EFFECT OF CONSERVATION WITH METAPERIODATE AND GLUTARALDEHYDE ON THE IMMUNOGENIC PROPERTIES OF BLOOD VESSELS

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Experiments on guinea pigs and rabbits showed that treatment of human femoral vessels with solutions of metaperiodate and glutaraldehyde sharply reduces (to one-tenth) the content of water-soluble proteins in tissue homogenates, depresses the sensitizing and anaphylactogenic activity of the extracts, and leads to inertia in the precipitation test with homologous antiserum against native tissue.

KEY WORDS: *blood vessel; conservation; sensitization; anaphylaxis, precipation.*

The problem of ideal prosthetic material for peripheral vessels has not yet been finally solved. The use of allogeneic and xenogeneic vessels, despite their evident advantages, is limited by the appearance of late degenerative changes, which leads to thrombi, rupture, and to the development of an aneurism of the graft [5, 8, 10, 11]. According to observations made by several workers [13, 15], these complications are due to the immunologic reaction of the host to the foreign tissue. In turn, the intensity of the immune response largely depends on the immunogenic properties of the graft. This suggests that a reduction in the antigenic activity of the grafted tissue would considerably improve the result of the clinical use of biological vessels. In recent years several types of conservation of blood vessels aimed at preserving the normal structure of the tissue and reducing its antigenic activity, have been studied intensively [1-4, 12].

The object of the present investigation was to study the immunogenic properties of human vessels preserved with solutions of sodium metaperiodate and glutaraldehyde [9] and to compare them with the antigenic properties of intact human blood vessels. This method of conservation has been studied in connection with the use of heart valves and has been widely applied in plastic cardiac surgery [6, 7].

EXPERIMENTAL METHOD

Human femoral vessels, both intact and preserved with solutions of sodium mataperiodate in conjunction with glutaraldehyde [9] were studied. To discover the antigenic and immunogenic properties fragments of blood vessels were carefully washed to remove the preservative in physiological saline, homogenized in a mortar with quartz sand and used to prepare saline extracts (1:5), which were used in the subsequent experiments. The protein concentration in the extract was determined by Lowry's method.

The sensitizing properties of extracts of native and preserved tissues were studied in active anaphylaxis tests on 68 guinea pigs weighing 225-250 g. The animals were sensitized with combinations of antigen with Freund's complete adjuvant by subcutaneous injection of the extract in a dose of 150 µg protein at 4 points. After 24 days anaphylactic shock was induced in the animals by intravenous injection of 1000 µg, and the severity of the shock in the groups for comparison was assessed by the anaphylactic index.

Qualitative analysis of the antigenic composition of the blood vessels before and after preservation was carried out by radial immunodiffusion in agar gel with antisera obtained by triple subcutaneous immunization of rabbits with a corresponding antigen: 15 mg protein/kg body weight in Freund's complete adjuvant.

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TABLE 1. Antigen Activity of Tissue Extracts of Native and Preserved Human Blood Vessels

Type of sensitizing antigen	Severity of shock in response to reacting injection of antigen	
	AGN	AGP
AGN	4,05/20	0,13/15
AGP	1,91/23	0/10

Legend. 1. AGN) extract of native vessels; AGP) extract of preserved vessels. 2. Numerator of fraction represents mean anaphylactic index (severity of shock assessed on a 5-point system); denominator represents number of animals in group.

EXPERIMENTAL RESULTS

The production of anaphylactic shock revealed that sensitization of the guinea pigs with homogenates of native blood vessel tissue led to a marked immunologic response of the recipient animals, characterized by a severe anaphylactic response to the reacting injection of specific proteins (Table 1).

Most guinea pigs developed severe signs of shock 1-2 min after injection of the reacting dose of homologous antigen, and in 40% of cases the animals died. The anaphylactic index was 4.05. The sensitizing activity of extracts of the preserved blood vessels was significantly lower than the activity of the native tissue.

After injection of the reacting dose of native tissue into guinea pigs sensitized with antigens of preserved blood vessels only 6 of the 23 animals developed an intensive shock reaction. The anaphylactic index was 1.91. In this series of experiments none of the animals died.

For a comparative study of the ability of extracts of blood vessels to induce anaphylactic shock, a reactiving injection of homologous antigen was given to sensitized animals. These experiments showed that, unlike native extracts, extracts of preactivity could be detected in only 2 of the 15 animals in response to intravenous reacting injection of preserved antigens after sensitization with native tissue. The anaphylactic index was 0.13.

Similar results were obtained in a study of the immunogenic properties of the tissues of native and preserved vessels in experiments with immunization of rabbits. These experiments showed that saline extracts of native blood vessels led to the formation of different antibodies, clearly detectable in the precipitation test with homologous antigens in a dilution of 1:64-1:128, in the animals. The antigens formed four precipitation arcs in the reaction with homologous antiserum.

Extracts of preserved tissues were inert in these tests. No precipitation could be obtained during the investigation of antigens of preserved tissues even in cross reactions with native antiserum.

These investigations showed that treatment of blood vessels with sodium metaperiodate and glutaraldehyde significantly reduces the immunogenic properties of the blood vessels. This effect is evidently due to denaturation of insoluble proteins [9] and to a decrease in the content of water-soluble proteins.

Determination of the protein concentration in the saline extracts of the blood vessels showed that preservation of the tissues by sodium mataperiodate and glutaraldehyde leads to a tenfold decrease in the content of water-soluble tissue proteins. The protein content in the extract after preservation averaged 542 µg/ml, compared with 5260 µg/ml in native tissue.

The results suggest that the clinical use of blood vessels treated with sodium metaperiodate and glutaraldehyde should not be followed by the development of a response of transplantation immunity.

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EFFECT OF DIMETHYLSULFOXIDE ON TRANSFORMATION OF *Bacillus subtilis in vitro*

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Transformation of *Bacillus subtilis* was carried out in the presence of dimethyl sulfoxide and polyethylene glycol. The frequency of transformation of *B. subtilis* was increased by 0.1% dimethyl sulfoxide but was not appreciably changed by polyethylene glycol. It is suggested that the increase in frequency of transformation was due to the effect of dimethyl sulfoxide on permeability of the cell membrane or to changes in membrane deoxyribonuclease activity.

KEY WORDS: *dimethyl sulfoxide; transformation; deoxyribonuclease.*

The transfer of genetic material by the transformation method is of considerable interest to the study of the role of DNA in the transmission of hereditary properties of bacterial cells, changes arising in the DNA molecule after exposure to various factors, and changes in the permeability of the bacterial cell membrane [3]. Substances capable of modifying the permeability of biomembranes include hormones, enzymes, antibiotics, dimethyl sulfoxide (DMSO), etc. [5, 8, 13]. It will be recalled that trypsin, for example, leads to an increase in the frequency of transfer of the lac operon during hybridization of *Escherichia coli* and the agent of dysentery [2].

The object of the present investigation was to study the effect of DMSO on the frequency of transformation of *B. subtilis*, for it can be postulated that DMSO, through its effect on the permeability of the cell membrane, may modify the frequency of transformation.

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