BIOLOGICAL ACTIVITY OF THE POLYSACCHARIDE GLUCAN

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Many bacterial polysaccharides, when administered parenterally, increase the resistance of animals to pathogenic agents. Different polysaccharides possess this property to a different degree depending on their origin and composition. The problem of the relationship between the biological action of these polysaccharides and their composition and structure is therefore one of considerable interest.

Most work has been carried out on the study of the biological activity of endotoxins—polysaccharide complexes isolated from pathogenic Gram-negative microorganisms (Salmonella, Bacillus pyocyaneus, Escherichia coli, etc.). These complexes, incorporating various sugars and their derivatives, also lipids, have an intricate structure. For these reasons we have investigated the biological activity of a polysaccharide, the structure of which is known. We used the polysaccharide glucan, isolated from bakers' yeast [1], and also its partial hydrolysis product "hydrolyzed glucan." Both substances are glucose polymers and they differ in the nature of the bonds between the glucose residues in their molecule [2].

EXPERIMENTAL METHOD

The biological action of glucan was studied on a model developed and used in earlier work [3-6]. Special experiments on rabbits* showed that glucan, in a dose of 5 and 200 μg, has no pyrogenic action. The ability of glucan to increase the resistance of animals to bacterial infection was tested in experiments on albino mice, which received an intramuscular injection of 200 and 400 μg glucan in 0.2 ml physiological saline 24 h before inoculation with a pathogenic strain of E. coli (No. 145). Mice of the control group received an injection of the same volume of physiological saline at the same time. Observations on the animals continued for 5 days.

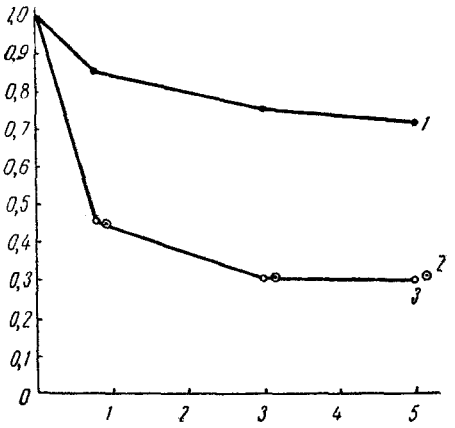
EXPERIMENTAL RESULTS

The experimental results were expressed by the survival index (ratio between the number of surviving mice and total number of mice in the experiment).

Results showing the rate of survivial of the mice inoculated with a pathogenic strain of E. coli (No. 145), after previously receiving an injection of 200-400 μg glucan or 200-400 μg "hydrolyzed glucan," and of control animals are given in the figure. It is clear that the survival rate of the mice receiving glucan was higher than that of the control group. On the 3rd day after inoculation the survival indices of the mice receiving glucan and of the controls were 0.75 and 0.3 respectively. This difference is statistically significant (T=2.5). It also follows from the figure that the injection of "hydrolyzed glucan" had no effect on the animals' resistance to infection. The survival indices of the mice receiving hydrolyzed glucan before infection and of the control animals were exactly the same.

The ability of glucan to increase the resistance of the organism to bacterial infection was also investigated in chick embryos. A finely divided suspension of 200 μg glucan in physiological saline was injected into the allantoic sac of 13-day chick embryos. Physiological saline was injected into control embryos. From 18 to 24 h later the

*Pyrogenicity was investigated by G. Ya. Kivman and A. V. Kosolapova, at the Institute of Pharmacology and Chemotherapy of the AMN SSSR.



Survival rate of mice inoculated with E. Coli, strain No. 145.
1) Mice receiving glucan; 2) receiving "hydrolyzed glucan"; 3) controls. Along the axis of ordinates—survival index; along the axis of abscissas—days after inoculation.

embryos were inoculated with a lethal dose (1000 bacterial cells) of a culture of Staphylococcus aureus (strain No. 596). Observations were made for 1 week. In the control group all 50 infected embryos died 3 days after inoculation. The survival rate of the embryos infected with S. aureus and receiving 200 μg glucan may be inferred from the fact that of the total number of 47 embryos in the experimental group all 47 were still alive on the 3rd day after injection of glucan, while on the 7th day 21 embryos survived.

The adminstration of 200 μg glucan to 13-day embryos thus led to a sharp fall in their mortality rate after infection with a lethal dose of S. aureus. Similar results were obtained with embryos infected with a pathogenic strain of E. coli.

The high rate of survival of the mice and chick embryos after different infections indicates that a single injection of glucan 24 h before infection leads to a marked increase in natural resistance.

We have previously shown [1] that glucan has the property of fixing properdin and inactivating the 3rd component of complement in blood serum. The effect of this polysaccharide in the serum properdin concentration in vivo was investigated in albino mice. Glucan was injected intraperitoneally in the form of a 1% suspension in physiological saline in a dose of 100 mg/kg body weight. Control animals received an injection of the same volume of physiological saline. Five mice each from the experimental and control groups were sacrificed 3 h after the injection and thereafter daily for 8 days. The blood serum of the mice of each group was pooled and kept at -20° until the end of the experiment. On the 8th day the properdin in all the sera was titrated by Pillemer's method [7].

Three hours after injection of glucan the properdin titer was observed to have fallen 50% of its initial level (in some cases, to values smaller than the limit of sensitivity of the method). After 48 h the normal properdin titer was restored, while on the 5th-7th day in some experiments the blood properdin concentration was raised. These findings indicate that glucan fixes properdin both in vitro and in vivo.

The study of the blood of mice various intervals after injection of glucan (in a dose of 200 μg intraperitoneally) showed that the animals developed leukopenia during the first 6-8 h after the injection. This was most marked 2-4 h after the injection, when the number of leukocytes in the peripheral blood fell to 52-57%. After 6-8 h the leukocyte count in the experimental animals was 72-80% of the control value. After 12-13 h practically no leukopenia was present, and after 24 h a moderate leukocytosis was observed: the leukocyte count was 40% above the control level. The leukocytosis was maintained for 72-96 h, and at the end of the 4th or 5th day after injection the normal leukocyte count was restored.

The intial leukopenia followed by a leukocytosis is a biphasic reaction simlar to that observed after administration of zymosan, acetoxan, prodigiosan, and other biologically active polysaccharides. The effect of glucan on the phagocytic activity of the reticulo-endothelial cells of the mice was determined by a method described earlier [4] using intravenously injected thorotrast as the object of phagocytosis. The number of cells exhibiting phagocytosis (per unit of surface) was counted in histological preparations, and their relative load of phagocytosed thorotrast particles determined. Glucan was injected intrperitoneally in a dose of 400 μg to each mouse, made up in 0.2 ml of physiological saline, 24 h before injection of the thorotrast. The mice of the control group received an injection of the same volume of physiological saline. The mean results of 6 experiments (60 mice altogether) are shown in the table.

The results in the table show that glucan had a stimulating action on the phagocytic activity of the cells of the reticulo-endothelial system, causing an increase both in the total number of cells exhibiting phagocytosis and in the relative number of Kupffer cells in which phagocytosis was intensive.

The effect of glucan on inflammation was studied by means of a method described previously [6]. Acute aseptic inflammation of the ears was produced in mice 4 h after injection of glucan intraperitoneally (200-400 μg per mouse) or subcutaneously (2μg per mouse). Fifteen minutes after application of the irritant (carbol xylol) to the surface of the ear the animals were given an intraperitoneal injection of trypan blue, after which the rate at

Effect of Glucan on Phagocytic Activity of Cells

Substance	No. of Kupffer cells with thorotrast particles per unit area of liver section (mean data)	Percentage of Kupffer cells with different degrees of loading with thorotrast		
		high	moderate	low
Physiological saline.	12.9	7	35	58
Glucan.	17.8	19	51	30

which it passes from the blood vessels into the tissue of the inflamed ear was noted in the experimental and control (not receiving glucan) animals. In addition, the intensity of staining of the inflamed tissues with trypan blue was noted in the mice of both groups. Experiments were undertaken on 108 mice (68 experimental and 40 control). Of the total number of experimental mice, 48 received glucan subcutaneously. In 28 of these animals injection of glucan was accompanied by marked lessening of exudation in the focus of inflammation: the delay before dye began to pass into the inflamed tissues was more than doubled, the intensity of staining was reduced by more than one-third. In 12 mice this effect was present to a moderate degree: the delay before dye began to pass into the tissues was increased by more than 50%, and the intensity of staining reduced by one-third or more. The results in eight mice were indistinguishable from the controls.

Among the 20 mice receiving glucan intraperitoneally, six gave a weakly positive effect and in 14 animals glucan had no effect whatever.

The results of these experiments demonstrate that glucan, when injected subcutaneously, has a definite anti-exudative action, by preventing the development of disturbances of permeability of the blood vessels in an inflammatory focus. This action has also been demonstrated in other polysaccharides of bacterial origin [6]. However, with the experimental technique which we adopted, the intraperitoneal injection of glucan had no such effect. Similar results were obtained in earlier experiments with zymosan, agar-agar, and several other polysaccharides of high molecular weight.

SUMMARY

Experiments on rabbits showed that glucan has the property of causing changes in the body characteristic of many bacterial polysaccharides: a reaction of the leukocytes, an increase in the phagocytic activity of the reticuloendothelial system, and an increase in the resistance of mice and chick embryos to bacterial infection. "Hydrolyzed" glucan does not possess these properties. Earlier studies [2] showed that "hydrolyzed" glucan differs in its chemical structure from the original glucan: its molecule contains no β=1:6 bond. The associated decrease in molecular weight and change in the configuration of the molecule apparently result in the loss of biological activity of this polysaccharide.

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