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Inhibition of Transformation of Streptococci by Antibodies

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It has been reported (Nava, Galis, and Beiser, Nature **197**:903, 1963) that immunization of rabbits with competent pneumococci stimulates the production of antibodies which inhibit transformation. Similar data reported below show that the antigenic structure of transformable streptococci in the competent state differs from the antigenic structure of cells in the noncompetent state.

Competence in cultures of streptococci is due to the action of a competence-provoking protein which changes the cell surface to allow deoxyribonucleic acid (DNA) uptake (Pakula and Walczak, J. Gen. Microbiol. **31**:125, 1963; Pakula and Hauschild, Can. J. Microbiol., *in press*). The competence-provoking protein produced by the highly transformable group H *Streptococcus*, strain Challis, was used to convert to competency cultures of strain Wicky, a *Streptococcus* of the same serological group. The latter strain does not produce measurable amounts of the enzyme and cannot, therefore, be transformed in a natural way with any significant efficiency.

Competent and noncompetent cells from cultures of both strains were prepared as described before (Pakula and Walczak, J. Gen. Microbiol. **31**:125, 1963). The cells were used to immunize rabbits for 6 weeks. Each week, three 1-ml injections were given on successive days followed by a resting period of 4 days. The rabbits were bled 10 days after the last injection. Since rabbit sera may occasionally inhibit transformation, globulin fractions were prepared as described by Strauss et al. (Proc. Soc. Exptl. Biol. Med. **105**:184, 1960).

Transformation of the drug-sensitive recipients, Challis and Wicky, to streptomycin resistance was carried out with DNA extracted from a streptomycin-resistant mutant of strain Challis. To do this, 0.25 ml of a preparation of the competence-provoking factor was added to 2-ml amounts of a 2.5-hr culture. After 15 min, each tube received 0.1 ml of globulin solution. The globulins were allowed to act for 30 min. To this mixture were added 5 μg/ml of DNA and, after 15 min, deoxyribonuclease. The cultures were then grown 110 min to allow phenotypic expression of the acquired streptomycin resistance and were assayed for the number of transformants.

The inhibitory effect of globulins against competent cells on transformation is shown in Table

TABLE 1. *Effect on transformation of serum globulins of rabbits immunized with competent and noncompetent streptococci*

Globulins against	Transformants per ml	
	Strain Challis	Strain Wicky
Competent Challis cells.....	3,200	6,400
Noncompetent Challis cells..	540,000	890,000
Competent Wicky cells.....	7,400	13,200
Noncompetent Wicky cells...	398,000	724,000
Control*.....	380,000	763,000
Control†.....	560,000	980,000

* Normal globulin.
† No globulin added.

TABLE 2. *Removal of antibodies inhibiting transformation by adsorption of globulins against competent cells with homologous cells*

Globulins against competent Wicky cells adsorbed with	Transformants per ml of strain Wicky		
	Expt 1	Expt 2	Expt 3
Competent Wicky cells...	246,000	340,000	264,000
Noncompetent Wicky cells.....	8,200	10,800	9,100

1. Globulins against noncompetent cells do not inhibit transformation.

Cells were also treated with globulins prior to the addition of the competence-provoking factor. However, this procedure did not prevent conversion of cells to the state of competency. The re-

sults, therefore, indicate that the DNA-specific receptor sites on the cell surface (Hotchkiss, Proc. Natl. Acad. Sci. U.S. **40**:49, 1954) were blocked by antibodies to competent cells.

A single adsorption of globulins against competent cells with competent cells resulted in a significant reduction of the inhibitory effect on transformation, whereas adsorption with noncompetent cells had no such effect (Table 2). Consequently, the factor in globulins inhibiting transformation appears to be an antibody to an antigen specific for the state of competency.