trolled by the immune response. Moreover, the absence of demonstrable lytic complement in the CSF throughout the course of the experimental infection in monkeys (*14*) indicated a lack of an important mechanism for immune control of the infection.

Other factors which are believed to affect the course of infection are fluctuations in the virus-induced antigens on the cell surface (*17*), an excess of antibody against viral nucleoprotein over antibody against viral membrane antigens (*18*), and inhibition of cell-mediated immune lysis by blocking factors in the CSF and serum (*19*).

Studies of the specificity and function of antibody and lymphocyte effector cells in the course of experimental SSPE infection in monkeys should provide results that will serve as a basis in evaluating therapeutic approaches to be used in the primate model.

PAUL ALBRECHT
Bureau of Biologics,
Division of Virology,
Food and Drug Administration,
Bethesda, Maryland 20014
THEODORE BURNSTEIN
Department of Veterinary Medicine,
Purdue University,
West Lafayette, Indiana 47907
MICHAEL J. KLUTCH
JOHN T. HICKS, FRANCIS A. ENNIS
Bureau of Biologics,
Division of Virology,
Food and Drug Administration

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Lysogeny in Pneumococci Freshly Isolated from Man

Abstract. *Twelve strains of encapsulated pneumococci isolated from patients with pneumococcal disease were examined for the presence of bacteriophage. Four of the strains yielded phage lytic for a noncapsulated indicator strain of pneumococcus. Three of the newly isolated bacteriophages differ serologically from pneumococcus bacteriophages described previously. The ability to yield lytic phage was lost by two of the lysogenic pneumococcal strains on repeated subculture.*

DNA-mediated transformation, first discovered in the pneumococcus (*1*), remains the only reported mechanism of gene transfer in this organism. While results from phage-mediated gene transfer studies have contributed greatly to the understanding of genetic mechanisms in other bacterial systems, no bacteriophages lytic for pneumococci had been described until recently (*2*). The recovery of temperate pneumococcal phages has not been recorded. In the course of testing the sensitivity of various pneumococcal strains to some of the ω phages (*3*), attempts were made to induce lytic phage from 24 laboratory strains of encapsulated pneumococci by growing the organisms in the presence of mitomycin C (MC); the experiments were uniformly unsuccessful. However, it seemed possible that pneumococci freshly isolated from patients in whom the organism was causing disease might be lysogenic. I have examined strains isolated from 12 patients with pneumococcal disease and have recovered active phage from four of the strains.

A pneumococcus, type 8, was recovered from the blood of a patient with bacteremic pneumococcal pneumonia. Colonies from a blood-agar plate streaked with the original blood-broth culture were inoculated into Neopeptone beef-heart infusion broth containing horse blood (2 percent), and the culture was incubated overnight at 37°C. A subculture in casein hydrolyzate (CH) medium (*4*) was grown at 37°C to visible turbidity [2×10^7 to 4×10^7 colony-forming units (cfu) per milliliter] and divided. To one portion, MC was added to a final concentration of 0.1 μg/ml, and both cultures were incubated further. The turbidity of the control culture increased steadily while the turbidity of the culture with MC (protected from light by aluminum foil) increased for about 1½ hours and then began to decrease. Complete lysis did not occur, but at 2½ hours the tubes were chilled and centrifuged to sediment the bacteria. The supernatants were filtered through GA-6 (Gelman) filter disks. Samples (0.1 ml and 0.01 ml) of the filtrates were mixed with 0.1 ml of a young culture (about 2×10^7 cfu/ml) of a noncapsulated pneumococcus, strain 213 [R36A *str-r* (*5*)], grown in tryptic soy broth (Difco). The mixtures were incubated at 37°C for 10 minutes to permit adsorption and plated by the soft-agar overlay technique (*6*). After overnight incubation of the plates at 34°C, examination revealed three possible plaques from samples of the MC-treated culture. Each was picked with a Pasteur pipette into 0.5 ml of MI broth containing 2 percent blood; 0.1 ml of fresh culture of strain 213 was added to each tube, and the mixtures were replated as before. One of them yielded several thousand plaques, and the phage could then be propagated easily in liquid or on solid medium. Attempts to reisolate phage from subcultures of the type 8 pneumococcus were unsuccessful although cultures grown with MC continued to exhibit partial lysis as compared to control cultures. Electron micrographs of phage preparations revealed entities (Fig. 1) resembling the phages described by others (*1*). Electron micrographs of a two-times concentrated preparation of the original MC-treated culture and of a subsequent MC-treated culture showed particles that re-

Table 1. Neutralization of phage by rabbit antiserum to ω2 phage.

Phage	K value*
ω2	38.7
ω3	22
ω8	26
K3²†	27.5
K11	36.8
K12	35
K19	2.5
Diplophage	17.7, 15.3
Induced 8‡	22
474, 481, and 496	§

*K value determined according to Adams as follows: 0.9 ml of antiserum, diluted 1:90, was incubated with 0.1 ml of phage for 15 minutes at 37°C. The mixtures were immediately diluted 100-fold, and 0.1-ml samples were assayed for phage. In control samples, 0.9 ml of tryptic soy broth replaced the antiserum. For phage K19, antiserum was used at a dilution of 1:9. †K phages were isolates from human throats. ‡The phage induced from pneumococcus, type 8. §The method is not applicable for these phages. See Table 2.

sembled phage heads. No tails were seen (Fig. 1, inset).

Three cultures of pneumococcus, types 6A (strain 474), 19A (strain 481), and 19F (strain 496), isolated from pediatric patients with otitis media (*7*), were grown at different times in CH medium with and without MC. All three strains yielded phage lytic for strain 213 in both control and MC-treated cultures. The recovery from the treated cultures was 10 to 20 times that from controls. The strains differed in their capacity to maintain the lysogenic state in culture. Strain 474 when first tested yielded 10^3 plaque-forming units (pfu) per milliliter in the control culture and at least ten times that number in the MC-treated culture. Upon retesting a second serial subculture, no phage was recovered from control cultures and only 300 pfu/ml from the MC-treated cultures. No lytic phage was recovered from a third serial subculture. Strains 481 and 496, however, continued to yield phage from the fifth serial subculture, about 10^5 pfu/ml in control cultures and from 1×10^6 to 4×10^6 pfu/ml in MC-treated cultures. No further subcultures have been tested. It has been found that phage can be recovered from overnight cultures in Neopeptone infusion broth containing 2 percent rabbit blood; 600 pfu/ml were recovered from the supernatant of strain 481 and 2500 pfu/ml from strain 496. On blood agar plates, the colonies of strains 481 and 496 have the same appearance as those of nonlysogenic strains of the same capsular type.

Electron microscopic examination of the phage isolated from strain 474 shows particles indistinguishable from those of other pneumococcal phages. The phage from strain 496, however, may have shorter, thicker tails. More concentrated preparations of all of the phages must be examined before final conclusions about morphological differences can be made. However, three of the phages recovered from lysogenic pneumococci are antigenically different from ω phages, *Diplophage* (*2*), or phages isolated in this laboratory from throat swabbings. Neutralization tests carried out with rabbit antiserum to ω2 and ω3 show cross-reactivity with the phage induced from the type 8 pneumococcus but none with phages 474, 481, or 496 (Tables 1 and 2). The phages also differ from each other in stability; preparations of phages 8 and 496 retain their titers for several weeks at 4°C whereas phages 481 and 474 are difficult to maintain.

Other freshly isolated pneumococcal strains have not yielded phage. Among the negative strains are a pneumococcus type 8 isolated from the spinal fluid of a patient with pneumococcal meningitis; two strains of pneumococcus type 3 recovered from transtracheal aspirates of patients with pneumococcal pneumonia. One type 3 strain, lysed in the presence of MC, and possible phage heads were seen in electron microscopy. The other type 3 strain exhibited no lysis with MC and no phage; five additional strains from children with otitis media, including two other strains of type 19F, two of type 14, and one of type 23 yielded no phage although two of the strains lysed completely in the presence of MC. These results can be explained in at least three ways: The negative strains may not carry phage, they may carry defective phage, or the indicator strain used may not be a host for the putative phage.

Capsulated pneumococci appear to be invulnerable to infection by all phages that have been tested (*3*, *8*). This suggests either that the presence of phage in the four lysogenic strains results from rare, chance infection of fully capsulated strains, or that the phages influence capsule production.

Table 2. Neutralization tests for phages 474, 481, 496, and induced 8 with rabbit antiserum.

Phage	Incubation mixture: Antiserum (final dilution)	Plaques (No.)	Remaining activity (%)
	Experiment 1		
ω3	ω3, 1:100	129	13
	None	980	
474	ω3, 1:100	449	100
	None	420	
481	ω3, 1:100	154	92
	None	168	
496	ω3, 1:100	333	91
	None	364	
	Experiment 2		
ω3	ω3, 1:100	181	23.5
	None	770	
Induced 8	ω3, 1:100	245	48.2
	None	508	
	Experiment 3		
ω3	ω2, 1:55	3	0.26
	None	1145	
474	ω2, 1:10	373	100
	None	357	
496	ω2, 1:10	123	88
	None	140	

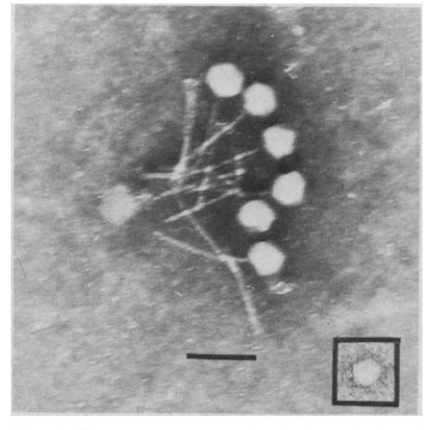

Fig. 1. Electron micrograph of phage isolated from pneumococcus type 8. Phage was prepared on solid medium, with strain 213 as host. The plate washings (2 ml) in tris buffer (tris-HCl, 50 m*M*, *p*H 7.8; Mg^{2+}, 10 m*M*) were centrifuged at 5000 rev/min at 4°C to remove cellular debris; the supernatant was centrifuged at 42,000 rev/min for 30 minutes in a Spinco model L ultracentrifuge. The supernatant from the ultracentrifugation was discarded and the pellet was allowed to elute overnight in the refrigerator in 1 ml of tris buffer. The eluate was centrifuged again at 5000 rev/min to clear, and this supernatant was used for electron microscopy without further purification (inset). Possible phage head from CH culture of pneumococcus type 8 treated with MC, concentrated as above by ultracentrifugation. This preparation yielded no active phage. The preparations were stained on grids with 2 percent sodium silicotungstate and examined in a Siemens 1A electron microscope. The bar represents 0.1 μm.

HARRIET P. BERNHEIMER
State University of New York, Downstate Medical Center, Brooklyn 11203

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7. The pneumococcal isolates were received from Dr. V. M. Howie, Huntsville, Ala. Aspirates taken to relieve pressure in the ears of ill children were streaked on blood agar plates: pneumococcal colonies were transferred to blood agar slants, and sent to Dr. Gerald Schiffman, Department of Microbiology, Downstate Medical Center. In Dr. Schiffman's laboratory, the slants were incubated for a few hours, and the pneumococci were transferred to Neopeptone beef-heart infusion broth containing 2 percent defibrinated rabbit blood. I received these cultures from Dr. Schiffman, stored them in the refrigerator, and transferred each culture to fresh blood broth the evening before I intended to test the strain. All pneumococcal type designations are according to the Danish system of nomenclature.
8. H. P. Bernheimer, in preparation.
9. I thank S. Scribani for all electron microscopy. Supported by NIH grant AI 05173 and career scientist award from the Irma T. Hirschl Trust.

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Complete Reversal of Experimental Diabetes Mellitus in Rats by a Single Fetal Pancreas

Abstract. *Complete reversal of streptozotocin-induced diabetes mellitus in adult rats will follow transplantation of a single fetal pancreas if the organ is first grown in a normal syngeneic carrier before transfer to the diabetic recipient. Careful control of the blood sugar of diabetic recipients may enhance the function of a single donor organ and thus improve histocompatibility matching.*

Under appropriate conditions transplanted fetal pancreases can completely reverse streptozotocin-induced diabetes in adult rats in addition to their having many other advantages as donor tissues (*1*). Initially, pancreases were removed from embryos at the optimal time of gestation (16 to 17½ days) and placed under the kidney capsule of syngeneic adult male rats previously made diabetic 3 to 4 days prior to the transplantation through streptozotocin injection. Recipients were treated with 4 units of NPH insulin (neutral protamine Hagedorn) injected daily for 4 days after transplantation and then with 3 units for an additional 4 days to partially control the diabetes during establishment of the fetal pancreas. Under these conditions, four or more pancreases were required to reverse completely the diabetic state. Transplantation of three fetal pancreases resulted in less than complete reversal and two transplants only alleviated the diabetic state significantly in 50 percent of the recipients.

In the present study, by evaluating the importance of blood sugar control following pancreas transplantation, we have found that a single fetal pancreas can completely reverse streptozotocin-induced diabetes mellitus. This was accomplished by culturing the fetal organ in vivo in a normal syngeneic carrier for a period of time before transplanting it into a diabetic host. The results suggest that control of the diabetic state may be very important for further growth and optimal development of function of the beta cells when the fetal pancreas is transplanted into diabetic recipients.

Pancreases were removed from embryos (16 to 17½ days old) of an inbred strain of Lewis rats (Microbiological Associates). One or two pancreases were immediately placed beneath the capsules of the right kidneys of normal syngeneic adults (primary transplants). At weekly intervals the kidneys, containing the undisturbed pancreases, were transplanted with vascular anastomosis into uninephrectomized syngeneic adult male rats (secondary transplant) made diabetic by intravenous injection of streptozotocin (62.5 to 65 mg per kilogram of body weight) 3 to 7 days previously. Urine volumes and glucose concentrations were measured daily, and plasma glucose concentrations were measured weekly, throughout the experiment beginning 24 hours after the injection of streptozotocin. Diabetes was of equal severity in control and all experimental groups. At 12 to 30 weeks after the secondary transplantation, the pancreases were removed from the kidney surface, and the recipients were monitored to assure that the observed reversal of diabetes was due to insulin secretion from the transplanted fetal pancreas rather than to spontaneous recovery of the host pancreas. The removed pancreatic tissues were immediately frozen (in acetone and Dry Ice) and stored at −20°C for determination of insulin content by radioimmunoassay according to the double antibody method.

Secondary transplantation of one or two fetal pancreases into adult diabetic rats did not significantly affect the diabetic state after 1 or 2 weeks in a normal carrier rat, whereas after 3 weeks in a normal carrier, the diabetic state was completely reversed (Fig. 1). Mean plasma glucose concentrations in ten diabetic rats of this group was 412 ± 3 mg/dl before transplantation (Fig. 2A). Daily urine volumes of these rats were 50 ± 6 ml prior to transplantation and decreased to normal levels of 11 ± 0.4 ml 4 weeks later. Glucose excretion in the urine fell from 1.20 ± 0.05 g/day to 0.08 ± 0.009 g/day over the same period. Additional evidence for complete reversal of the diabetic state was provided by the steady gain in body weight (2 g per day) in the experimental animals in contrast to the weight loss (2 g per day) in untreated diabetic controls. The glucose disappearance rate after intra-arterial injection of 0.5 mg of glucose per gram of body weight was 3.2 ± 0.5 percent per minute, a value not significantly different from normal rats of the same strain (2.9 ± 0.2 percent per minute). The mean concentration of immunoreactive insulin in the plasma of the rats with transplants before glucose injection was 50 ± 7 microunits per milliliter, a value barely elevated ($P < .05$) above normal (27 ± 2 microunits per milliliter). The mean increase in plasma insulin in response to glucose was 53 ± 11 microunits per milliliter, statistically not significantly different from the response in normal rats (39 ± 4 microunits per milliliter). Plasma glucose, urine volume, and glucose remained normal in this group of rats for more than 10 weeks until removal of the pancreas transplants. After removal of the transplants (Fig. 2A) the mean plasma glucose concentration reached 255 ± 16 mg/dl at 1 week and 390 ± 8 mg/dl at 2 weeks, when the mean urine volume reached 62 ± 4 ml/day and urine glucose 1.6 ± 0.6 g/day.

Secondary transplantation of fetal pancreases after an initial period of 4 or 5 weeks in a normal carrier rat was not only less effective in reversing diabetes but led to greater variability in the results (Fig. 1). Of eight diabetic rats receiving a 4-week-old secondary transplant of one fetal pancreas (Fig. 2B), only one manifested complete reversal of the diabetic state (plasma glucose 140 mg/dl) and the other seven were partially reversed (mean plasma glucose 277 ± 7 mg/dl from 3 to 5 weeks). At 6 weeks after secondary transplantation there was an abrupt spontaneous failure of response in all eight of these animals, the mean plasma glucose concentration reaching 414 ± 9 mg/dl compared to 413 ± 4 mg/dl before transplantation. The daily urine volume in the seven rats during the period of partial reversal of diabetes was 44 ± 7 ml/day, and with recurrence of severity at 6 weeks reached 137 ± 9 ml/day. Urine glucose content followed a similar pattern increasing from 1.1 ± 0.3 g/day during the period of partial reversal of diabetes to 11.0 ± 0.7 g/day follow-