Transduction of Capacity to Produce Staphylococcal Penicillinase. (26726)

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Resistance of *Staphylococcus aureus* to penicillin seems to be due to 2 apparently different mechanisms. Resistance in staphylococci isolated from natural sources is related to capacity to produce the enzyme, penicillinase(1) while resistant staphylococci isolated *in vitro* by selection from large populations of cells do not produce this enzyme (2).

The present report concerns an investigation of phage-mediated transduction of capacity to produce penicillinase by *S. aureus*. Transduction was first described by Zinder and Lederberg who observed this type of genetic exchange in the genus *Salmonella*(3). The phenomenon was observed in the genus *Escherichia* by Morse(4); in the genus *Shigella* by Lenox(5); and in the genus *Staphylococcus* by Ritz(6) and later by Morse(7).

Methods. The strains of *S. aureus* employed were isolated during epidemiological studies of staphylococcal infections in nurseries at Ohio State University Hospital. The Standard International Typing Series of staphylococcal bacteriophages employed were received from Dr. John E. Blair. Cultures of *S. aureus* were maintained on brain heart infusion (BHI) agar slants containing 7.5% NaCl at 4°C as were suspensions of bacteriophages.

The bacteriophages were propagated on penicillinase producing strains of *S. aureus* using the method of Swanstrom and Adams (8). The agar medium (P and D) used for propagation and titration of bacteriophage was nutrient broth containing 0.25% K_2HPO_4 and 0.2% dextrose; the basal layer contained 1.5% agar, the seed layer 0.5% agar. P and D broth was used to harvest and to prepare dilutions of bacteriophages, and to wash and suspend bacterial cells. Maximum amounts of phage were obtained after incubation for 8 to 10 hr at 37°C. Phage lysates were rendered free of bacteria by passage through an 02 Selas filter.

The bacteriophage used in most experiments was phage 80, propagated on a strain of *S. aureus* designated as U-40. One ml of a lysate containing 2×10^{10} plaque forming units of this phage, designated as 80/U-40, was mixed in a 15×150 mm culture tube with 1 ml of a broth suspension of the sensitive acceptor strain, C-72, which had been grown on a BHI agar slant for 18 hours at 37°C. The bacterial inoculum was adjusted to give a multiplicity of infection of 1.0. The suspension of phage and acceptor cells was shaken gently during incubation for one hour at 37°C. The transduction mixture then was centrifuged, the supernatant fluid discarded and the bacterial pellet resuspended in 2 ml of broth. After a second centrifugation, the pellet was resuspended in 0.9 ml of P and D broth. Using sterile glass rods, 0.05 ml samples of the suspension of cells were streaked onto the surface of BHI agar plates containing 0.12 unit of penicillin per ml of medium. Plates were incubated at 37°C for 18 hours before being scored for the number of penicillinase producing colonies.

Results. Exposure of a penicillin sensitive strain of *S. aureus* to a bacteriophage, propagated on a strain producing penicillinase, resulted in the appearance of colonies that had acquired the capacity to produce penicillinase. These colonies were readily detected by their large size and the satellite of penicillin-sensitive "persisters" in the area where penicillin had been inactivated by penicillinase. From 3 experiments, a total of 144 pure cultures were isolated from different colonies that had acquired the penicillinase marker. When tested for 12 distinctive physiological characteristics, the transduced clones were similar to the acceptor strain except for resistance to penicillin. The origin of the variants as a consequence of mutation and selection was refuted by the failure of massive inocula (2×10^{10} cells) of untreated suspensions of the acceptor strains to yield colonies on the selective me-

dium. Furthermore, attempts to isolate penicillinase-producing variants of the acceptor strain by the gradient plate technic(9) were not successful.

The rate of transduction was 1-2 transduced cells per 10^7 plaque forming units. Agitation of the suspension of phage and acceptor strain during the initial incubation period increased the yield of transduced cells 3 to 5-fold. Varying the time of the initial incubation period from 5 minutes to 2 hours revealed that one hour resulted in maximum numbers of transduced cells.

With the strains of *S. aureus* that were tested, 0.12 unit of penicillin per ml of medium yielded the largest number of transduced colonies that could be accurately scored. Concentrations only slightly greater than 0.12 unit caused a marked decrease in detectable transduced cells. Lower concentrations permitted the emergence of an undesirable background growth and the common 1st step mutants which were resistant but failed to produce penicillinase. Although low concentrations of penicillin were necessary for primary expression of transduced cells, subcultures grew readily in the presence of 250 units of penicillin per ml of medium.

When tested by the routine typing procedures, the donor strain U-40 was sensitive to lysis by phages 80 and 81 and mutants of phages 42B, 47C, 44A and 52. Three of the mutants, 42B, 47C, 52, were used successfully to transduce the penicillinase marker. The frequencies of transduction, using phages 42B and 47C, were considerably lower than those obtained with phages 52 and 80. Although phages 80 and 81 are considered to be closely related, all attempts to obtain transduction with phage 81 were unsuccessful.

Since the mutant phages 42B, 47C, and 52 and phage 80 were also highly lytic for recipient strain, C-72, transduced cells could not be detected on media on which lysis occurred. However, BHI or heart infusion agar (Difco) inhibited lysis by the above phage but not the transduction process and either medium could be employed for detection of transduced cells.

TABLE I. Bacteriophage Type of Acceptor Strains of *Staphylococcus aureus.*

Strain	Bacteriophage type
C-72	29/52A/79/80 (42B, 47C, 52)*
W-26	29/79/80 (52)
CH-50	80 (52)
M-1	29/52A/55/79/80 (52)

* (42B, 47C, 52) mutants of original phage.

Of 19 penicillin sensitive strains tested, 4 were capable of acting as recipients of the penicillinase marker following exposure to phage 52/U-40 or 80/U-40. The 4 acceptor strains were sensitive to lysis by bacteriophage in Group I. Most of the strains which failed to accept the penicillinase marker were sensitive to lysis by phage in Group III, and a few by phages in Groups I and III. The phage types of the acceptor strains are shown in Table I.

Discussion. The exchange of genetic material among strains of *S. aureus* by transduction opens the way for a genetic analysis of this important species. If transduction can be expanded among the staphylococci, an analysis using this technic will supply essential information on the problem of differentiation of species and strains. Furthermore, more precise and detailed information on the chemical nature, modes of action, and interrelationship of the toxins and enzymes of *S. aureus* coupled with an analysis at the genetic level could provide the ultimate solution to the problem of pathogenesis.

There has been widespread and rapid emergence of penicillinase-producing strains of *S. aureus* since the advent of penicillin therapy. However, the ultimate source of these strains is completely unknown. Fairbrother feels that the great number of strains which produce penicillinase represent original wild types that have persisted since the time when the natural habitat of the staphylococci was the soil(10). However, it is not unlikely that transfer of genetic material *via* bacteriophage occurs in a mixed population of staphylococci in nature and this could readily explain the divergence of penicillinase-producing strains that have been observed.

Summary. Transduction of capacity to

produce the enzyme penicillinase among strains of *S. aureus* was accomplished. Four of 19 sensitive strains were capable of accepting the penicillinase marker. The 4 acceptor strains were sensitive to lysis by phages in Group I. Four different phages were capable of participating in the transduction process.

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Embryonic Survival in Relation to Number and Size of Implantation Swellings in the Rabbit *Oryctolagus cuniculus.** (26727)

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The mechanisms involved in implantation of blastocysts are different from those required for embryonic or fetal survival. The attachment of blastocysts depends on 3 principal mechanisms related to myometrial muscle, adhesions and invasion; these operate in sequence but with some overlap(1). Embryonic survival depends on other factors such as genetic makeup(2). age of female output of unknown substances in the dam's blood(3) and age of gametes at fertilization (4). Hormonal requirements of implantation are also different from those of embryonic survival(5). It has been shown experimentally that deficiency in progesterone can cause embryonic death at some stages of pregnancy(6).

In polytocous mammals, embryonic mortality is usually followed by reabsorption. With a very large number of implantations complete reabsorption of the litter during pregnancy is most common among younger females(7). In the rabbit, embryonic loss is distributed in a specific pattern; 7% immediately after implantation, 66% during midpregnancy and 23% during late pregnancy (8).

The purpose of this experiment is to examine embryonic survival in relation to number of implantations and diameter of uterine swelling at implantation sites. The ova transfer technic was used to obtain rabbits with different numbers of implantations within the physiologic range of the species (normal implantation number in the rabbit 5 to 15).

Materials and methods. A total of 56 pubertal and 49 adult rabbit does of New Zealand White, California, and crossbreds were used in this experiment. Average weights were 6.3 lb for pubertal does and 7.5 lb for adults. All rabbits were brought from one local breeder who used a mild degree of inbreeding. Prior to the experiments each doe was isolated for 16 days. Twelve fertile and vasectomized bucks were also available.

Donors. The pubertal does used as donors were superovulated by gonadotrophic hormones. Pregnant mare serum was used for the priming doses and HCG was used to induce ovulation. Soon after the ovulation-inducing injection, each donor was mated to

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