BIOGLYCANS AS IMMUNOMODULATORS*

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A review is given of facts relating to the search for and study of bioglycan immunomodulators which are polysaccharides and glycoconjugates of natural origin and possess the capacity for stimulating or inhibiting the immunity of Man and animals to various diseases. The structural-chemical characteristics and biological properties of the following classes of bioglycan immunomodulators are discussed: bacterial lipopolysaccharides and peptidoglycans of marine organisms. Particular attention is devoted to marine invertebrates as an important source of active bioglycan immunomodulators. On the basis of information that has been accumulated on this question, it is suggested that the capacity for producing bioglycans with pronounced immunostimulating activity is a common property of marine invertebrates.

In recent decades, the search for regulators of immune processes has attracted the intense interest of a number of research workers. Substances exhibiting a regulating influence on the immune system have obtained the general name of immunomodulators. They include: immunostimulators, which intensify the immune response of the organism; immunoadjuvants, which are capable of enhancing the action of other substances on the immune system; and immunodepressants, which inhibit various immune processes and weaken or suppress the immune response of the organism. The stimulation of the immune system is very important in the prophylaxis and treatment of various diseases, while immunodepressants play a decisive role in the transplantation of organs and tissues with the aim of overcoming the barrier of immunological incompatibility.

Immunomodulators are substances of very different chemical natures and at the present time it is difficult to deduce any common factor in the search for them. An important place among them is occupied by bioglycans, by which we shall understand polysaccharides and glycoconjugates of natural origin (Fig. 1).

As stimulators of the immune system, the greatest interest is presented by the following groups of bioglycans:

lipopolysaccharides of Gram-negative bacteria and blue-green algae;
bacterial peptidoglycans;
glycans of fungi and lichens;
polysaccharides of yeasts; and
bioglycans of marine organisms.

The best known of the immunodepressants among the bioglycans is carragheenan — a sulfated polysaccharide derived from red algae.

Bacterial lipoglycans and peptidoglycans had already become an object of search for immunomodulators by the end of the fifties and the beginning of the sixties. The other groups of bioglycans have long attracted attention primarily as antitumoral preparations. In actual fact, many of them possess a well-marked antitumoral action. However, these preparations do not exhibit cytotoxic activity and show no direct action on tumor cells. In this connection, it has been shown that bioglycans act on malignant neoplasms not directly but by the normalization and activation of immune processes directed against the tumors. This has permitted first the hypothesis and then the experimental proof of the fact that bioglycans raise the resistance of the organism not only to malignant neoplasms but also to the most diverse dis-

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eases in combating which the immune system plays a fundamental role. Depending on many factors — the structure of the bioglycan, the method, time, and point of introduction into the organism, the nature of the disease, etc. — bioglycans act on different components of the immune system — the macrophages, the T- and B-lymphocytes, the antibody-forming cells, or complements — and, consequently, have an influence on the cell and humoral immunity systems.

Let us consider the individual groups of bioglycan immunomodulators in more detail.

BACTERIAL LIPOPOLYSACCHARIDES

We shall not dwell in detail on the many-sided action of the bacterial lipopolysaccharides (LPSs) on the immune system; it has been described fairly well and is widely known. The main disadvantage for the therapeutic use of the LPSs is their extremely high toxicity.

In this connection, the most promising for practical use has proved to be prodigiosan — a practically nontoxic LPS from the nonpathogenic bacterium *Bacterium prodigiosum*. It was isolated in 1962 by Z. V. Ermol'eva et al., and its physiological action was studied in detail by them [1] and by other groups of workers [2]. This showed that prodigiosan greatly increases the resistance of the organism to bacterial and viral infections. In addition, it appreciably affects the growth of tumors.

The intraperitoneal and intravenous administration of prodigiosan to animals with various experimental tumors (Ehrlich's carcinoma, sarcoma 180, Walker's carcinosarcoma, etc.) causes inhibition of the growth of the tumors. The optimum doses are 0.3-2.0 mg/kg live weight. An increase in the dose does not lead to an intensification of the antitumoral action. Prodigiosan leads to an increase in the level of protective reactions of the tumor-bearing animals and, in particular, greatly intensifies the phagocytic activity of the macrophages.

The chemical structure of prodigiosan has not so far been established. It consists of an LPS which contains 15-20% of lipid A; glucosamine, glucose, galactose, and mannose residues have been identified in the carbohydrate component.

In addition to prodigiosan, antitumoral activity is revealed by a large number of LPSs from the most diverse microorganisms [3], and although the immunostimulating activity of the majority of these LPSs has not yet been studied, by analogy with prodigiosan it may be assumed that the antitumoral activity of the LPSs is connected with an intensification of the immune processes of the tumor-bearing organism. As a confirmation of this can be taken information on the use as stimulators of the immune system of LPSs from the Gram-negative bacteria *Brucella abortus*, *Bordetella pertussis*, and *Serratia marcescens* [2, 3].

A basically new source of seriologically active LPSs has proved to be the blue-green algae. According to a modern classification, they belong to the bacteria and compose an individual class of cyanobacteria. In actual fact, they occupy an intermediate position between lower plants and microorganisms. In the fine structure of their cell membrane, the blue-green algae or cyanobacteria are similar to Gram-negative bacteria [5]. From this arose the idea of the possible presence of LPSs in the external membrane of the cells of the blue-green algae. And, in actual fact, in 1970 an LPS in many respects similar to the LPSs of Gram-negative bacteria was isolated from the blue-green alga *Anacystis nidulans* [6].

Then LPSs were detected in a number of other blue-green algae, and in 1979 the first review devoted to this question appeared [7]. We have isolated and characterized LPSs from blue-green algae of the genus *Phormidium* and from *Microcystis aeruginosa* [9]. Quite recently LPSs have been isolated from eight strains of *Synechococcus* cyanobacteria [10] and from four strains of *Synechocystis* [11]. The LPSs of the blue-green algae have a number of peculiar features. In particular, the majority of the specimens studied are characterized by the absence, or a low content, of 2-keto-3-deoxyoctonic acid (KDO) and of D-glycero-L-mannoheptose. The absence of KDO can explain the increased resistance of the LPSs of the blue-green algae to cleavage into lipid A and a polysaccharide component under the conditions of mild acid hydrolysis, which is common for the LPSs of the Gram-negative bacteria. It is interesting that the amount of lipid A in the LPSs of blue-green algae is usually comparatively low and fluctuates in the range below 10%. Furthermore, in lipid A the β-hydroxymyristic acid that is an obligatory component of the lipid A of the LPSs of Gram-negative bacteria is not infrequently absent. However, the presence of glucosamine and of various fatty acids (including hydroxy acids) serves as a confirmation of the presence of lipid A in the LPSs of the blue-green algae.

The LPSs of the blue-green algae are distinguished by a low toxicity, which is a very valuable property and opens up prospects for their use in practical medicine. According to the limited amount of information available, their serological activity is comparatively low, but the influence of the LPSs of the blue-green algae on the components of the immune system has clearly been studied inadequately at the present time.

BACTERIAL PEPTIDOGLYCANS

Information on the immunomodulatory properties of the peptidoglycans (PGs) and of their soluble fragments has been obtained from investigations in the last two decades which have shown that live mycobacteria can intensify the immune response of the organism to foreign antigens. In the well-known complete Freund's adjuvant [12], the cells of mycobacteria greatly enhance the humoral response to any soluble foreign antibody.

The first step in determining the structure of the adjuvant from killed mycobacteria was made in 1958 by French workers [13] led by E. Lederer. They showed that the chloroform-soluble wax D from mycobacteria possesses adjuvant activity. Later [14, 15] it was established that wax D forms the skeleton of the cell membrane and consists of a PG linked to an arabinogalactan, which is esterified with mycolic acid (Fig. 2).

In 1959, the Bulgarian worker I. G. Bogdanov [16] showed that a crude preparation from the lactic acid bacillus *Lactobacillus bulgaricus* possesses a pronounced antitumoral activity. Subsequently, it was established that the active principle of the preparation is a peptidoglucan from the cell membrane of the bacterium [17].

In 1963, Mathé et al. (cited in [18]) were the first to make successful use of the cells of the bovine tuberculosis bacillus *Mycobacterium bovis* (better known as BCG, bacillus Calmette-Guérin) in order to stimulate the immune system after the preliminary chemotherapy of patients with acute lymphoid leukemia.

At the same time, the immunostimulating activity of the cell membranes of anaerobic Gram-positive microorganisms of the genus *Corynebacterium* (according to a modern classification, *Propionibacterium*) was established [19]. The cell membranes of a number of other, mainly Gram-positive, bacteria also proved to be active immunostimulators and adjuvants [18-20]. In all cases, the active principle is represented by the PGs of the cell membrane.

In the course of the following decades, the structure of the PGs was studied intensely and the minimum fragment responsible for their adjuvant activity was determined [21, 22].

In 1972 [15] it was found that lysozyme, which cleaves purified cell membranes of *Mycobacterium smegmatis*, gives a soluble adjuvant (mol. wt. 20 thousand daltons) which is a PG linked to an arabinogalactan (Fig. 2).

Similar compounds have been obtained by the aqueous extraction of defatted cells of *M. tuberculosis* with subsequent precipitation by ammonium sulfate and chromatography on DEAE-cellulose [23], and also by the hydrogenolysis of defatted BCG cells [24]. An active PG fraction has also been isolated from defatted cells of *Nocardia opaca* [25].

It was soon shown that the neutral polysaccharide is not a necessary component for adjuvant activity: In actual fact, the soluble PGs deprived of the neutral arabinogalactan and obtained by different methods [22] did not differ in activity from the purified water-soluble adjuvants of the mycobacteria and Gram-negative bacteria.

After the structure of the PGs had been established, it was found that the repeating subunit (Fig. 2) of the majority of PGs is capable of replacing mycobacteria in the complete Freund's adjuvant. As can be seen from Fig. 2, the third amino acid of the repeating subunit is meso-diaminopimelic acid — which is found in the PGs of mycobacteria, *Lactobacillus plantarum*, and Gram-negative bacteria — or lysine — in *Staphylococcus aureus*. The absence of an amide group in the D-glutamic acid residue and the presence of an acetyl or glycolyl residue in the amino group of muramic acid does not affect activity. The terminal D-alanine residue in the tetrapeptide likewise has no fundamental value for activity. However, the glycopeptide structure is necessary, since the free disaccharide and the free tetrapeptides have no activity. It was then found that the N-acetyl-D-glucosamine and the third amino acid residues can also be eliminated without changing the activity, but the N-Ac-Mur-L-Ala fragment proved to be inactive. In this way it was shown that the muramyldipeptide (MDP) is the minimum subunit of the PG retaining adjuvant activity (Fig. 3). This was shown almost simultaneously by French [26] and Japanese [27] workers.

MDP has been successfully used as a powerful adjuvant for the most diverse antigens such as ovalbumin, bovine serum albumin, etc. [28]. It is capable of enhancing the efficacy of vaccines against many diseases, which is apparently connected with a stimulation of nonspecific immunity.

Several hundreds of derivatives and analogs of MDP have been synthesized [22]. They have been studied as immunomodulators. At the present time, the dependence of the physiological action on elements of the structure and mechanism of the action of MDP and its analogs is being elucidated. The immunostimulating action of MDP is connected directly with the activation of the T- and B-lymphocytes. In addition, MDP and its active analogs have a substantial influence on many functions of macrophages. Nevertheless, the mechanism of the molecular interaction of MDP with the receptors of immunoactive cells remains obscure [22].

The opinion has been expressed [22] that MDP and its analogs will be useful in medical practice as:

adjuvants of vaccines for man and agricultural animals;

stimulators of nonspecific immunity in combination with tumoral and bacterial antigens against the most diverse diseases; and

stimulators of the fight of the organism against strains resistant to antibiotics.

The use in clinical medicine of PG and cell walls is complicated by a series of side effects, including, in the first place, allergic reactions [29].

POLYSACCHARIDES OF FUNGI AND LICHENS

A fairly intensive study of the polysaccharides of fungi and lichens has been performed for a long time exclusively in the light of their potential antitumoral activity [3]. And only comparatively recently has it been found that the antitumoral activity of these compounds is connected directly with their action on the immune system of the tumor-bearing organism [3, 18].

Then, in the fifties, it was found that crude aqueous extracts of the most diverse species of *Basidiomycetes* possess a pronounced activity against experimental tumors [30-33]. Subsequently, polysaccharides were isolated from these extracts and it was established that it is precisely these that are the active principles of these fungi [3]. Polysaccharides were isolated from the fruiting bodies, mycelium, and culture liquid of a large number of fungi (Table 1).

The greatest attention of research workers has been attracted by lentinan and pachymaran [23, 18]. Lentinan was first isolated by Japanese workers [34] in 1969 from the edible fungus *Lentinus edodes*, which is grown in the Far East and is popular in Japan. It was shown that lentinan consists of a β-1,3-D-glucan and possesses a high antitumoral activity [34, 35]. It was soon established [36-38] that the antitumoral activity of lentinan is due to its stimulation of the T-lymphocytes. Lentinan exerts no influence whatever on the humoral immune responses [38]. In active doses (up to 5 mg/kg) lentinan is practically nontoxic. The optimum doses for experimental animals are 1-2 mg/kg. However, it must be mentioned that high doses or the prolonged administration of lentinan may cause an appreciable lowering of the generation of the T-cytotoxic lymphocytes [39]. Lentinan is a typical immunomodulator. The molecular weight of lentinan (about 1 million daltons) plays a fundamental role in the manifestation of its physiological activity. The degradation of lentinan leads to a fall in its antitumoral effect, and fragments of low molecular weight exhibit no activity whatever [40].

The structure of lentinan was studied by Japanese workers [34, 41] using the ordinary methods of carbohydrate chemistry. The investigations showed that lentinan (Fig. 4) consists of a branched glucan with main and side chains constructed of β-1,3-D-glucopyranose residues. In each five glucose residues of the main chain there are two points of branching. The side chains are attached to the main chain by β-1,6-glucosidic bonds. The presence of a small number of β-1,6 bonds in the side chains is possible. A certain proportion of the side chains consists of single glucose residues attached to the main chain by β-1,6 bonds.

It is interesting to note that the elimination of the side chains with the aid of periodate oxidation and subsequent hydrolysis leads to practically no loss of antitumoral activity. The lentinan fragment obtained suppresses the growth of sarcoma 180 by 90%, which is comparable with the activity of lentinan itself.

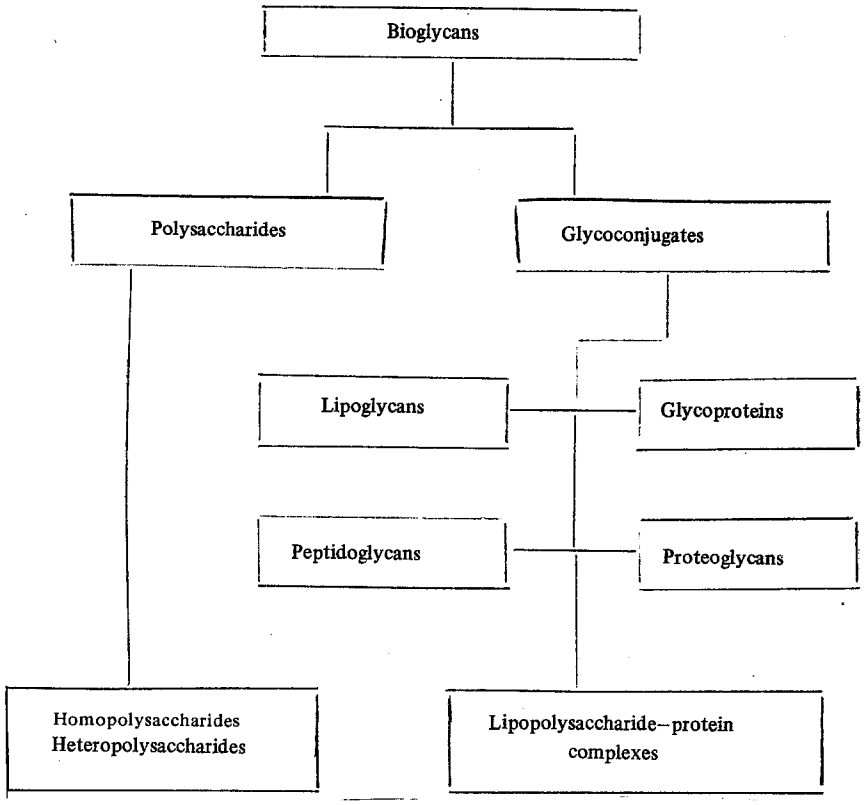


Fig. 1. Bioglycans.

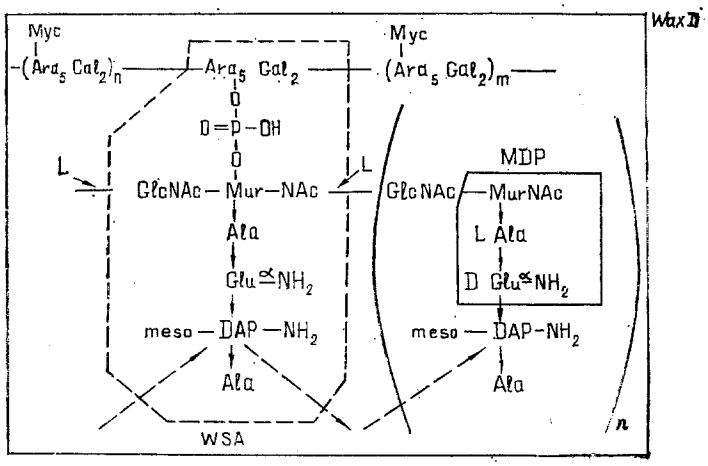


Fig. 2. Two mycobacterial cell membranes: WSA – water-soluble adjuvant; MDP – muramyldipeptide; Myc – mycolic acid; L – lysozyme.

The alkali-soluble, water-insoluble glucan known as pachyman was isolated as long ago as the last century [42, 43] from the fungus *Poria cocos* (Table 1), which is parasitic on exposed roots of coniferous trees. In 1957, Warsi and Whelan [44] showed that pachyman (Fig. 5) has a main carbohydrate chain consisting of β-1,3-linked D-glucopyranose residues. It was established later that side chains constructed of β-1,6-linked D-glucopyranose residues [45, 46] were attached to the main chain of pachyman by 1,2- and 1,4-bonds [45]. The degree of polymerization is 690. Biological trials conducted by Japanese workers [47] have shown that pachyman does not possess antitumoral activity while pachymaran, which is obtained by eliminating the side chains of pachyman by periodate oxidation followed by mild hydrolysis proved to be extremely effective against sarcoma 180 in mice. The hypothesis has been put forward that, like lentinan, pachymaran possesses antitumoral activity as the result of its stimulation of cellular immunity [36, 48].

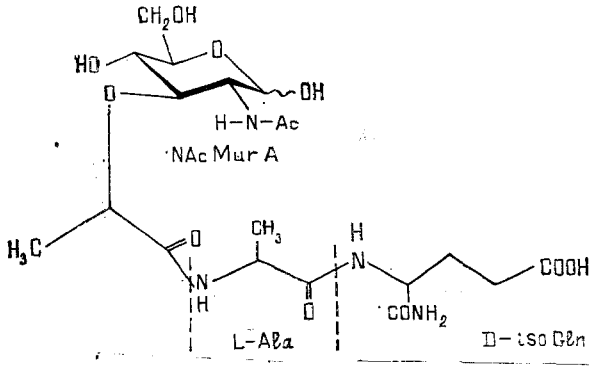


Fig. 3. The muramyldipeptide (MDP).

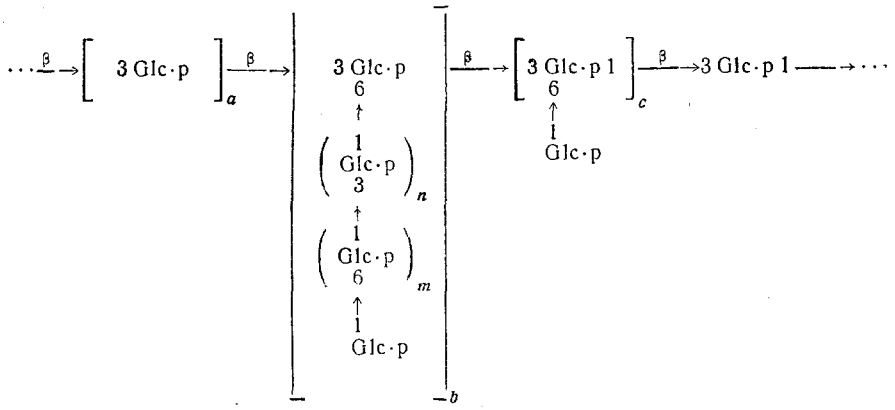


Fig. 4. Lentinan.

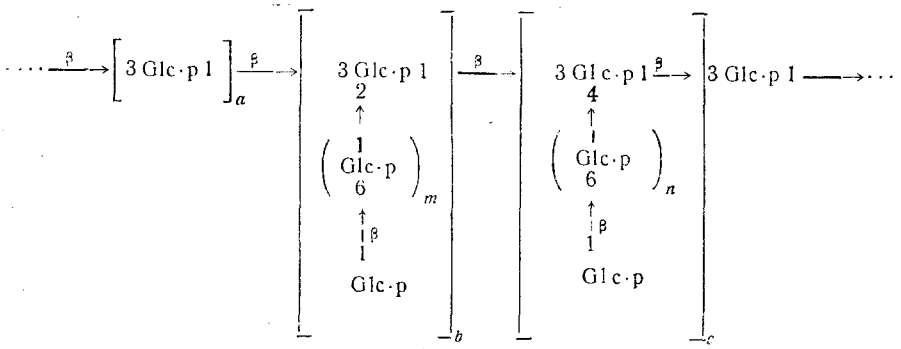


Fig. 5. Pachyman.

TABLE 1. Glucans of Fungi

Source	Glucan	Glucosidic bonds	Activity	
			inhibition, %	survival rate
Lentinus edodes	Lentinan	β-1,3; β-1,6	100	10/10
Auricularia auricula-judae	—	β-1,3; β-1,6	95	6/9
Poria cocos	Pachyman	β-1,3; β-1,6 (1,2; 1,4)	0	0/8
Poria cocos	Pachymaran	β-1,3	88	2/6
Coriolus versicolor	Coriolan	β-1,3; β-1,6	77,5	4/8
Schizophyllum commune	Schizophyllan	β-1,3; β-1,6	98,5	7/10
Sclerotium glucanicum	Scleroglucan	β-1,3; β-1,6	91,6	7/10

In 1973, the same group of Japanese workers [37] detected the appearance of activity in pachyman after it had been treated with urea. This leads to a modification of the higher structure of pachyman with no change in its basic structure. The active antitumoral preparation obtained as the result of this was called U-pachyman. Another pachyman derivative — carboxymethylpachymaran [49] — proved to be active against a whole series of tumors, particularly sarcoma 180 and adenocarcinoma MM-102. Unlike pachyman, these two derivatives of it are soluble in water. No toxic effects whatever are observed when pachymaran, U-pachyman, and carboxymethylpachymaran were administered in doses active against sarcoma 180 (5 mg/kg intraperitoneally each day for 10 days).

Other polysaccharides that have now been isolated from edible and basidial fungi have structures similar to those of lichenan and pachyman (Table 1). They are all β-1,3-D-glucans and possess antitumoral activity due to their stimulation of the cellular immunity of the tumor-bearing organism.

Among the sources of these substances must be mentioned the basidial fungi belonging to the family Polysporaceae: *Coriolus versicolor* [50-52], *Fomes fomentarius* [53], *Grifora umbellata* [54, 57], *Ganoderma applanatum* [58, 59]; Mucronoporaceae: *Phellinus linteus* [58], *Schizophyllum commune* [60, 64]; and to the family Sclerotiniaceae: *Sclerotium glucanicum* [65, 66], *Sclerotinia sclerotiorum* [67], and *Monilinia fructigena* [68]. Among edible fungi we must also mention the fungus *Auricularia auricula-judae* from the fruit bodies of which a β-1,3-D-glucan similar to lentinan has been isolated quite recently [69, 70]. In addition, a similar glucan has been isolated from the Gram-negative bacterium *Alcaligenes faecalis* var. *myxogenes* [71, 72]; it is known as curdlan. An antitumorally active glucan has also been isolated from the flagellate protozoan *Astasia longa*; this has been called astasian [73].

It is interesting that the edible fungus *Tremella fuciformis*, which is grown in Japan and China, has become a source of an unusual polysaccharide possessing pronounced activity against sarcoma 180 [74]. Unlike the others, this polysaccharide is constructed of residues of D-xylose, D-mannose, and D-glucuronic acid [74, 75] (Fig. 6). This molecule is based on a carbohydrate chain of α-1,3-bound D-mannopyranose residues. The side chains consist of β-1,2-bound D-xylopyranose residues and single D-glucopyranosyluronic acid and D-xylopyranose. The side chains are attached to the mannopyranose residues of the main chain by 1,2-bonds [76]. The polysaccharide is acetylated, containing acetyl groups in position 4 and (or) 6 of the mannose residues and in position 2 and (or) 4 of the glucuronic acid residues [77].

As already mentioned, all the antitumoral polysaccharides of fungi exhibit immunostimulating activity [3, 18, 78]. The antitumoral action of these polysaccharides is due to an increase in the nonspecific resistance of the tumor-bearing organism. The fact that fungal polysaccharides possess a higher antitumoral activity at lower doses shows a connection of this dose-dependent activity with immune processes in the tumor-bearing organism [3, 34]. The inhibition of immune processes in the animal organism leads to a fall in the antitumoral action of the polysaccharides [3, 78]. The antitumoral influence of glucans of the lentinan type is completely absent in animals deprived of the thymus. This confirms the direct action of the polysaccharides on the T-cells [78].

In healthy animals, the glucans show no increase in the immune indices, but in diseased animals the weakened immune processes are restored to the normal level. A specific immunological feature of the glucans of the lentinan type is their restoration of lowered immune reactions in diseased animals with malignant neoplasms [78].

A very interesting fact has been recently found by Japanese workers [79]. They have shown that carboxymethyl derivatives of β-1,3-D-glucans possessing antitumoral activity are capable in concentrations of 10^{-2}-10^{-6} mg/ml of giving a positive limulus test with an amebocytic lysate of the xiphosuran *Limulus polyphemus* similar to that of the immunoactive bacterial LPSs. This fact may be used subsequently for the rapid revelation of glucans possessing antitumoral and immunostimulating activity.

Various glucans such as lichenan, isolichenan, and pustulan isolated from lichens have also proved to be active against solid sarcomas in mice [3]. The lichen glucans are, as a rule, linear polysaccharides (Fig. 7).

Lichenan, the main source of which is Iceland moss *Cetraria islandica* [80] has a linear β-1,3:β-1,4-D-glucan structure. In isolichenan from the same source and evernan from *Evernia* spp. the same bonds but in the α-configuration are present between the D-glucopyranose residues. Pustulan from *Gyrophora esculenta* is a β-1,6-D-glucan [80, 81], while the carbohydrate

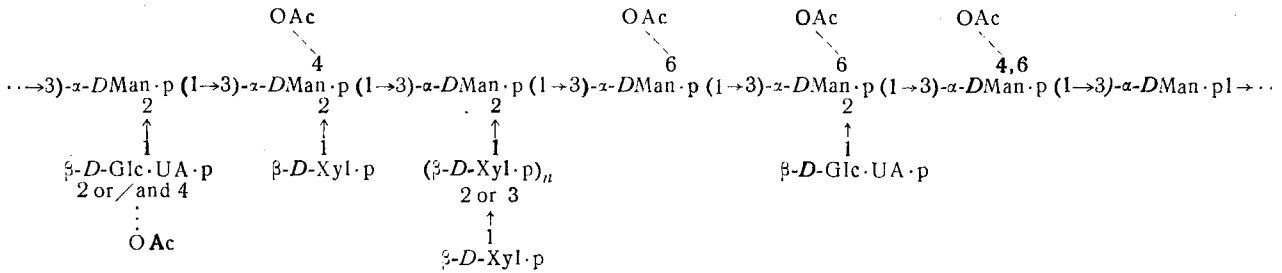


Fig. 6. The polysaccharide of *Tremella fuciformis*.

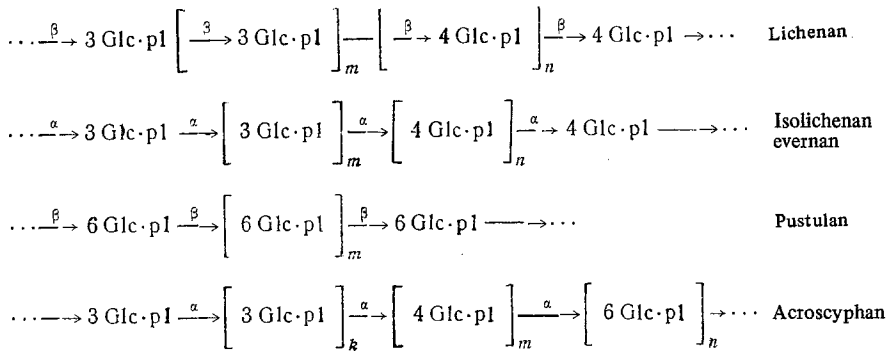


Fig. 7. Lichen glucans (structure).

TABLE 2. Lichen Glucans (sources, activities)

Glucan	Source	Degree of polymerization	Activity	
			inhibition, %	survival rate
Lichenan	Cetraria spp., Parmelia spp.	80—400	100	8/8
Isolichenan	Usnea spp., Cetraria spp., Alectoria spp.	34—43	100	9/9
Pustulan	Umbilicaria spp., Lasallia spp.	120	99	6/8
Evernan	Evernia spp.	70	100	10/10
Acroscyphan	Acroscyphus sphaerophotoi. Sphaerophorus globosus	n. d.	—	—

chain of acroscyphan from *Acroscyphus sphaeropholoides* is constructed of α-1,3-, α-1,4-, and α-1,6-bound D-glucopyranose residues [82, 83].

The glucans of the types given are found in a whole series of lichens, and they all possess antitumoral activity [3, 82-87] (Table 2). In a number of cases, lichens have yielded active polysaccharides, the carbohydrate chains of which contain, in addition to glucose, residues of other monosaccharides, especially mannose and galactose [85, 88, 89].

Since the polysaccharides of lichens do not possess phytotoxic activity, the hypothesis has been put forward of their immunostimulating activity [85, 86, 90]. It has been established, for example, that lichenan has a pronounced stimulating influence on the reticuloendothelial system [90]. There is no doubt that a more detailed study of the action of lichen polysaccharides on the components of the immune system of a tumor-bearing organism is necessary, although it is natural to assume a high analogy with the β-1,3-D-glucans of fungi in this respect.

YEAST POLYSACCHARIDES

Of the active polysaccharides of yeasts, zymosan has attracted the greatest attention [2, 3, 18].

Since the time of the first investigation of zymosan carried out by Pillemer and Ecker in 1941 [91], a large number of studies on the structure and physiological activity of this substance have been performed.

As early as 1941, Pillemer and Ecker [91] established that the insoluble fraction from yeasts possesses well-marked anticomplementary properties. The term "zymosan" was proposed in 1953 by Pillemer et al. [22] to denote the polysaccharide complex of the yeast cell wall. Zymosan became known as a nonspecific stimulator of the immune system of the organism following a report of Rowley [93] that after the administration of zymosan the resistance of mice to infection by *E. coli* increased. Immunization by zymosan increases the resistance of animals to the implantation of tumors and it suppresses the metastasis of Ehrlich's ascitis tumor and lowers the number of tumor nodes in the lungs [94]; it stimulates the immune protection of the organisms against sarcoma 180 [95]; and it increases the resistance of animals to various infections [96]. At the same time, depending on the experimental conditions, zymosan may also exhibit an immunodepressive action [97] and it is, therefore, a true immunomodulator.

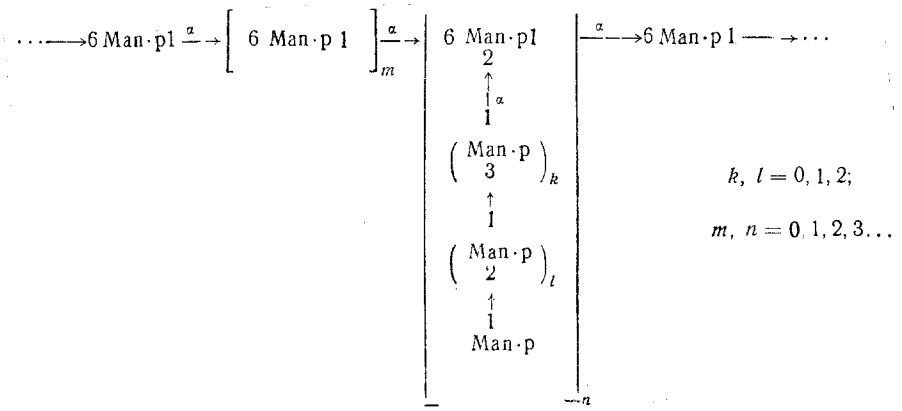


Fig. 8. Structure of yeast mannan.

Numerous investigations have been devoted to the antitumoral activity of zymosan [1-3, 18, 98, 99]. The antitumoral activity is connected with the action of the polysaccharide complex on the immune system of the tumor-bearing organism [2, 18, 100]. Zymosan increases the number and activity of the B-lymphocytes of the peripheral blood [101]. Yeast polysaccharides act as stimulators for the accumulation of antibody-forming cells [102]. Zymosan exhibits a stimulating action on the T-lymphocytes [103]: There is a statement on the activation by zymosan of T-lymphocyte killer cells [104]. The injection of antilymphocytic serum lowers the antitumoral effect of zymosan, which serves as a confirmation of its action on tumors through the activation of T-killer cells. Zymosan stimulates the activity of macrophages in the lungs, spleen, and liver, mobilization of the macrophages being observed at the point of injection of the zymosan [105-108]. The majority of workers have expressed the opinion that the main effector link in the antitumoral action of zymosan is formed by the macrophages of the tumor-bearing organism activated by the polysaccharide [99, 109, 110].

The study of zymosan as a polysaccharide complex has shown that it includes a glucan and a mannan [111]. It has been found that the mannan forms a complex with protein [111, 112]. The existence of glucan–mannan–protein complexes as the basis of the yeast wall has also been reported [113]. Depending on the method of isolation and fractionation, it is possible to isolate a mannan or a glucomannan, or both components [114]. The fragments obtained possess pronounced antitumoral activity. They are usually obtained by treating an aqueous extract of yeast cells with Fehling's solution, and the glucan component is isolated by alkaline extraction of the residue. A glucan is also an active component of the yeast cell wall [94, 115, 116].

A large number of studies has been devoted to establishing the structure of the yeast glucan and mannan [112, 117-124]. They have shown that the glucans and mannans isolated from various yeasts have similar structures.

The main carbohydrate chain of the yeast mannans (Fig. 8), consists of α-1-6-linked D-mannopyranose residues. The mannans from various sources differ from one another by the number and length of the side chains, which are constructed of α-1,2- and -1,3-linked D-mannopyranose residues [112, 121-123].

Glucans are distinguished by considerable homogeneity [118-120] in relation to solubility and structure. At the present time, several groups of yeast glucans have been isolated and characterized in accordance with their solubilities in alkali and acetic acid:

α- and β-1,3-D-glucans soluble in alkali;

branched β-1,3-D-glucans insoluble in alkali and in acid;

β-1,6-D-glucans insoluble in alkali but soluble in acid.

It is not excluded that the yeast cell wall in the native state contains a single complex branched glucan containing all the above-mentioned types of glucosidic bonds (Fig. 9). In the process of isolation, as the result of treatment with alkali and acid, fragments of it are formed which are also responsible for the great heterogeneity of the preparation.

In the process of fractionation, all the fragments of the complex glucan–mannan–protein complex were isolated and then characterized. They are of undoubted interest, since they

possess high physiological activity and open up the possibility of subsequently elucidating the relationship between structure and activity, including immunomodulating activity. It is possible that the glucomannans and galactomannans isolated from pathogenic fungi and studied by N. P. Elinov et al. [125] may be nonspecific stimulators of the immune system against various diseases.

BIOGLYCANS OF MARINE ORGANISMS

Marine organisms as a source of the most diverse biologically active substances have been studied from all aspects in the last few decades [126]. As in the case of other objects, a specially directed search for immunomodulator bioglycans in marine organisms has arisen only in recent years. For a long time investigations were directed to the search for antitumoral substances of the most diverse nature [126].

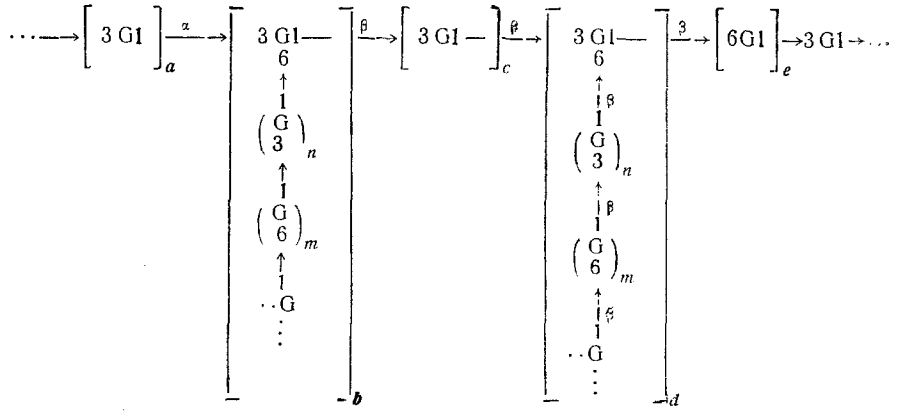
It is well known that neoplastic formations are found comparatively rarely in marine invertebrates [127]. Consequently, in invertebrates a special protection against neoplasms must exist which may be due to antitumoral compounds and phagocyte stimulators as the bases of their immunity. This circumstance has been made the basis of a search for preparations with antitumoral and (or) immunostimulating activity using marine organisms as the objects of this search.

In an excellent review, Li, Goldin, and Hartwell [128] have given an exhaustive analysis of the first investigation devoted to the search for physiologically active biopolymer preparations in the most diverse marine organisms. Let us briefly mention the main points of these studies. In 1964, Schmeer of Szent-Györgyi's laboratory [129] isolated for the first time from the widely distributed edible bivalve mollusc *Mercenaria mercenaria* a carbohydrate-containing biopolymer preparation which he called mercenene and which possesses pronounced inhibiting action on the growth of tumors: sarcoma 180 and Krebs-2 carcinoma in mice. In a series of papers, Schmeer et al. in the period up to 1970 discussed the results of a study of aqueous extracts of a number of marine invertebrates as potential sources of biopolymers with antitumoral activity. In addition to mercenene they studied aqueous extracts from the oyster *Ostrea virginica*, the whelk *Busycon canaliculatum*, a snail *Helix* sp., and a squid *Loligo* sp., All the extracts exhibited antitumoral activity to some degree or other; the smallest activity was found in an extract from the whelk and the highest activity was possessed by mercenene which was also studied in more detail. It is interesting to note that the active principle in mercenene is destroyed on boiling for 25 min and its activity falls at temperatures above 50°C but is retained completely at 37°C. Mercenene possesses no toxicity, which may show a direct action of the substance on mouse tumors through their immune system. In the summer months, the amount of mercenene in *M. mercenaria* is 8-9 times greater than its amount in the remainder of the year [129, 130]. This is connected with the fact that the amount of mercenene in the mollusc depends on the temperature of its living environment: The largest amount is detected in molluscs kept in warmed seawater [130]. In 1979, it was established that mercenene is a bioglycan consisting of a polysaccharide component, phosphate, and unidentified material [131].

In 1969 [132], in a study of the polysaccharide composition of a number of invertebrates of the Sea of Japan, we showed that the components of the polysaccharide fractions were glucans and heteropolysaccharides containing glucose residues as the main component. It is well known [2, 3] that many glucans from various sources possess an immunostimulating activity against tumors.

Definite interest as an immunomodulator is possessed by carragheenan, a sulfated galactan from a number of red algae of the family Rhodophyta. This polysaccharide is well known as a toxin for macrophages [133]. It is widely used in experiments where the inactivation of macrophages is required. Furthermore, carragheenan blocks the activation of complement [134]. It has recently been found [135] that it suppresses the proliferation of T-lymphocytes. All these facts indicate an immunodepressive activity of carragheenan [136, 137]. Some authors even warn of the danger of the daily consumption of carragheenan as a food product [138, 139].

On the other hand, it has been shown that carragheenan may have a stimulating effect on the B-lymphocytes and thereby activate the synthesis of immunoglobulins and enhance the humoral response of the immune system [140, 141].



General structural scheme

Types of Glucans

d_2, $d_3 \cdots = d$ for structures 2, 3, etc.

No. of the structure	Type of structure	Units of the chain	Solubility		Degree of polymerization
			alkali	CH_3COOH	
1	α—1,3*	$c, d, e = 0$ $b = 10$ $m, n = 0, 1, 2$	+	+	200
2	β—1,3	$a, b = 0$ $d_2 \ll d_3$; $e_2 \gg e_3$	+	+	500—800
3	β-1,3	$a, b = 0$; $d_3 \gg d_2$ $(m, n) \gg (m, n)_2$ $e \sim 3\%$	—	—	1500—1800
4	β—1,6	$e \sim 100$; $a, b = 0$ $c + d = 14\%$	—	+	140—200

*The presence of up to 7% of α-1,4 cb is possible.

Fig. 9. Glucans: G represents glucopyranose, and a, b, c, d, e, m, n represent the number of units in the chains.

These facts relating to carragheenan suggest that other sulfated bioglycans of marine invertebrates may act as immunomodulators.

Performing a broad program of searching for compounds active against neoplastic growth, a group of Japanese authors [142] in 1978 used as the source of an antitumoral biopreparation a bivalve mollusc that is common in the Sea of Japan — the scallop *Patinopecten yessoensis*. The freshly collected scallops were boiled at 100°C in their shells for 10 min, and after the removal of the shells they were subjected to aqueous extraction. As a result of subsequent purification and treatment with ultrasound, a heat-stable fraction was obtained which inhibited the growth of sarcoma 180 in mice by 93.7% and of Erhlich's carcinoma by 73.5%. In a number of cases the complete disappearance of the tumors was observed. It was shown that the heat-stable active principle consists of a glycoprotein with a molecular weight of about 100 thousand daltons which does not lose its activity on treatment with pronase. It was established by special experiments that the active extract obtained possesses no direct inhibiting action on tumor cells and is therefore not cytostatic. From this it may be concluded that the preparation acts on tumors through the immune system of tumor-bearing animals. The structural study of the preparation isolated is continuing [142].

In recent years, we [143-146] have performed a systematic study of marine invertebrates as potential sources of bioglycans possessing an immunomodulating action. This has led to results in many directions which cannot always be compared with one another, but they all confirm the conclusion that marine invertebrates contain bioglycans possessing an immunostimulating action. It is not excluded that this property is common for invertebrates and is connected with the necessity for stimulating phagocytosis. All the preparations isolated and studied are distinguished by low toxicity (more than 0.5 g/kg) and possess no direct cytotoxic

TABLE 3. Influence of Mytilan on the Immunoreactivity of Intact Mice

Dose, mg/kg	Phagocytic number	Phagocytic index, %	No. of antibody-forming cells, %
0 (control)	5	100	100
10	12	220	216
50	17	306	255
100	15	290	244

TABLE 4. Influence of Mytilan on the Phagocytosis of Infected Animals

Microorganism	Dose of mytilan, mg/kg	Phagocytic index, %	Phagocytic number	Percentage completion
Yersinia pseudotuberculosis	0	20±4	1,4±0,1	0
	100	93±1,5	5,4±1,2	58±19
Salmonella typhimurium	0	8±0,4	0,9±0,1	0
	100	78±2	3,4±0,1	63±22
Staphylococcus aureus	0	14±4	1,1±0,1	0
	100	50,5±3,5	3,4±0,6	27±5

action. At the same time, they exhibit a pronounced capacity for activating phagocytosis and increasing the index of antibody-forming cells (AOCs) by a factor of 1.5-2.

A preliminary chemical study of the preparations obtained has shown that they include polysaccharides and accompanying protein components; they have a low content of nucleic acid impurities. Thus, all the preparations studied are bioglycans. Glucose has been detected as the main monosaccharide component in their hydrolysates, and other monosaccharides are present in only small amounts. There is no doubt that the preparations isolated require further fractionation and purification with the aim of obtaining the most active fraction. Such fractionation and a more detailed study has been carried out up to the present only with extracts from the mussel *Crenomytilus grayanus* [143] and the soft coral *Pseudopterogorgia americana* [144]. The preparations from mussels were called mytilan and those of soft coral coralan.

As a study of the influence of mytilan on the components of the immune system of animals has shown, they intensify phagocytic activity and the chimeral factors of immunity (Table 3) [145, 146]. An influence of phagocytic activity is also shown in a quantitative increase of the population of cells participating in phagocytosis (P. No. — the phagocyte number) and also in the enhancement of their activity (PI — phagocytic index). In an investigation of the dynamics of the AOCs in the spleen of a mouse injected with mytilan and ram erythrocytes, a statistically significant increase in the number of AOCs by a factor of 2-2.5 was found (Table 3) [146]. It is interesting that the titer of the hemolysins in the blood also rose by a factor of 2-4 [146]. This fact may be of great importance in the preparation of high-titer hemolytic sera. In *in vivo* experiments on mice of the CBA line it was found that the absolute number of cells of a peritoneal exudate (CPE) increased when mytilan was injected and rose with an increase in its concentration from 50 to 200 mg/kg (Fig. 10) [146].

Great interest was presented by the study of the influence of mytilan on the immunobiological reactivity of the organisms on animals with experimental infections caused by the following microorganisms: *Yersinia pseudotuberculosis*, *Salmonella typhimurium*, and *Staphylococcus aureus* [145, 146]. Mytilan was administered to the animals in a dose of 100 mg/kg a day before infection with a lethal dose of bacterial culture. This led to a doubling of the mean lifetimes (MLTs) of the animals. Furthermore, while all the control animals died, on the administration of mytilan, a considerable percentage of survivors was observed.

When mytilan was administered to mice and guinea pigs, the phagocytic activity of the leukocytes with respect to all the above-mentioned microorganisms changed. The administration of the preparation enhanced the absorptive activity (PI) and the number of phagocytes (P. No.) for all the infected animals (Table 4). In addition, while in the infected animals a total incompletion of phagocytosis was observed, for those to which mytilan had been administered the percentage completion was extremely considerable (Table 4).

The chemical study of mytilan has shown that it is composed mainly of a D-glucan, since glucose is the main carbohydrate component of a hydrolysate. Other monosaccharides were detected in smaller amounts. In addition to the polypeptide component, a small amount of protein (about 5%) was detected in mytilan. Judging from the results obtained, the substance is a complex high-molecular-weight aggregate.

Considerable interest is presented by the study of corallan [144], a bioglycan from the soft coral *P. americana* (type Coelenterata) widely distributed in the waters of the Caribbean basin. The corallan isolated from it possesses a high physiological activity and, in particular, it is capable of stimulating the immune system against various diseases [144].

Corallan $[\alpha]_D^{20} - 70.7°$ (in water), is a glycoprotein including polysaccharides and protein components. The total carbohydrate content in corallan is 50% and the protein content is about 20%. In addition the substance includes about 10% of mineral impurities not removed by dialysis. In a corallan hydrolysate arabinose, galactose, fucose, and glucose have been identified in a molar ratio of 35.6:33.3:24.5:6.6. In addition, an unidentified glucuronic acid (9% in corallan), about 2% of hexosamines, and more than 6% of sulfate groups have been detected. Thus, the polysaccharide component of corallan is a sulfated glycuronoglycan.

On treatment with Cetavlon, corallan precipitated almost completely, which confirms its acidic nature and is one of the proofs of its homogeneity. Attempts to fractionate corallan with the aid of gel filtration and ion-exchange chromatography have been unsuccessful, which confirms its homogeneity, and the coincidence of the peaks of the protein and the polysaccharide on elution curves may serve as an indication of a bond between the carbohydrate and protein components of corallan. This bond proves to be stable even under the conditions of treating corallan with alkali in the presence of tetrahydroborate. This method is used for the cleavage of carbohydrate–protein bonds.

Corallan is distinguished by a high viscosity of its solutions, and only extremely dilute aqueous solutions can be used for its study. The structural study of corallan is being continued at the present time.

Thus, although insufficient information has yet been accumulated on the bioglycans of marine invertebrates, it may be assumed with some confidence that *a common property of marine invertebrates is the production of bioglycans possessing the capacity for stimulating various components of the immune system of the organism and, primarily, phagocytosis.* This property is directed to enhancing the protective mechanisms of marine invertebrates. If this is the case, the production of such bioglycans should be enhanced in the periods of the greatest vital activity of marine invertebrates and under various unfavorable effects, which probably takes place and in a number of cases has been recorded experimentally [130].

In addition to marine invertebrates, definite interest for the search for active immunomodulator bioglycans is presented by sharks in which, as is well known [127], neoplasms arise very rarely. More than 200 species of such sharks have been detected. The causes of this phenomenon have not yet been investigated in detail, but it has been reported [127] that various organs and tissues of the hammerhead shark *Sphyrna lewini* contain macromolecular glycoproteins (mol. wt. not more than 40 million daltons) that are readily soluble in water and exhibit a pronounced indirect action on tumors. It is not excluded that other species of shark may also contain similar active bioglycans capable of affecting the immune system of a tumor-bearing organism.

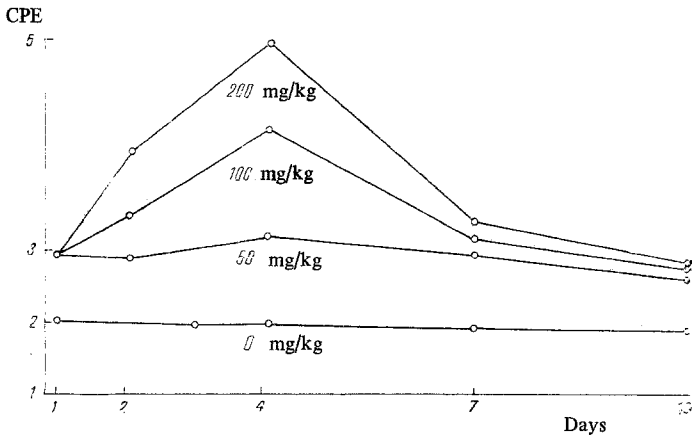


Fig. 10. Influence of mytilan on the CPE in mice of the CBA line.

Thus, marine organisms are valuable objects in the search for active immunomodulator bioglycans.

The information given indicates the great attention that has been devoted, particularly in recent years, to the search for and study of immunomodulator bioglycans [1-3, 18, 78]. Many investigations have been devoted particularly to the elucidation of the interrelationship between the structure of the bioglycans and their physiological action. However, it does not yet appear possible to draw clear conclusions. There is no doubt that the primary structure plays a fundamental role in the manifestation of its activity [3, 78]. In this connection the study of primary structure is of prime interest. A not unimportant role is played by the size of the molecule and the molecular mass of the functioning compound. However, bioglycans close in primary structure and in molecular weight not infrequently differ appreciably in their activity.

In this connection, the hypothesis has been expressed that the higher structures of the bioglycans are important in the manifestation of immunomodulating activity by them [18, 78]. It is interesting to note that, to all appearances, in the interaction of a bioglycan with the elements of the immune system, a deciding role is played by the conformation of the molecule at a given moment — at the moment of interaction. And here it is possible to speak of a dynamic structure, a dynamic conformation of the bioglycan. The functioning structure of the bioglycan is most probably dynamic and changes as a function of the time and conditions of interaction with the elements of the immune system. The opinion expressed is confirmed by the following observations. Schmeer [129] has reported that mercenene isolated from the mollusc *M. mercenaria* in the winter is considerably less active than that isolated in the summer. We have observed a similar situation for mytilan from mussels: The activity of the substance isolated in July-September was considerably higher than that of preparations obtained in other months. This is undoubtedly connected with differences in the vital activity of the molluscs: In the summer months it is considerably higher. But it is not excluded that this is also reflected in the conformation of the functioning bioglycan, since in terms of their main chemical characteristics the preparations obtained at different times differ little from one another. Of course, the available information is extremely inadequate, but it is possible to work purposefully in this direction.

Unfortunately, at the present time the fine mechanisms of the interaction of bioglycans with the receptors of the cells of the immune system sre still very far from being completely understood. Little information has accumulated on the higher structures of the bioglycans. But the answer to the question is being delayed particularly because of the inadequacy of the information on the structures of functioning receptors, although it is known even now that they are glycoproteins. Basic studies are necessary to elucidate their structure and conformation, Finally, a carefully developed mathematical apparatus is required for creating a mathematical model of the interaction of the active bioglycans with the receptors of various cell structures of the immune system of the organism.

French workers [22] have directed attention to and have warned against those potential dangers that may arise in the unreasonable use of immunomodulator bioglycans. These are, in the first place, the sensitization of the organism to the immunomodulator itself or to external antigens and autoantigens the action of which is enhanced by a given bioglycan. The enhancement of autoimmune processes is particularly dangerous.

Nevertheless, the important role of bioglycans as immunomodulators is not a matter of doubt. The search for new immunomodulator bioglycans and the study of their structure and mechanism of their action on the immune system are of fundamental interest. This opens up the possibility of performing a series of experiments of fundamental importance for elucidating the molecular bases of immunity. In addition, preparations of immunostimulator bioglycans and adjuvants can be used successfully for the prophylaxis and treatment of many severe diseases, including malignant tumors. And, finally, the weakly toxic immunodepressant bioglycans are of great value as a basis for the transplantation of organs and tissues that is becoming more and more common. The search for them is only just beginning but it is undoubtedly promising.

Thus, for chemists, biochemists, and physicians there is very much more work to be done on the study of the immunomodulator bioglycans.

LITERATURE CITED

1. É. V. Ermol'eva and G. E. Vaisberg, The Stimulation of the Nonspecific Resistance of the Organism and Bacterial Polysaccharides [in Russian], Moscow (1976).
2. K. P. Balitskii and I. G. Veksler, The Reactivity of the Organism and the Chemotherapy of Tumors [in Russian], Kiev (1975).
3. R. L. Whistler, A. A. Bushway, and P. P. Singh, Adv. Carbohydr. Chem. Biochem., 32, 235 (1976).
4. J. G. Holt (editor), The Shorter Bergey's Manual of Determinative Bacteriology, VIIIth ed., Williams and Wilkins, Baltimore (1977).
5. G. Drews, in: The Biology of the Blue-Green Algae, N. G. Carr and B. A. Whitton, eds., Blackwell Scientific Publications, Oxford (1973), pp. 96-116.
6. G. Weise, G. Drews, B. Jann, and K. Jann, Arch. Microbiol., 71, 89 (1970).
7. J. Weckesser, G. Drews, and H. Mayer, Annu. Rev. Microbiol., 33, 215 (1979).
8. **L. V. Mikheyskaya [Mikheiskaya], R. G. Ovodova,** and Yu. S. Ovodov, J. Bacteriol., 130, 1 (1977).
9. L. V. Mikheiskaya, R. G. Ovodova, and Yu. S. Ovodov, Khim. Prir. Soedin., 146 (1981).
10. W. Schmidt, G. Drews, J. Weckesser, I. Fromme, and D. Borowiak, Arch. Microbiol., 127, 209 (1980).
11. W. Schmidt, G. Drews, J. Weckesser, and H. Mayer, Arch. Microbiol., 127, 217 (1980).
12. J. Freund, Adv. Tubercul., 7, 130 (1956).
13. R. G. White, L. Berustock, R. G. Johns, and E. Lederer, Immunol., 1, 54 (1958).
14. I. Azuma, Y. Kishimoto, Y. Yamamura, and J. F. Petit, Jpn. J. Microbiol., 15, 193 (1971).
15. A. Adam, R. Ciorbaru, J. Petit, and E. Lederer, Proc. Natl. Acad. Sci. USA, 69, 851 (1972).
16. I. G. Bogdanov, Symposium on Antibiotics, Prague, Abstracts of Papers (1959), pp. 120-121.
17. I. G. Bogdanov, P. G. Dalev, A. I. Gurevich, M. N. Kolosov, V. P. Mal'kova, L. A. Plemyannikova, and I. B. Sorokina, FEBS Lett., 57, 259 (1975).
18. G. H. Werner, R. Maral, F. Floch, and M. Joanne, Bull. Inst. Pasteur, 75, 5 (1977).
19. A. R. Prevot, B. N. Halpern, G. Biozzi, C. Stiffel, D. Monton, J. C. Morard, I. Bouthillier, and C. Decrensefond, C. R. Acad. Sci. Paris, Ser. D, 257, 13 (1963).
20. E. M. Hersch, G. M. Mavligit, J. U. Gutterman, and S. P. Richman, in: Cancer, E. F. Becker, ed., Vol. 6, Plenum, New York–Oxford (1977), pp. 425-532.
21. E. Lederer, J. Med. Chem., 23, 819 (1980).
22. A. Adam, J.-F. Petit, P. Lefrancier, and E. Lederer, Mol. Cell. Biochem., 41, 27 (1981).
23. D. Miglioze-Samour and P. Jolles, Biochem. Biophys. Res. Commun., 66, 1316 (1972).
24. I. J. Hiu, Nature New Biol., 238, 241 (1972).
25. R. Ciorbaru, A. Adam, J. F. Petit, E. Lederer, C. Bona, and L. Chedid, Infect. Immun., 11, 257 (1975).
26. F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, Biochem. Biophys. Res. Commun., 59. 1317 (1974).
27. S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisani, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, Biken J., 18, 105 (1975).
28. F. Audibert, L. Chedid, P. Lefrancier, and J. Choay, Cell. Immunol., 21, 243 (1976).
29. B. Heyner and E. T. Rietschel, Microbiology, 344 (1977).
30. A. Catero, S. B. Azevedo, A. E. Arêaleão, et al., Acta Univ. Intern. Contra Cancrum., 7, 448 (1951).
31. Z. T. Mankowski, Proc. Soc. Appl. Bacteriol., 83, 25 (1955).
32. Z. Mankowski, I. C. Diller, and M. E. Fisher, Cancer Res., 17, 382 (1957).
33. E. H. Lucas, R. L. Ringer, et al., Antibiot. Chemotherapy, 7, 1 (1957).
34. G. Chihara, Y. Maeda, J. Hamuro, T. Sasaki, and F. Fukuoka, Nature (London), 222, 687 (1969).
35. G. Chihara, J. Hamuro, Y. Y. Maeda, Y. Arai, and F. Fukuoka, Cancer Res., 30, 2776 (1970).
36. Y. Y. Maeda and G. Chihara, Nature (London), 229, 634 (1971).
37. Y. Y. Maeda, J. Hamuro, Y. O. Yamada, K. Ishimura, and G. Chihara, in: Immunopotentiation. Ciba Foundation Symposium No. 18, Elsevier, Excerpta Medica, Amsterdam (1973), pp. 257-286.
38. G. Dennert and D. Tucker, J. Natl. Cancer Inst., 51, 1727 (1973).

39. J. Hamuro, M. Rollingholf, and H. Wagner, Cancer Res., 38, 3080 (1978).
40. T. Sasaki, N. Takasuka, G. Chihara, and Y. Y. Maeda, Gann, 67, 191 (1976).
41. T. Sasaki and N. Takasuka, Carbohydr. Res., 47, 99 (1976).
42. M. P. Champion, C. R. Acad. Sci. Paris, 75, 1526 (1872).
43. E. Fisher, Hedwigia, 30, 61 (1891).
44. S. A. Warsi and W. J. Whelan, Chem. Ind. (London), 1573 (1957).
45. G. C. Hoffmann, B. W. Simson, and T. E. Timell, Carbohydr. Res., 20, 185 (1971).
46. H. Saito, A. Misaki, and T. Harada, Agric. Biol. Chem., 32, 1261 (1968).
47. G. Chihara, J. Hamuro, Y. Y. Maeda, Y. Arai, and F. Fukuoka, Nature (London), 225, 943 (1970).
48. Y. Y. Maeda, J. Hamuro, and G. Chihara, Int. J. Cancer, 8, 41 (1971).
49. J. Hamuro, Y. Yamashita, Y. Ohsaka, Y. Y. Maeda, and G. Chihara, Nature (London), 233, 486 (1971).
50. H. Ito, J. Fujii, S. Naruse, M. Sugiura, and T. Miyazaki, Mie Med. J., 22, 103 (1972).
51. T. Miyazaki, T. Yadomae, M. Sugiura, H. Ito, K. Fujii, S. Naruse, and M. Kunihisa, Chem. Pharm. Bull., 22, 1739 (1974).
52. H. Ito, S. Naruse, and M. Sugiura, Folia Pharmacol. Jpn., 72, 77 (1976).
53. H. Ito, M. Sugiura, and M. Miyazaki, Chem. Pharm. Bull., 24, 2575 (1976).
54. T. Miyazaki and N. Oikawa, Chem. Pharm. Bull., 21, 2545 (1973).
55. T. Miyazaki, N. Oikawa, H. Yamada, and T. Yadomae, Carbohydr. Res., 65, 235 (1978).
56. T. Miyazaki, N. Oikawa, T. Yadomae, H. Yamada, Y. Yamada, Hong-Yen Hsu, and H. Ito, Carbohydr. Res., 69, 165 (1979).
57. Y. Ueno, Y. Okamoto, R. Yamauchi, and K. Kato, Carbohydr. Res., 101, 160 (1982).
58. T. Sasaki, Y. Arai, T. Ikekawa, G. Chihara, and F. Fukuoka, Chem. Pharm. Bull., 19, 821 (1971).
59. T. Usui, Y. Iwasaki, K. Hayashi, T. Mazuno, M. Tanaka, K. Shinkai, and M. Arakawa, Agric. Biol. Chem., 45, 323 (1981).
60. N. Komatsu, S. Okubo, S. Kikumoto, K. Kimura, G. Saito, and S. Sakai, Gann, 60, 137 (1969).
61. N. Komatsu, S. Okubo, and S. Kikumoto, J. Antibiot. (Tokyo) Ser. B., 23, 157 (1970); Chem. Abstr. 73, 129539 (1970).
62. S. Kikumoto, T. Miyama, S. Yoshizumi, S. Fujimoto, and K. Kimura, Nippon Nogei Kagaku Kaishi, 44, 337 (1970); Chem. Abstr., 74, 61776g (1971).
63. K. Tabata, W. Ito, T. Kojima, S. Kawabata, and A. Misaki, Carbohydr. Res., 89, 121 (1981).
64. T. Sato, T. Norisuye, and H. Fujita, Carbohydr. Res., 95, 195 (1981).
65. J. Johnson, S. Kirkwood, A. Misaki, T. E. Nelson, J. V. Scaletti, and F. Smith, Chem. Ind. (London), 820 (1963).
66. P. P. Singh, R. L. Whistler, R. Tokuzen, and W. Nakahara, Carbohydr. Res., 37, 245 (1974).
67. S. Sakai, C. Takada, T. Kamasuka, K. Monioki, and J. Sugayama, Gann, 59, 507 (1968).
68. S. A. Archer, J. R. Clamp, and O. Migliore, J. Gen. Microbiol., 102, 157 (1977).
69. Y. Sone, M. Kakuta, and A. Misaki, Agric. Biol. Chem., 42, 417 (1978).
70. A. Misaki, M. Kakuta, T. Sasaki, M. Tanaka, and H. Miyaji, Carbohydr. Res., 92, 115 (1981).
71. T. Sasaki, N. Abiko, Y. Sugino, and K. Nitta, Cancer Res., 38, 379 (1978).
72. T. Sasaki, N. Abiko, K. Nitta, N. Takasuka, and Y. Sugino, Eur. J. Cancer, 15, 211 (1979).
73. N. I. Sukhareva-Nemakova, V. I. Brusovanik, N. M. Kalenik, and A. B. Silaev, in: Urgent Questions of Modern Oncology [in Russian], Moscow, No. 4 (1975), p. 220.
74. S. Ukai, K. Hirose, T. Kiho, C. Hara, T. Irikura, T. Kanechika, and Y. Hasegawa, Chem. Pharm. Bull., 20, 2293 (1972).
75. S. Ukai, K. Hirose, T. Kiho, and C. Hara, Chem. Pharm. Bull., 22, 1102 (1974).
76. S. Ukai, K. Hirose, T. Kiho, and C. Hara, Chem. Pharm. Bull., 25, 338 (1977).
77. T. Kiho, C. Hara, and S. Ukai, Chem. Pharm. Bull., 29, 225 (1981).
78. G. Chihara and Kobunshi, High Polym. Jpn., 26, 117 (1977).
79. A. Kakinuma, T. Asano, H. Torii, and Y. Sugino, Biochem. Biophys. Res. Commun., 101, 434 (1981).
80. F. Fukuoka, M. Nakanishi, S. Shibata, Y. Nishikawa, T. Takeda, and M. Tanaka, Gann, 59, 421 (1968).
81. Y. Nishikawa, T. Takeda, S. Shibata, and F. Fukuoka, Chem. Pharm. Bull., 17, 1910 (1969).
82. M. Takeda, M. Funatsu, S. Shibata, and F. Fukuoka, Chem. Pharm. Bull., 20, 2445 (1972).

83. I. Yokota, S. Shibata, and H. Saito, Carbohydr. Res., 69, 252 (1979).
84. K. Takahashi, T. Takeda, S. Shibata, M. Inomata, and F. Fukuoka, Chem. Pharm. Bull., 22, 404 (1974).
85. Y. Nishikawa, K. Ohai, K. Takahashi, G. Kurono, F. Fukuoka, and M. Emori, Chem. Pharm. Bull., 22, 2692 (1974).
86. Y. Nishikawa, K. Yoshimoto, R. Horiuchi, K. Michishita, M. Okabe, and F. Fukuoka, Chem. Pharm. Bull., 27, 2065 (1979).
87. K. Takahashi, T. Kon, I. Yokota, and S. Shibata, Carbohydr. Res., 89, 166 (1981).
88. A. A. Zeidaka and Kh. F. Bass-Sharkhar, Zh. Prikl. Khim. Biokhim. Mikrobiol., 13, 359 (1977).
89. Kh. F. Bass-Sharkhan and A. A. Zeidaka, in: New Immunoregulating Drugs and Immunological Methods [in Russian], Riga (1978), p. 9.
90. Y. Nishikawa and H. Ohino, Chem. Pharm. Bull., 29, 3407 (1981).
91. L. Pillemer and E. Ecker, J. Biol. Chem., 137, 139 (1941).
92. L. Pillemer et al., J. Immunol., 71, 331 (1953).
93. D. Rowley, Lancet, 1, 232 (1955).
94. S. M. Navashin, I. P. Fomina, and T. G. Terent'eva, Dokl. Akad. Nauk SSSR. 158, 981 (1964).
95. W. T. Bradner, D. A. Clarke, and C. C. Stock, Cancer Res., 18, 347 (1958).
96. J. S. Kiser, H. Lindh, and G. C. DeMello, Ann. N.Y. Acad. Sci., 66, 312 (1956).
97. G. Mathé and J. Bernard, C. R. Acad. Sci. Paris, 150, 1768 (1956).
98. H. F. Oettgen and W. W. Gallmeier, Dtsch. Med. Wschr., 93, 1157 (1968).
99. I. C. Diller, Z. T. Mankowski, and M. E. Fisher, Cancer Res., 23, 201 (1963).
100. K. Pfordte, Z. Naturforsch., 216, 56 (1966).
101. Kh. M. Veksler, Zh. U. Timoshenko, A. I. Keish, I. M. Remez, V. A. Sevast'yanova, and O. V. Popova, in: Nonspecific Stimulators of the Reactivity of the Organism and Their Use in Oncology [in Russian], Riga (1977), p. 104.
102. M. A. Kashkina and N. P. Elikov, Zh. Mikrobiol., Épidemiol. Immunobiol., No. 5, 86 (1977).
103. V. É. Liepa, S. A. Avertsev, and Kh. F. Bass-Sharkhan, in: Immunological Homeostasis and Immunoregulating Therapy [in Russian], Riga (1979), p. 133.
104. I. G. Veksler, Byull. Éksp. Biol. Med., No. 8, 64 (1980).
105. N. R. Di Luzio, Adv. Exp. Med. Bull., 73, 412 (1976).
106. N. R. Di Luzio, R. McNamee, E. Jones, J. A. Cook, and E. O. Hoffman, in: The Macrophages in Neoplasia, M. A. Fink, ed., Academic Press, New York (1976), p. 181.
107. N. R. Di Luzio and J. C. Pisano, J. Reticuloendothel. Soc., 7, 731 (1970).
108. A. F. Blyuger, Kh. M. Veksler, Ya. D. Keish, P. M. S"yakete, O. V. Popova, Kh. F. Bass-Sharkhan, and G. K. Zakenfel'd, in: New Immunoregulating Drugs and Immunological Methods [in Russian], Riga (1978), p. 5.
109. J. A. Cook, D. Taylor, C. Cohen, E. O. Hoffman, J. Rodriguez, V. Malshet, and N. R. Di Luzio, J. Reticuloendothel. Soc., 22, 21 (1977).
110. H. Mashiba, M. Gojobori, and K. Matsunaga, Gann, 68, 703 (1977).
111. D. H. Northcote, Pure Appl. Chem., 7, 669 (1963).
112. C. E. Ballou and W. C. Raschke, Science, 184, 127 (1974).
113. G. H. Fleet and D. J. Manners, J. Gen. Microbiol., 94, 180 (1976).
114. S. Suzuki, M. Suzuki, H. Hatsukaiwa, H. Sunayama, T. Suzuki, M. Uchiyama, F. Fukuoka, M. Nakonishi, and S. Akiya, Gann, 60, 273 (1969); S. Suzuki, H. Natsukaiwa, H. Sunayama, M. Uchiyama, F. Fukuoka, M. Nakanishi, and S. Akiya, Gann, 60, 65 (1969).
115. S. J. Riggi and N. R. Di Luzio, Fed. Proc., 20, 265 (1961).
116. I. C. Diller, M. E. Fisher, and D. Gable, Proc. Soc. Exp. Biol. Med., 117, 107 (1964).
117. D. J. Bell and D. H. Northcote, J. Chem. Soc., 1944 (1950).
118. R. J. Yu, C. T. Bishop, F. P. Cooper, F. Blank, and H. F. Hasenclever, Can. J. Chem., 45, 2264 (1967).
119. D. J. Manners, A. J. Masson, and J. C. Patterson, J. Gen. Microbiol., 80, 411 (1974).
120. D. J. Manners and M. T. Meyer, Carbohydr. Res., 57, 189 (1977).
121. W. C. Raschke, K. A. Kern, C. Antalis, and C. E. Ballou, J. Biol. Chem., 248, 4660 (1973).
122. C. E. Ballou, K. A. Kern, and W. C. Raschke, J. Biol. Chem., 248, 4667 (1973).
123. T. Matsumoto, M. Takanohashi, Y. Okubo, M. Suzuki, and S. Suzuki, Carbohydr. Res., 83, 363 (1980).
124. P. A. J. Gorin and J. F. T. Spencer, Adv. Carbohydr. Chem., 23, 367 (1968).
125. N. P. Elinov, "The antigenic determinants of yeasts," in: Proceedings of the 4th International Symposium on Yeasts, Vienna, Part II (1974), pp. 35-48.

126. O. G. Sakandelidze and R. E. Kipiani, Biologically Active Substances of Hydrobionts — A New Source of Drugs [in Russian]. Kishinev (1979).
127. G. R. Pettit, Biosynthetic Products for Cancer Chemotherapy, Vol. 1, Plenum Press, New York—London (1977), pp. 165-175.
128. C. P. Li, A. Goldin, and J. L. Hartwell, Cancer Chemother. Rep., Part 2, 4, 97-129 (1974).
129. M. R. Schmeer, Science, 144, 413-414 (1964).
130. A. Hegyeli, Science, 146, 77-79 (1964).
131. A. C. Schmeer, Physiol. Chem. Phys., 11, 415-424 (1979); Fed. Proc., 37, 317-323 (1978).
132. Yu. S. Ovodov, R. G. Gorshkova, S. V. Tomshich, and M. N. Adamenko, Comp. Biochem. Physiol., 29, 1093-1098 (1969).
133. N. A. C. Allison, J. S. Warington, and M. Birbeck, J. Exp. Med., 124, 141-149 (1966).
134. G. E. Davies, Immunology, 8, 291-295 (1965).
135. J. A. Bash and J. R. Vago, J. Reticuloendothel. Soc., 28, 213-221 (1980).
136. V. M. Rumjanek, S. R. Watson, and V. S. Slijivic, Immunology, 33, 423-432 (1977).
137. A. W. Thomson, Biomedicine, 28, 148-160 (1978).
138. A. W. Thomson, E. F. Fowler, V. S. Slijivic, and L. Brent, Lancet, 1034 (1980).
139. A. W. Thomson, L. Brent, and E. F. Fowler, Lancet, 671 (1981).
140. P. C. Quan, J. P. Kolb, and G. Lespinats, Cell. Immunol., 37, 1-9 (1978).
141. J. P. Kolb, P. C. Quan, M. F. Poupon, and C. Desaymard, Cell. Immunol., 57, 348-360 (1981).
142. T. Sasaki, N. Takasuka, and N. Abiko, J. Natl. Cancer Inst., 60, 1499-1500 (1978).
143. Yu. S. Ovodov, R. G. Ovodova, and Yu. N. Loenko, The Chemistry and Biochemistry of Carbohydrates [in Russian], Pushchino (1982), pp. 4-5.
144. V. I. Molchanova, R. G. Ovodova, I. Perera, and Yu. S. Ovodov, Khim. Prir. Soedin., 677 (1982).
145. T. S. Zaporozhets, R. G. Ovodova, Yu. N. Loenko, and N. N. Besednova, Zh. Mikrobiol. Épidemiol. Immunobiol., No. 2, 106-107 (1981).
146. T. S. Zaporozhets, Yu. N. Loenko, R. G. Ovodova, N. F. Timchenko, N. G. Prokof'eva, and N. N. Besednova, Antibiotiki, No. 6, 460-465 (1981).