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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TABLE 1. Effect of Dimethyl Sulfoxide on Frequency of Appearance of trp^+ Transformants of *B. subtilis*

Ingredients	Frequency of appearance of trp^+ transformants of B. subtilis	
	$(M \pm m) \times 10^{-2}$	P
B. subtilis + DNA	0,240±0,097	—
B. subtilis + DNA + 0,1% DMSO	0,797±0,220	<0,05
B. subtilis + DNA + 1% DMSO	0,566±0,157	0,1

EXPERIMENTAL METHOD

Transforming DNA was isolated from cells of *Bacillus subtilis* strain 23 EMB by the method of Ephrati-Elizur et al. [7], modified by Bresler [1]. The protein concentration in the DNA preparations was determined by Lowry's method [10] and their viscosity was determined on a low-gradient capillary viscosimeter with extrapolation to zero gradients. The molecular weight of the DNA preparations was calculated by Spitkovskii's equation [4]. Cells of *B. subtilis* strain 168-2, unable to synthesize leucine and tryptophan independently (leu^-trp^-) were used as the recipient. Transformation experiments were carried out with the aid of a modified Spizizen's method [6]. DMSO was added to competent recipient cells along with DNA.

EXPERIMENTAL RESULTS

To study the effect of DMSO on genetic transformation of *B. subtilis* DNA preparations isolated from the donor strain *B. subtilis* 23 EMB, in a concentration of 0.2 mg/ml, and a Soviet preparation of DMSO were used. DMSO was added to the transformation mixture in a final concentration of 0.1, 1, and 10%. These concentrations were chosen from the results of experiments to determine the survival of *B. subtilis*, in which the effect of different concentrations of DMSO was studied. In the concentrations mentioned above DMSO caused death of 0, 10, and 66% of cells respectively.

The results of the study of the effect of DMSO on transformation of *B. subtilis* are given in Table 1. They show that DMSO in concentrations of 0.1 and 1% stimulated the transformation of *B. subtilis*; the stimulating action of a concentration of 0.1% was stronger than that of a concentration of 1%.

For comparison with DMSO in the transformation experiments polyethylene glycol was used. According to data in the literature this compound, if used in hybridization experiments with somatic cells, causes them to shrink and subsequently to agglutinate [12]. A preliminary series of experiments was carried out in order to study the action of this compound on the survival rate of *B. subtilis*. The results of these experiments showed that polyethylene glycol, in final concentrations of 200, 100, 50, 25, and 12.5 mg/ml, caused death of 65, 55, 55, 40, and 20% of cells respectively. With these results in mind, in the next experiments polyethylene glycol was used in concentrations of 100 and 50 mg/ml. The results indicate that polyethylene glycol had no marked stimulating action on the transformation of *B. subtilis*.

The results of these experiments suggest that the increase in frequency of transformation caused by DMSO may be due to an increase in the permeability of the cell membranes; this effect may also be indirect, through a change in the activity of membrane deoxyribonuclease (DNase), which is said [9] to break up one strand of DNA into oligonucleotides and thereby facilitate the penetration of the complementary segment into the cell. DMSO may perhaps increase the activity of this DNase, for the work of Monder [11] has shown that DMSO increases the activity of purified pancreatic DNase and of microbial DNase (streptodornase).

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SUPPRESSOR ACTIVITY OF SPLEEN CELLS IN DRUG-INDUCED IMMUNOLOGIC TOLERANCE

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Tolerance to sheep's red blood cells (SRBC) was induced in (CBA × C57BL/6)F_1 mice by a single intraperitoneal injection of 6×10^9 SRBC followed by injection of 100-200 mg/kg cyclophosphamide 44-46 h later. Spleen cells of tolerant mice, obtained at various times (12-26 days) after induction of tolerance, when injected into intact syngeneic recipients, did not depress their immune response to SRBC. Unlike intact mice, tolerant mice were unable to produce suppressor cells after a single immunization with SRBC. Only if three additional injections of large doses (6×10^9) of SRBC were given to the tolerant mice did their spleen cells acquire the ability to inhibit the immune response on injection into normal mice. It is postulated that the absence of suppressor cells on induction of immunologic tolerance by means of cyclophosphamide is due to clonal elimination. Suppressor cells may arise in tolerant animals under the influence of intensive antigenic stimulation, leading to deepening of the state of tolerance as a result of additional injections of SRBC.

KEY WORDS: *immunologic tolerance; cyclophosphamide; suppressor cells.*

Recent investigations have shown that suppressor cells play a role in the mechanism of some forms of immunologic tolerance [6, 10]. Suppressor T cells have also been found in drug-induced tolerance, although attempts by some workers to detect their presence were unsuccessful [5, 7, 9].

It was shown previously that during immunization of mice with a sufficiently high dose of sheep's red blood cells (SRBC) T suppressors inhibiting the immune response of intact syngeneic recipients appeared among their spleen cells (SC) [3, 11].

The object of this investigation was to study suppressor activity of mouse SC in tolerance induced to SRBC with the aid of cyclophosphamide (CP).

EXPERIMENTAL METHOD

Experiments were carried out on adult (CBA × C57BL/6)F_1 male mice weighing 22-28 g (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR). Tolerance was induced by intraperitoneal injection of 6×10^9 SRBC followed 44-46 h later by injection of

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