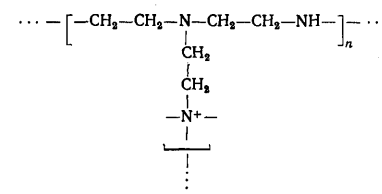
On the basis of studies by a number of investigators, Ambrose et al.[31] showed by electrophoresis that the negative charges arising on the surfaces of normal and cancer cells are of different magnitudes. Subsequently these workers[32] confirmed this by experiments with cancer cells from mouse kidneys and lungs. They tested synthetic polycations as antitumour preparations *in vitro*. Polyethyleneimine ... $-[-CH_2CH_2NH-]_n$ ..., polyvinyl-4-butylpyridine, and polyhexamethylenediguanidine ... $-[-(-CH_2-)_6-\underset{\underset{NH}{\|}}{C}-NH-\underset{\underset{NH}{\|}}{C}-NH-]_n-$... were tested. It was found that only polyethyleneimine exhibits a fairly marked antitumour activity. The highest efficiency is obtained at a low concentration of the test polymers. The agglutination of tumour cells in such experiments confirms the hypothesis that the cancer cell has a higher negative charge than the normal cell. According to these investigators, this can be explained by a decrease on the surface of cancer cells of the number of Ca^{2+} ions, which play the role of an intercellular adhesive. A decrease of the number of these ions on the surface of cancer cells impairs the intercellular linkages. Polycations which combine with the negatively charged surface neutralise the surface charge. This increases the adhesion between the cells and causes the agglutination of the tumour cells[33].



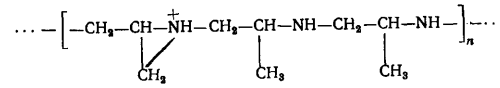
It has been shown that the units of sialic acids are responsible to a greater extent than phosphate residues for the formation of the negative surface charge of cancer cells[33, 34].

In accordance with the latest research, Moroson[34] believes that, apart from the Coulombic cytostatic binding of cancer cells described above, polycations in general enhance the immune response of the organisms to cells transplanted from the outside. Polycations of the following types were investigated *in vivo* and *in vitro*:

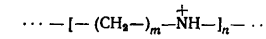
Polyethyleneimines with molecular weights of 600, 1800, 60 000, and 100 000:



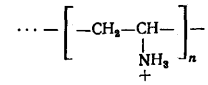
Polypropyleneimine with a molecular weight of 10 000 to 15 000:



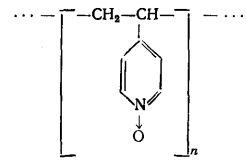
A polyamine with a molecular weight of 30 000 – 50 000:



Polyvinylamine with a molecular weight of 10 000 to 300 000:



Poly(vinylpyridien *N*-oxide) with a molecular weight of 100 000:



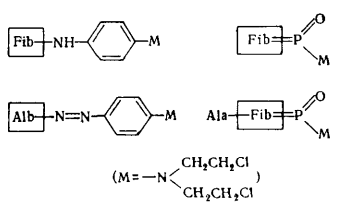
These polymers have basic properties and in the neutral form each of the four nitrogen atoms carries a positive charge. The mechanism of the polymerisation of the ethyleneimine [aziridine] monomer includes a stage involving the formation of a quaternary immonium ion and then interaction with yet another ethyleneimine molecule. Polyethyleneimine molecules with a higher molecular weight (in excess of 100 000) has been obtained by treatment with ethylene chloride.

Apart from inhibiting the growth of tumours by direct electrostatic addition, some investigators believe that polycations are capable of transporting powerful cytostatic substance to the cancer cells. In his experiments, Woodman[35]

employed for this purpose chitosan (a copolymer of hexosamine and *N*-acetylhexosamine), polyethyleneimine, polypropyleneimine, diethylaminodextran, "polybrene" [poly-1, 5-dimethyl-1, 5(diaza-undecamethylene dimethobromide)], and other polymers. These high-molecular-weight compounds are capable of forming complexes with iododeoxycytidylic acid and thus transporting iododeoxyuridine — an active metabolite. The presence of polycations probably facilitates the passage of the active metabolite through the membrane of the cell after its enzymatic decomposition (cell dephosphorylation[8]) or even the entire polycation – metabolite complex may pass into the cell. As a result, the activity of nuclear deoxyribonuclease is inhibited.

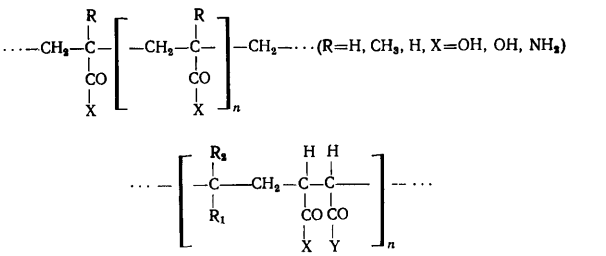
Ryser[36] and Larsen[37] observed an increase of the transmitting capacity of a cell membrane model (thiolated gelatin) in the presence of polycations. Larsen and Thörling[38] therefore explained their observation of the anticancer inhibiting effect of diethylaminodextran and the sodium salt of sulphonated dextran by the facilitation of the passage of the macromolecule through the cell membrane.

The possibility of the transport of cytostatic agents of another type with the aid of polycations is also of great interest. Experiments with complexes of methotrexate, chlorambucil, etc. and polyethyleneimine proved unsuccessful, since these complexes dissociate in 0.015 M NaCl. Nitrogen mustard gas, linked covalently to albumin (a natural polyelectrolyte) as well as a phenylalanine – mustard gas polymer did not exhibit the expected antitumour activity[39]. However, the diphenylamine analogue of mustard gas and some of its derivatives exhibited a high chemotherapeutic selectivity. These preparations proved to be effective in relation to certain mouse tumours[40]. Some derivatives of nitrogen mustard gas linked to biopolycations of the type of albumin, globulin, and fibrinogen showed a high latent activity[40]



Regelson and coworkers[41] discovered that many synthetic heparinoid polyanions exhibit a comprehensive antitumour activity. Regelson attributes this activity to the anticancer activity of heparin itself, which is an inhibitor of cell division. Heparin is a sulphomucopolysaccharide—a natural anionic hydrophilic polymer, which acts as a coagulator. While the causes of the anticancer activity of polycations are known comparatively accurately, for polyanions there are numerous likely mechanisms and hypotheses. It has been suggested that polyanions bind Ca^{2+} and Mg^{2+} ions, keeping them on the surface of the cell, which promotes cohesion among the cells. As mentioned above, cancer cells are distinguished by impaired intermolecular adhesion owing to a lower coverage of the surface by Ca^{2+} or Mg^{2+} ions. Polyanions can also combine with various biopolycations (enzymes), which results in a decrease or complete disappearance of the activity of enzymes, promoting uncontrolled cell division. Polyanions also inhibit the activity of nuclear deoxyribonuclease of cancer cells.

Regelson investigated the sodium salt of polyethylenesulphonic acid (molecular weight 15 000 – 35 000)[42] as the first analogue of heparin and demonstrated its anticancer activity on various types of tissue culture. Later, in order to confirm this hypothesis, he investigated the anticancer activity of other polyanions — polyacrylic acid and ethylenemaleic acid anhydride[43], both pure and hydrolysed. Polyanions in the amidoacid form have also been examined. They differ by the density of electrical charges:



(R_1=H; H; H: H; H; H; CH_3; CH_3
R_2=H; H; H; H; CH_3; CH_3; CH_3; CH_3
X=OH; NH_2; NH_2; NH_2; ONH_4; NH_2; ONa; NH_2
Y=OH; ONH_4; OH; NH_2; ONH_4; ONH_4; ONa; NH_2)

It was found that the amidoacid form of the copolymer of ethylene and maleic anhydride exhibits a broad spectrum of antitumour activity, while the hydrolysed form inhibits most effectively the growth of sarcoma 180 (equally toxic doses were compared). The highest effectiveness is, however, achieved when the carboxamide groups are distributed along the main copolymer chain. The enhanced anticancer activity of the cobalt, copper, and nickel salts of the copolymer of ethylene and maleic anhydride against Walker sarcoma in mice was also discovered by Hodnett et al.[44]

Another polyanion, a pyran copolymer, investigated by Merigan, Regelson and coworkers[45–48], exhibits an interesting antitumour activity. To facilitate its elimination from the organism (with urine), it is convenient to employ a polymer with a molecular weight of 17 000. It was established that the pyran copolymer induces the formation in the organism of an active interferon, giving rise thereby to a broad spectrum of carcinolytic activity[49]. Interferon, which is a low-molecular-weight protein, exhibits a universal antiviral activity. It appears in the cells of many animals and man as a result of the action of virusus and viral nucleic acids. Its activity against carcinogenic viruses has been demonstrated[49].

The monomeric pyran ring is formed in the polymer chain after the copolymerisation of divinyl ether with maleic anhydride (in a pure form and with a 50% admixture of the amidoacid form) or by the interaction of methyl vinyl ether with maleic anhydride (also in a pure form and with a 50% admixture of the amide). Other methods of synthesising this ring are also known, for example, by the reaction of maleic anhydride and styrene. It has been established that methylation of 50% of the carboxy-groups does not block the formation of interferon, while amidation sometimes does have a blocking effect. The capacity of these copolymers to induce the formation of interferon depends on the steric accessibility of the carboxy-groups at the instant of their addition to cancer cells. Copolymers without free carboxy-groups are inactive[45].

Finally, special mention should also be made of the search for carcinolytic substances directly related to Szent-Gyorgyi's interesting hypothesis concerning the essential nature of the cancer cell[50]. According to his view, the cells of a living organism are in a state of equilibrium in the presence of free methylglyoxal. By virtue of its high activity, only traces of methylglyoxal are required for normal cell reproduction. The division of normal cells begins as a result of the liberation of the enzyme glyoxalase,

which inactivates methylglyoxal. When the cell is capable of binding glyoxalase, division ceases at the required instant. If the cell loses this capacity, it changes, according to Szent-Gyorgyi's idea, from a normal to a cancer cell. Possibly special administration of glyoxalase-inactivated methylglyoxal from the outside might have a definite therapeutic effect. Polymeric carriers have the ability to maintain a constant therapeutic concentration of methylglyoxal liberated on gradual decomposition of the polymer chain. Experimental evidence for the anticancer activity of methylglyoxal and its derivatives has been published in a number of studies[51, 52].

Despite the vigorous development of the applications of polymers in all branches of medicine and particularly their recent use in chemotherapy, carcinolytic activity has been demonstrated or even investigated for only a small number of polymeric preparations. The reason for this is probably lack of an exhaustive explanation of the mechanism of carcinogenesis and of the control of cell division as well as the mechanism of the antitumour activity of various polymeric substances. This hinders the search for new preparations and forces investigators to study mainly the available commercial polymers with suggested carcinolytic activity.

There is no doubt that polymers constitute an exceptionally promising field for the search for antitumour agents. They are particularly suitable for the application of the principle of latent activity (by employing carrier polymers with marked carcinolytic properties). Complexes with an ionic linkage or polymers modified by a covalently linked agent might transport the active form in a combined state to the cancer cell, where, as a result of a chemical change (decomposition, reduction, oxidation), the cytostatic activity against malignant formations may be liberated. Vast possibilities are also inherent in the use of synthetic polycations as covalent or ionic carriers of alkylating agents. Thus, there are many possibilities in polymer cancer chemotherapy which have not yet been utilised.

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