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Biologically Active Cellulose Derivatives

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The review deals with the synthesis and applications of biologically active cellulose derivatives. The synthesis of cellulose derivatives with antimicrobial and haemostatic (arresting blood flow) properties, the possible fields of application of antimicrobial and haemostatic cellulose materials, the synthesis of graft copolymers of cellulose and biologically active proteins (enzymes and antigens), and medical applications of cellulose derivatives containing ionogenic or complex-forming groups are considered.
The bibliography includes 197 references.

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I. INTRODUCTION

One of the rapidly developing fields in the chemistry of macromolecular compounds concerns the synthesis of new polymers and the chemical modification of known natural and synthetic polymers carried out to obtain biologically active substances. In particular the use of polysaccharides for such syntheses is of considerable interest. At the present time both insoluble and water-soluble biologically active polymers have been obtained on their basis. For the synthesis of water-insoluble polymers, it is often useful to employ cellulose—a readily available and cheap fibre-forming polysaccharide. The synthesis of biologically active cellulose derivatives and the preparation of biologically active cellulose materials constitutes one of the most interesting and promising aspects of the general problem of the chemical modification of cellulose [1]. The principal types of biologically active cellulose derivatives and their applications are considered below.

II. CELLULOSE DERIVATIVES WITH ANTIMICROBIAL PROPERTIES

The development of methods for the synthesis of cellulose derivatives with antimicrobial properties makes it possible to create a new type of cellulose fibre—antimicrobial fibre; as will be shown below, this can be made into articles which can be employed effectively in medicine and certain branches of industry. Studies on the synthesis of biologically active cellulose derivatives by chemical addition of bactericidal and fungicidal substances to the cellulose macromolecules have developed extensively during the last decade. This method yields fibrous materials, the antimicrobial activity of which is retained after they have been used for a long time. The methods for the determination of the antimicrobial activity of cellulose derivatives (in the form of fibrous materials) have been considered by Virnik and Mal'tseva [2].

One of the main questions arising in the development of methods for the preparation of antimicrobial cellulose fibres containing chemically combined bactericidal substances is that of which type of chemical bond between the bactericidal substance and the polymer ensures an antibacterial activity of the material. To elucidate this problem, a study was made *in vitro* of the antibacterial activity of modified cellulose fibres containing bactericidal substances of the acridine series, halogenophenols, halogens, and silver and copper ions, linked to the functional groups of the modified cellulose macromolecules in different ways [3-7]. Tables 1 and 2 present data for the antibacterial activity of cellulose derivatives with different structures.

Table 1. Effect of the structure of the cellulose derivative containing a chemically combined bactericidal substance on the antibacterial activity of the material [4-6].

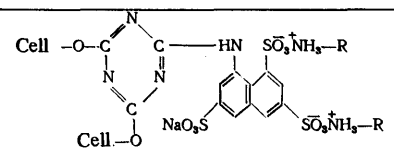
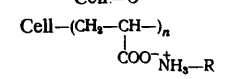
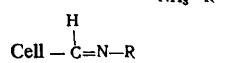
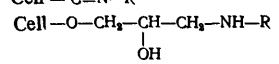
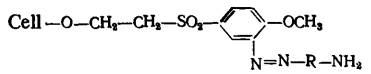
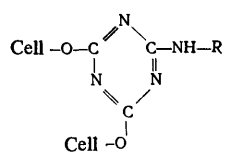
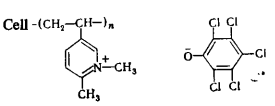
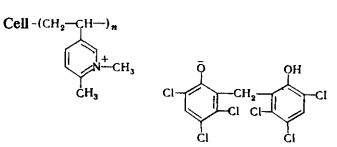
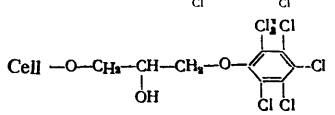
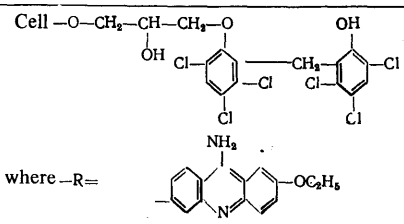
No.	Schematic structure of cellulose derivative	Antibacterial activity
1		+
2		+
3		+
4		−
5		−
6		−
7		+
8		+
9		−

Table 1. (contd.)

No.	Схе Schematic structure of cellulose derivative	Antibacterial activity
10		—

The results show that only those cellulose derivatives in which the bactericidal substance is attached to the polymer by ionic, labile covalent, or coordinate linkages possess antibacterial activity. An example of compounds of this type is provided by cellulose derivatives containing 6,9-diamino-4-ethoxyacridine (Table 1, Nos. 1 and 2) or halogenophenols (Table 1, Nos. 7 and 8). The second type includes cellulose derivatives containing 6,9-diamino-4-ethoxyacridine (Table 1, Nos. 3) or the residue of an *N*-halogenoamino-compound (Table 2, Nos. 5 and 6). Polymers of the third type include complexes of a graft copolymer of cellulose and poly-2-methyl-5-vinylpyridine (or polyvinylpyrrolidinone) with iodine (Table 2, Nos. 7 and 8). Compounds with a stable covalent bond between the cellulose derivative and the bactericidal substance do not exhibit antibacterial activity.

The antibacterial activity of cellulose derivatives in which silver ions are linked to various functional groups increases with the number of silver ions split off from the functional groups of the polymer in an aqueous medium.

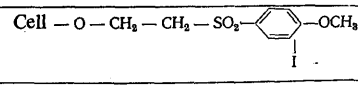
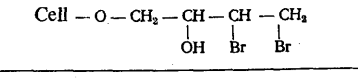
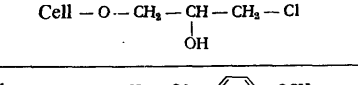
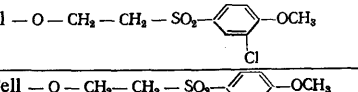
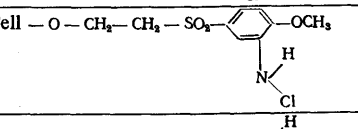
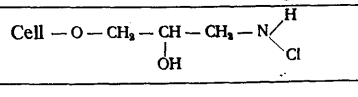
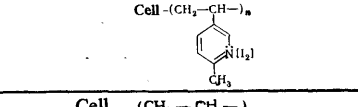
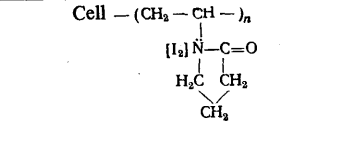
It has been shown [7] that, for the same iodine content, materials obtained from graft copolymers of cellulose and polyvinylpyrrolidinone have more pronounced antibacterial properties than materials obtained from a graft copolymer of cellulose and poly-2-methyl-5-vinylpyridine. This finding can be explained by the fact that owing to the *–I* effect of the carbonyl group in the graft polyvinylpyrrolidinone, the electron density of the nitrogen atom is lower than in the poly-2-methyl-5-vinylpyridine graft copolymer and therefore the stability of the complex formed with iodine by grafted polyvinylpyrrolidinone is also lower.

The sum total of the data quoted above lead to the conclusion that the antibacterial activity of the cellulose derivatives investigated is due to the fact that bactericidal substances interact with the microbial cell after being split off from the polymer carrier.

Cellulose derivatives with antimicrobial properties have been synthesised on the basis of two main principles: (1) by treating cellulose with bactericidal and fungicidal substances containing reactive functional groups capable of interacting with the hydroxy-groups of cellulose or (2) by a preliminary chemical modification of cellulose in order to introduce into its macromolecules reactive functional groups which are subsequently used to combine with bactericidal and fungicidal substances.

The bactericidal and fungicidal substances employed in the synthesis of antimicrobial cellulose derivatives have been mainly (1) metal ions, (2) quaternary ammonium bases, (3) antibiotics, (4) halogens, (5) phenol derivatives,

Table 2. Effect of the structure of the cellulose derivative containing chemically combined halogens on the antibacterial activity of the material [5].

No	Schematic structure of cellulose derivative	Antibacterial activity
1		—
2		—
3		—
4		—
5		+
6		+
7		+
8		+

(6) organometallic compounds, and (7) compounds of the nitrofuran series.

It has been suggested [8] that antimicrobial cellulose derivatives containing metal cations and quaternary ammonium bases be obtained by treating cellulose with compounds having the general formula A—B—A, where A $\overset{\oplus}{X}[BC^+]$ are groups capable of reacting with the cellulose hydroxyls (for example, N=C=O, CHO, and $CH\!-\!CH_2$), B is the $\diagdown O \diagup$ residue of an aliphatic, aromatic, or heterocyclic compound, X is an ionogenic group, (for example, COO^- and SO_3^-), and $[BC^+]$ is a cation with antimicrobial properties (for example, Ag^+, Hg^{2+}, Cu^{2+}, Zn^{2+}, and R_4N^+). Salts of hydroxymethyl derivatives of levulinhydantoin, *N*-(carboxymethylaminomethyl)melamine, and sulphomethylurea [8] as well as salts of 1,3-di(hydroxymethyl)-5-sulphoethyltetrahydro-1,3,5-triazin-2-one, 5-(*m*-carboxyphenyl)-1,3-di(hydroxymethyl)tetrahydro-1,3,5-triazin-2-one, and 5-carboxymethyl-1,3-di(hydroxymethyl)tetrahydro-1,3,5-triazin-2-one [9] can be used as compounds of this kind. To synthesise derivatives with antimicrobial properties, cellulose (in the form of a fabric) is treated for a short time at 20°C with an aqueous solution of the salt of one of the above compounds containing an acid catalyst, after which the reaction is carried out at 100–150°C.

Antimicrobial derivatives of cellulose containing metal and quaternary ammonium cations and also antibiotics with basic groups have been synthesised from a graft copolymer

of cellulose and poly(acrylic acid) or poly(methacrylic acid)[10,11], a weakly substituted carboxymethyl ether of cellulose[12,13], 2-carboxy-2-chloroethyl ether of cellulose[14], cellulose sulphate[15], cellulose treated with 2,4-dichloro-6-[*N*-(3′,6′,8′-trisulpho-1′-naphthylamino)]-1,3,5-triazine[11,16,17] or dyed with active dyes containing ionogenic groups[17,18] and cellulose phosphate[19] and xanthate[20]. In most of the above studies ionogenic groups were initially introduced into the cellulose macromolecule and then used to combine with the bactericidal substance. An example is provided by the synthesis of antimicrobial cellulose derivatives on the basis of the graft copolymer of cellulose and poly(acrylic acid):

$$\text{Cell}-(CH_2-\underset{\underset{COONa}{|}}{CH}-)_n + [BC^+]\,A^- \rightarrow \text{Cell}-(CH_2-\underset{\underset{COO^-[BC^+]}{|}}{CH}-)_n + Na^+A^-,$$

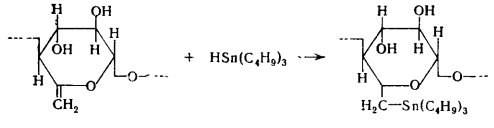
where $[BC^+] = Ag^+$, Cu^{2+}, R_4N^+, or NH_3^+ - neomycin and $A^- = NO_3^-$, SO_4^{2-}, or Cl^-. The addition of bactericidal cations to the carboxy-groups of viscose fibre has been described[21,22].

Graft copolymers of cellulose with poly-2-methyl-5-vinylpyridine, polyvinylpyrrolidonone, and other polymers containing lactam rings have been used to synthesise antimicrobial cellulose materials containing iodine[6,23,24]. Cellulose derivatives containing chemically combined antimicrobial substances belonging to the group of chlorinated phenols have been synthesised on the basis of the graft copolymer of cellulose and poly-2-methyl-5-vinylpyridine[6,11].

An organotin derivative of cellulose was synthesised for the first time by the reaction of ethynyldeoxycellulose and triphenyltin bromide according to the following scheme[25]:

$$\{C_6H_7O_2(OH)_{3-x}(C{\equiv}CNa)_x\}_n + BrSn(C_6H_5)_3 \rightarrow \{C_6H_7O_2(OH)_{3-x}[C{\equiv}C-Sn(C_6H_5)_3]_x\}_n$$

The synthesis of tin-containing deoxy-derivatives of cellulose, cellulose ethers, and graft copolymers of cellulose has now been achieved[26-28]. Tributylstannyldeoxycellulose has been synthesised by the radical addition of tributyltin hydride to 5,6-celluloseen according to the reaction



and by nucleophilic substitution via the reaction of cellulose iodotoluene-*p*-sulphonate or toluene-*p*-sulphonate with tributylstannyl-lithium[28].

When cellulose toluene-*p*-sulphonate was employed as the starting material, the reaction took place according to the mechanism

$$\{C_6H_7O_2(OH)_{3-x}(O-SO_2-C_6H_4-CH_3)_x\}_n + LiSn(C_4H_9)_3 \rightarrow \{C_6H_7O_2(OH)_{3-x}[Sn(C_4H_9)_3]_x\}_n$$

Tributylstannylthiodeoxycellulose has been obtained by treating mercaptodeoxycellulose with bis(tributyltin) oxide[28].

Tin-containing cellulose ethers have been synthesised from trialkyltin oxides and hydroxides and cellulose ethers the functional groups of which contain a mobile hydrogen atom [carboxymethyl, 2-hydroxy-3-mercaptopropyl, 2-(3′-mercapto-4′-methoxyphenyl), sulphonylethyl, and propargyl derivatives] and also by the radical addition of tributyltin hydrides to allyl and propargyl ethers of cellulose[26,28]. The reaction took place according to the following mechanisms:

$$\text{Cell}-O-CH_2-COOH + HOSnR_3 \rightarrow \text{Cell}-O-CH_2-C(=O)-O-SnR_3$$

$$\text{Cell}-O-CH_2-\underset{\underset{OH}{|}}{CH}-\underset{\underset{SH}{|}}{CH_2} + HOSnR_3 \rightarrow \text{Cell}-O-CH_2-\underset{\underset{OH}{|}}{CH}-\underset{\underset{S-SnR_3}{|}}{CH_2}$$

$$\text{Cell}-O-CH_2-CH_2-SO_2-C_6H_3(SH)-OCH_3 + HOSnR_3 \rightarrow \text{Cell}-O-CH_2-CH_2-SO_2-C_6H_3(S-SnR_3)-OCH_3$$

$$\text{Cell}-O-CH_2-C{\equiv}CH + HOSnR_3 \rightarrow \text{Cell}-O-CH_2-C{\equiv}C-SnR_3$$

$$\text{Cell}-O-CH_2-CH{=}CH_2 + HSnR_3 \rightarrow \text{Cell}-O-CH_2-CH_2-CH_2-SnR_3$$

$$\text{Cell}-O-CH_2-C{\equiv}CH + HSnR_3 \rightarrow \text{Cell}-O-CH_2-CH{=}CH-SnR_3$$

(R = alkyl residue)

Graft copolymers of cellulose and poly(trialkylstannyl acrylate) or poly(triarylstannyl acrylate) have been synthesised by the reactions of the graft copolymer of cellulose and poly(acrylic acid) with trimethyl-, triethyl-, and triphenyltin hydroxides and also with bis(tributyltin) oxide. The structures of some of the tin-containing cellulose derivatives synthesised were confirmed by Mössbauer spectroscopy[29]. Data on the effect of the structure and amount of the organotin compound chemically combined with cellulose on the antimicrobial properties of the polymer have been published[28,30].

The synthesis of tin-containing derivatives with the general formula cell $-O-SnR_3$ by treating cellulose (or its ether) with alkoxytrialkyltin or the potassium alkoxide derivative of cellulose with trialkyltin chloride has been described[31-33]. However, organotin compounds of the type R_3SnOR' are readily hydrolysed even by atmospheric moisture. It is noteworthy that a high antimicrobial activity of organotin and organomercury derivatives of cellulose can be achieved by introducing into the macromolecules of modified cellulose a very small amount of tin or mercury (0.1–0.2% of the weight of cellulose).

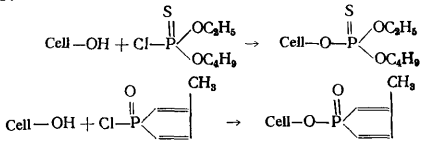
To synthesise organotin derivatives, cellulose ethers and esters containing the residues of unsaturated aliphatic, aromatic, or heterocyclic compounds [2-hydroxybut-3-enyl[34,35], allyl[36], benzyl[37], and 2-(3′-amino-4′-methoxyphenyl)sulphonylethyl[38] cellulose ethers as well as cellulose esters with methacrylic[39,40], sorbic[41,42], oleic[43,44], salicyclic[45,46], furancarboxylic[47,48], and cinnamic[44,47] acids] were mercurated (usually with mercury acetate). As an example one may quote the mercuration of cellulose methacrylate:

$$\text{Cell}-O-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_3}{|}}{C}=CH_2 \xrightarrow{Hg(OOC-CH_3)_2} \text{Cell}-O-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_3\ OH}{|}}{C}-\underset{\underset{HgOOC-CH_3}{|}}{CH_2}$$

Mercury-containing cellulose derivatives have also been synthesised by mercurating with mercury acetate cellulose dyed by active dyes[49], graft copolymers of cellulose with polyacrylonitrile and poly-2-methyl-5-vinylpyridine[50] as well as styrene and benzyl methacrylate[51]. In the preparation of antimicrobial bast fibres, there is no need for a preliminary modification of the fibres; in this case the initial bast fibres can be mercurated to impart to them antimicrobial properties[52,53], since the lignin macromolecules, which enter into the composition of such fibres, contain aromatic residues, which readily interact with mercury salts.

The synthesis of antimicrobial cellulose derivatives containing antimony from cellulose and antimony(III) acid chlorides or esters have been described [54]. The structures of the compounds synthesised may be represented schematically by the formulae Cell–O–$SbCl_2$ and Cell–$OSb(OC_2H_5)_2$. According to patent data [55-57], various phosphorus-containing cellulose derivatives have been synthesised by treating cellulose with phosphorochloridothioates, phosphorochloridothioites, phosphoroisothiocyanatidates, and phosphoroisothiocyanatidites and also with chlorophosphacyclopentenes in organic solvents.

The reactions of cellulose with *O*-n-butyl *O*-ethyl phosphorochloridothioate and 1-chloro-1-oxo-3-methylphosphacyclopentene proceed according to the following mechanisms:



Antimicrobial derivatives of cellulose containing residues of a nitrofuran bactericidal substance have been obtained by the reaction of partly hydrolysed cellulose triacetate with β-(5-nitrofuryl)acrolein [58,59]. According to the literature, esters of cellulose and unsaturated aliphatic acids [60-62] and also 2-carboxy-2-chloroethyl ether of cellulose [14] acquire antimicrobial properties on bromination or substitution with thiocyanate groups [14].

The properties and possible applications of antimicrobial cellulose fibre materials were investigated in detail in recent years. Under practical conditions, the most detailed studies have been made on the antimicrobial cellulose fibre materials containing chemically combined copper or silver ions and also hexachlorophene. It has been shown [30,63-66] that antimicrobial cellulose fibre materials containing various chemically combined bactericidal substances suppress the development of pathogenic microorganisms or postoperative infections. Experiments carried out under the conditions of the surgical clinic of the Antarctic station (Vostok) and also in undertakings of the food industry showed that, when clothing made of antimicrobial fibres containing chemically combined hexachlorophorene and silver or copper ions is used, the population of microorganisms on human skin and on the clothing itself is reduced severalfold compared with the values obtained when ordinary clothing is worn [67-69]. It has been shown [70,71] that, when surgical masks made of antimicrobial muslin containing chemically combined silver ions are worn, the frequency of infection after operative wounds by the microflora from the surgeon's nasopharynx is approximately three times smaller than under the conditions when a mask of ordinary muslin is worn. This suggests that articles made of antimicrobial fibres can be used as a means whereby the microbial population can be reduced and the risks of microbial infection in surgical clinics, in undertakings of various branches of industry, and also in prolonged expeditions can be lowered. Clothing made of antimicrobial fibres can be used not only under specific conditions when the risk of infection by microorganisms is great or the conditions of personal hygiene are difficult but also in ordinary life.

Modified cellulose materials containing chemically combined chlorophenols, organomercury and organotin compounds, or iodine are resistant to the action of mildew and suppress the development of pathogenic fungi causing fungal affections of the feet [30,72,73]. Such fibres can apparently be used to manufacture stockings, socks, and shoe insoles for use in the prophylaxis of common fungal affections.

It has been shown [74] that antimicrobial cellulose fabrics containing chemically combined copper or silver ions can be usefully employed for the packing of sterile surgical instruments. Packing made of antimicrobial fabrics ensures that the surgical instruments remain sterile for several months. The employment of such packing is particularly important for first aid posts and also in rural and mountainous localities.

At the present time antimicrobial materials containing chemically combined hexachlorophene and copper or silver ions have been recommended by the Ministry of Health of the USSR for use in medical practice and their manufacture has begun.

Antimicrobial fibres and non-woven filtering materials made from them can be usefully employed for the sterilisation of air supplied to the fermentation units in the manufacture of antibiotics and in the apparatus for the preparation of blood components [75-78]. In the filtration of air, micro-organisms are not only mechanically trapped by the filtering materials, but also destroyed by the action of the bactericidal substances in the fibres. Non-woven antimicrobial filtering materials ensure the necessary degree of purification and sterilisation of air over a period of several months and their employment increases the reliability of the operation of the filter. In contrast to the filtering materials made of the usual non-antimicrobial fibres, they do not require periodical sterilisation [76,77]. It has been shown [79] that antimicrobial cellulose fabrics containing silver ions bound to the carboxy-groups of grafted poly(acrylic acid) can be used to disinfect river water.

Antimicrobial cellulose materials will presumably prove useful also in other fields.

III. CELLULOSE DERIVATIVES WITH HAEMOSTATIC PROPERTIES

The problem of the arrest of blood flow in surgical operations, particularly of blood flow from small vessels, is important in surgery and still cannot be regarded as finally solved. The use of haemostatic preparations greatly simplifies the technique of the surgical operation and shortens its duration. Despite the positive results achieved when expensive and scarce haemostatic preparations made from donated blood are employed, the search for new haemostatic preparations, based on polysaccharides including cellulose rather than on proteins, is being continued.

Among haemostatic preparations obtained on the basis of cellulose, the so called monocarboxycellulose, synthesised at the end of the 1930's by oxidising cellulose with nitrogen oxides [80], has been investigated in greatest detail. According to the literature [81-84], during the Second World War oxidised cellulose already began to be used in surgical practice as a haemostatic preparation absorbable by the organisms.

Reviews [83,84] and other publications [85-95] have been devoted to monocarboxycellulose. On oxidation by nitrogen oxides, carboxy-groups are formed in the cellulose macromolecule mainly at the sixth carbon atom of the elementary unit. Apart from the main process involving the oxidation of primary hydroxy-groups, secondary hydroxy-groups are also oxidised, which results in the formation of keto- and then carboxy-groups with rupture of the pyran ring [89,90]. Some of the carboxy-groups react with the

hydroxyls in the same cellulose macromolecule (with formation of lactones) or with the hydroxyls of another macromolecule (with formation of esters)[88]. The products always contain a certain amount of nitrate groups. The presence in the oxidised cellulose macromolecule of elementary units containing simultaneously carboxy- and carbonyl groups is responsible for the low resistance of the oxidised cellulose macromolecule to the action of water at an elevated temperature[96]. The sterilisation of cellulose materials oxidised with nitrogen oxides by treatment in an autoclave (this sterilisation procedure is common in surgical clinics) is impossible, since treatment with steam leads to complete degradation of monocarboxycellulose. Haemostatic preparations made from monocarboxycellulose are sterilised by γ-radiation[97,98] or are prepared under sterile conditions[99].

It has been suggested[83,84,96,98] that the calcium salt of monocarboxycellulose, which is prepared by treating oxidised cellulose for a long time with an aqueous solution of calcium chloride, be employed as a haemostatic material. Experiments showed that the calcium salt is an effective haemostatic preparation. During recent years a number of patents and publications have appeared[98,100-104] in which new forms of haemostatic preparations made from oxidised cellulose or various improvements to the technological process for the manufacture of monocarboxycellulose have been proposed. The addition of various antimicrobial substances (tubazid, kanamycin, rivanol, norsulphazol, and streptomycin) to the functional groups of cellulose oxidised by nitrogen oxides and the use of the resulting compounds in surgical operations have been described[97,104-108]. According to the literature, the employment of absorbable haemostatic preparations with antimicrobial activity simplifies the technique of surgical operations and promotes the prophylaxis of post-operative complications. A prolonged action of the above medicinal substances is achieved at the same time.

Nowadays cellulose oxidised by nitrogen oxides is used in surgical practice but the wide-scale employment of this haemostatic preparation which can be absorbed by the organisms is difficult owing to the complicated apparatus required for the technological preparation of monocarboxycellulose and particularly its calcium salt and by the necessity to manufacture the haemostatic material under sterile conditions or the employment of γ-radiation for its sterilisation.

In the patent literature, it has been suggested that haemostatic preparations in the form of tablets consisting of salts of the carboxymethyl ether of cellulose[109] and also salts of cellulose sulphate and ethers of cellulose and aliphatic hydroxyacids be employed to make solutions for the impregnation of bandaging materials[110-112].

Several years ago, a new haemostatic cellulose material, non-absorbable in the organism, which consists of the calcium salt of the graft copolymer of cellulose and poly(acrylic acid), was proposed[113-117]. In contrast to the haemostatic preparations of monocarboxycellulose, this material is sterilised in an autoclave where its haemostatic properties and stability are hardly altered. The graft copolymer of cellulose and the calcium salt of poly(acrylic acid) was synthesised by three methods[113-117]: (1) by grafting poly(acrylic acid) to cellulose and subsequently treating the resulting graft copolymer with a solution of calcium hydroxide or the calcium salt of a weak acid: (2) by grafting poly(methyl acrylate) to cellulose and then hydrolysing the ester groups of the resulting copolymer with a solution of calcium hydroxide: (3) by grafting the calcium salt of poly(acrylic acid) to cellulose using calcium acrylate as the monomer.

The third method of preparation of the haemostatic material is the most rational, since, in contrast to acrylic acid and methyl acrylate, calcium acrylate is involatile, which greatly simplifies the technology of the synthesis. The synthesis of the graft copolymer of cellulose and poly(acrylic acid) using calcium acrylate as the monomer has been described in a number of studies[118-120]. Wide-scale clinical tests showed that the haemostatic material is useful in surgical practice and it has been recommended by the Ministry of Health of the USSR for use in medicine. Haemostatic muslin is now being manufactured by a continuous process[117].

IV. GRAFT COPOLYMERS OF CELLULOSE AND BIOLOGICALLY ACTIVE PROTEINS

Water-insoluble graft copolymers of cellulose and biologically active protein are of considerable theoretical and practical interest. The synthesis of graft copolymers of cellulose with enzymes and antigens has been most thoroughly investigated. These compounds are obtained by the interaction of cellulose derivatives containing various reactive groups with proteins. However, one must bear in mind that under these conditions several reactive groups of one cellulose macromolecule can interact with several functional groups of one protein macromolecule. There are at present no reliable data concerning the structures of these compounds and therefore we shall henceforth refer to them as graft copolymers.

Water-insoluble graft copolymers of cellulose and enzymes can be used as specific catalysts and can be removed from the reaction sphere at any instant. The employment of these copolymers permits the solution of many problems, which is impossible when the usual water-soluble enzymes are used, such as, for example, the isolation of pure products of the enzymatic decomposition of a substrate, the activation of the enzyme with subsequent complete removal of the activating substance, and sorption with subsequent isolation and investigation of enzyme inhibitors[121]. Water-insoluble graft copolymers of cellulose and antigens, which have been called immunosorbents, are used to sorb antibodies for their quantitative determination and isolation in a pure form with the aim of subsequent investigation of their structures and properties[122,123]. In the synthesis of water-insoluble graft copolymers containing antigens, it is more useful to employ hydrophilic polymers, particularly cellulose, and not hydrophobic synthetic polymers, for example polystyrene, since non-specific sorption of proteins on hydrophilic cellulose materials is much weaker than on hydrophobic synthetic polymers[123].

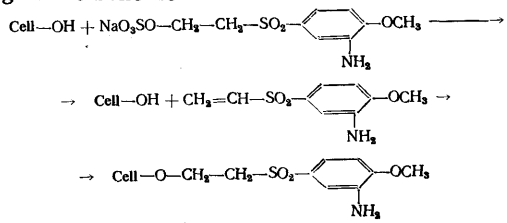
The initial cellulose derivatives employed in the synthesis of the above graft copolymers are cellulose ethers and esters containing aromatic amino-groups, the carboxymethyl ether of cellulose, the ether of cellulose and cyanuric acid chloride, the 3-chloro-2-hydroxypropyl ether of cellulose, the ester of cellulose and monobromoacetic acid, and cellulose derivatives containing epoxy-rings.

The synthesis of a graft copolymer of cellulose and a protein using as the starting material a cellulose derivative containing aromatic amino-groups was achieved for the first time at the beginning of the 1950's.[124] In this investigation the *p*-aminobenzyl ether of cellulose was diazotised and then introduced into the azo-coupling reaction with the antigen. Subsequently the ester of cellulose and

p-aminobenzoic acid and *m*-aminobenzyloxymethylcellulose were used, together with the *p*-aminobenzyl ether, for coupling with enzymes and antigens [124-137]. The cellulose derivatives used in the synthesis of graft copolymers were obtained by treating cellulose with *p*-nitrobenzoyl chloride, *p*-nitrobenzyl chloride, or *N*-(*m*-nitrobenzyloxymethyl)pyridinium chloride followed by reduction of the nitro-groups.

A simpler method for the synthesis of cellulose derivatives containing aromatic amino-groups was proposed in a study [138] where cellulose was treated in an aqueous medium in the presence of sodium carbonate with the sulphate esters of 2-amino-4-(β-hydroxyethyl)sulphonylanisole or 4-(β-hydroxyethyl)sulphonylaniline. 2-(3′-Amino-4′-methoxyphenyl)- and 2-(4′-aminophenyl)-sulphonylethyl ethers of cellulose were synthesised in this way.

The first of the above cellulose ethers was synthesised according to the scheme



The amino-derivatives of cellulose obtained were used to synthesise graft copolymers of cellulose with antigens and enzymes [139-143].

It has been shown [128,129] that the amount of antigen adding to cellulose can be increased sharply by employing a highly dispersed suspension of the cellulose ether containing aromatic amino-groups. The suspension can be obtained by the reprecipitation of the above ether from a cuprammonium solution. Later it was demonstrated [144] that this highly dispersed suspension can also be prepared by substituting the cellulose ether containing aromatic amino-groups with xanthate (in order to obtain a cellulose ether-ester soluble in alkali) with subsequent decomposition of the ester groups in the presence of sulphuric acid. The amounts of antibodies sorbed on the immunosorbents prepared in this way is 500–1000 mg per gramme of immunosorbent and the degree of non-specific sorption of the protein amounts to only 1 mg per gramme of immunosorbent [123].

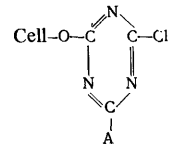
Recently it has been suggested that graft copolymers of cellulose and biologically active proteins be synthesised from 3-(4′-aminophenoxy)-2-hydroxypropyl [145,146] and *N*-[3′-(*p*-aminophenyl)butyryl]-2-aminoethyl [147,148] ethers of cellulose. However, one should note that these methods for the introduction of amino-groups into the cellulose macromolecule have no advantages over those described above.

One must also note that in immunosorbents where the protein is attached to the polymeric carrier via azo-groups a certain amount of antigen is gradually split off [149]. This is probably due to the formation during the synthesis of diazoamino-compounds, which are gradually cleaved during the storage of the immunosorbent.

The carboxymethyl ether of cellulose was synthesised for the first time for coupling with protein by Michael and Evers [150], the azide obtained from the hydrazide of the carboxymethyl ether of cellulose being introduced into the reaction. Later this method was employed to synthesise the graft copolymers of cellulose with various antigens and enzymes [130,133,151-158]. It was suggested [159-161] that the reaction between carboxymethylcellulose and protein be carried out in the presence of carbodi-imide derivatives. This method of synthesis for graft copolymers, which is simpler than the procedure involving the preparation of the azide of carboxymethylcellulose as an intermediate, has been used recently to synthesise various graft copolymers of cellulose and biologically active proteins [162-165].

The reaction between carboxymethylcellulose and protein can also be achieved in the presence of the Woodward reagent—*N*-ethyl-5-(3′-sulphophenyl)isoxazole [166].

The use of the ester of cellulose and monobromoacetic acid [167-171], the 3-chloro-2-hydroxypropyl ether of cellulose [143,149], and the ester of cellulose and cyanuric acid chloride with the general formula



where A = O–Cell, OH, NH_2, NH–CH_2COOH, etc. [143,149,172-175], as starting materials in the synthesis of graft copolymers of cellulose and biologically active proteins is of considerable interest. The synthesis of these cellulose derivatives is comparatively simple and the reaction between them and proteins can be achieved more simply than with cellulose derivatives containing aromatic amino-groups or carboxy-groups. A cellulose ether containing amino-groups has been treated [176] with an active dichlorotriazine dye (one of the chlorine atoms in the dye molecule reacted with the cellulose ether) and was then allowed to react with the protein (the second chlorine atom in the dye molecule reacted with the functional groups of the protein).

The graft copolymer of cellulose and poly(glycidyl methacrylate) [149], the 3-(4′-isothiocyanatophenoxy)propyl ether of cellulose, and the 3-allyloxy-2-hydroxypropyl ether of cellulose mercurated with mercury acetate [177] were also employed in recent years for coupling with biologically active proteins. In the last case, the mercapto-groups of the protein react with the mercury atoms attached to the alkyl residues of the cellulose ether. Cellulose has been treated [178,179] with cyanogen bromide in an alkaline medium and then a protein was coupled to the iminocarbonate groups formed. The synthesis of a graft copolymer of cellulose and a protein by treating the aminoethyl ether of cellulose and a protein with glutaraldehyde has also been described [180,181].

Likewise, the coupling of enzymes to the carboxymethyl and diethylaminoethyl ethers of cellulose and its phosphate ester via ionic linkages has been achieved [182]. When enzymes are coupled to cellulose by covalent linkages, their activity as a rule decreases. This may be due to the modification of the active centre of the enzyme, a change in the conformation of the enzyme macromolecule, and steric hindrance arising when the enzyme linked to cellulose interacts with the substrate (particularly a high-molecular-weight substrate).

It has been shown [130] that the activity of the graft copolymer of cellulose and its enzymes in relation to a polymeric substrate can be lower than in relation to low-molecular-weight model compounds. This phenomenon can probably be accounted for by steric factors. The same cause may be used to explain the finding [130] that the activity of chymocrypsin linked to carboxymethylcellulose increased with the degree of swelling of the carboxymethylcellulose and reached a maximum when a water-soluble carboxymethylcellulose was employed.

It has been shown in several studies that the addition of enzymes to cellulose not only permits the synthesis of specific catalysts which can be readily removed from the reaction sphere but also reduces the inactivation of the enzyme by external factors and its self-degradation [121,130,145,146,152,154,163]. Interesting results were obtained [137] in the immunisation of animals by the graft copolymer of cellulose and an antigen. In this case a greater amount of antibodies was formed in the animal organism than in immunisation by the soluble antigen. On repeated (after several months) immunisation of animals, to which the graft copolymer of cellulose and the antigen had been administered previously, with the soluble antigen, an extremely intense and prolonged synthesis of antibodies was observed. Their amount exceeded by a factor of 10–20 the amount of antibodies formed on repeated immunisation when the first immunisation was with the soluble antigen. Despite a number of attempts [134,161,167], hitherto it has not been possible to synthesise a graft copolymer of cellulose and an antibody capable of sorbing considerable amounts of the antigen [123].

Graft copolymers of cellulose and enzymes can be used in laboratory practice for preparative and analytical purposes (including clinical analyses), for the simulation of enzymatic processes in living organisms where the enzymes are frequently linked to cellular structures, and in industry and medicine [182]. According to the literature [182], the industrial manufacture of certain cellulose derivatives (bromoacetylcellulose, the *p*-aminobenzyl ether of cellulose, and the carboxymethyl ether of cellulose and its hydrazide), which are employed for coupling to biologically active proteins, has already begun.

As shown above, the synthesis and properties of graft copolymers of cellulose with enzymes and antigens has been investigated in greatest detail. However, bearing in mind that at the present time the advances in the chemistry of macromolecular compounds are being increasingly used for the solution of problems in biochemistry, biology, and medicine, one may expect that in the next few years it will be useful to synthesise water-insoluble graft copolymers of cellulose with other biologically active proteins and polymers incorporating polypeptide chains. The chemical fixation of the above polymers on cellulose should be achieved via functional groups which do not affect their biological activity. Therefore an increase of the number of cellulose derivatives containing various reactive groups, the development of simpler methods for synthesising graft copolymers of cellulose and proteins, and also detailed studies of the interaction of cellulose derivatives with biologically active proteins and of the effect of the structure of graft copolymers on their properties are of great interest.

V. MEDICAL APPLICATIONS OF CELLULOSE DERIVATIVES CONTAINING IONOGENIC OR COMPLEX-FORMING GROUPS

A much smaller number of studies have been made on the possibility of employing in medicine cellulose derivatives containing ionogenic or complex-forming groups. In the middle 1950's, an attempt was made to employ a plasma substitute solution of carboxymethylcellulose polymerised and substituted to different extents [183–185]. The optimum effect in experiments on animals was achieved when a 3% solution of the sodium salt of carboxymethylcellulose (degree of substitution about 0.8, degree of polymerisation 70–100) was used. However, these studies were not developed further because another polysaccharide (dextran) was found to be more useful for the preparation of a plasma substitute.

Cellulose sulphate as well as the sulphates of other polysaccharides are known to have anticoagulant properties (they prevent the clotting of blood); however, owing to its high toxicity, it has not been used for this purpose [186,187]. Cellulose phosphate can be employed as a filtering material for the extraction of calcium ions from the blood of donors for the purpose of its conservation [188–190]. In the presence of an excess of calcium in the organisms of patients, cellulose phosphate can be administered *per os* to sorb calcium ions in the intestine and to remove them from the organism [191–193].

Graft copolymers of cellulose with poly(acrylic acid) and poly-2-methyl-5-vinylpyridine (in the form of fabrics) have been used to prepare radioactive applicators for the treatment of skin diseases [194,195]. These materials are obtained by attaching to the acid and basic groups of the graft copolymers of cellulose the cations and anions of radioactive isotopes respectively. The addition of radioactive isotopic ions to the functional groups of graft cellulose copolymers ensures uniform emission from the entire surface of the radioactive applicator, which enhances its efficiency.

The antithrombogenic cellulose membranes for the dialysis of blood, obtained by coupling heparin to cellulose films with anion-exchange properties, have aroused considerable interest [196,197]. Cellulose materials with anion-exchange properties were obtained in the above investigations by treating cellulose with aziridine.

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Thus considerable advances have now been achieved in the synthesis of biologically active cellulose derivatives. Antimicrobial cellulose derivatives containing chemically combined bactericidal and fungicidal substances of different types have been synthesised. The effect of the structure of the cellulose derivatives containing chemically combined bactericidal substances on their antibacterial activity has been investigated. A study has been made of the properties and possible applications of antimicrobial cellulose fibre materials and their manufacture has begun. Methods have been developed for the synthesis of cellulose derivatives with haemostatic properties and their industrial manufacture has been achieved. Various procedures for the synthesis of the graft copolymers of cellulose and biologically active proteins (mainly enzymes and antigens) have been developed and some of the properties of such compounds have been investigated. Cellulose derivatives containing ionogenic or complex-forming groups have been used as independent biologically active compounds or as polymeric carriers for various biologically active substances.

One may expect that further development of studies in this field will yield interesting data and will make it possible to produce new biologically active polymeric materials based on cellulose.

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