JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 445-452
Vol. 100, No. 1

Spontaneously Occurring Bacterial Transformations in Mice

ELENA OTTOLENGHI-NIGHTINGALE
Department of Microbiology, Cornell University Medical College, New York, New York 10021

Received for publication 25 July 1969

In 1963, it was reported that deoxyribonucleic acid (DNA)-mediated transformations could take place spontaneously between genetically distinguishable pneumococci growing in a living host, the mouse. It was found in the present study that such transformations could be increased in frequency if infected animals were treated with a drug which kills only one of the two infecting strains. The frequency of in vivo transformations was also increased if the infection was prolonged. Interspecific transformation between pneumococci and streptococci without the addition of purified DNA was demonstrated both in vitro and in vivo. These results make it seem more likely that DNA-mediated transformations occur in nature.

Since Griffith's discovery of type transformation in pneumococci almost 40 years ago (6), a great deal of knowledge about genetic transformations in several bacterial genera has been accumulated. Most of this information has been obtained by studying transformation reactions produced by purified deoxyribonucleic acid (DNA) reacting with competent recipient cells. The occurrence of transformations under conditions approaching natural ones for the bacteria remained unknown. In 1960, the release of genetically active DNA by untreated pneumococcal cultures was discovered (10). Later, it was found that transformations could take place among genetically distinguishable pneumococcal strains growing in a living host, the mouse (12).

The present experiments elucidate some of the circumstances under which transformations occur spontaneously among bacteria in vivo.

MATERIALS AND METHODS

Pneumococcal strains. These strains have been fully described previously (12). The features of these strains pertinent to the present experiments are summarized in Table 1.

Streptococcal strains. Streptococcal strains D and NBS-1 belong to the Viridans group (1). Strains DSm^r and NSB-1Sm^r were made resistant to 1 mg of streptomycin per ml by transformation with DNA from a spontaneous streptococcal mutant. The mutant was isolated by spreading 10^{10} to 10^{11} sensitive organisms on the surface of blood-agar plates containing 10 mg of streptomycin per ml (1). The lethal dose of streptococci for mice is 3×10^9 colony-forming units (CFU) injected intraperitoneally (ip). These strains and a strain of *Streptococcus salivarius* were kindly supplied by C. M. MacLeod.

Transformation system. Transformation of R36NCSm^r by DNA from IIIS-IR6 produces pneumococci which are fully encapsulated type III cells and which form large, mucoid colonies typical of naturally occurring, virulent type III strains. The transformant is highly virulent for mice and is streptomycin-resistant so that it is easily distinguished from either parental strain and also has a selective advantage over either parent in the mouse (12; Table 1). The streptococcal strains can act as either donors or recipients of the drug-resistance markers which were used.

Transformation method. Mice of CFW and ICR strains, weighing 18 to 20 g, were injected ip or subcutaneously, or both ways. The cultures to be used in transformation experiments were incubated at 37 C for 12 to 16 hr in 1% neopeptone-beef heart infusion broth containing 2% fresh defibrinated rabbit blood and then were diluted appropriately into fresh broth. Separate syringes and needles were used for different bacterial strains. For control experiments, pancreatic deoxyribonuclease (1× recrystallized, Worthington Biochemical Corp., Freehold, N.J.), in a final concentration of 10 μg/ml, and $MgSO_4 \cdot 7H_2O$ (0.007%) were added to each culture before injection.

Detection of transformation. A loop of heart blood from each mouse (found dead or sacrificed) was streaked on Brain Heart Infusion Agar (BBL) containing 5% horse blood and the appropriate antibiotic(s). To detect small numbers of transformants, as much as 0.1 ml of heart blood was also inoculated into neopeptone-blood-broth containing 10 μg of pancreatic deoxyribonuclease per ml to prevent in vitro transformation from taking place (12). After overnight incubation at 37 C, the blood-broth cultures were streaked on horse blood-Brain Heart Infusion Agar containing the appropriate selective drug(s). The capsular type of the smooth colonies was identified immunologically by using the Quellung method and rabbit antisera.

Transformants producing type III capsule in large amounts are highly virulent, so that one transformed cell can cause the death of a mouse. The limit of resolution of the detection method for the less virulent transformants was considerably less and depended on the volume of heart blood or peritoneal fluid used. For one transformant to be picked up in 0.1 ml of blood, at least 30 to 50 transformants must be present in the circulation. Since the time at which transformation took place after infection is unknown, and the number of times the transformant divided is also unknown, the frequency of bacterial transformation in a given mouse could not be determined.

RESULTS

Effect of streptomycin treatment on the frequency of in vivo transformations. Each of a number of mice was injected ip with 10^7 to 2×10^7 CFU of IIIS-IR6 and 10^8 to 2×10^8 CFU of R36NCSm^r pneumococci (Table 1). Four hours later, each mouse received 2.5 mg of streptomycin sulfate (Eli Lilly & Co.) intramuscularly. Some animals were sacrificed, autopsied, and cultured 8 hr after infection; others were observed for as long as 1 week, at which time all of the surviving mice were sacrificed, autopsied, and cultured. The results are summarized in Table 2.

Streptomycin treatment increased the incidence of spontaneous transformations among pneumococci in mice. This observation was also reported by Conant and Sawyer (4). In our experiments, however, streptomycin did not prolong survival. All deaths occurred within 48 hr. In addition, we observed that transformants could be detected as early as 8 hr postinfection and that even at this early time streptomycin increased the incidence of transformation. The streptomycin did not act as a selective agent for spontaneous mutations of the IIIS-IR6 to resistance, since these mutants could be distinguished by their small capsule (Table 1). Streptomycin did not merely give a growth advantage to a few transformants, since, as can be seen from Table 2, the total number of transformants (rather than those found in animals which died) was almost twice as great as in the absence of streptomycin. In addition, as observed by Conant and Sawyer (4), streptomycin does not alter the transformation frequency in the presence of deoxyribonuclease. Smaller doses of streptomycin (0.25 mg per mouse) had very little effect.

In sufficient dosage, streptomycin, by killing the susceptible IIIS-IR6 pneumococci, probably caused the release of genetically active DNA in larger amounts and therefore increased the incidence of transformation in vivo. These results are in agreement with those on in vitro transformations reported previously (11).

In these cases, the pathogen is sensitive to the chemotherapeutic agent, but the chance of a fatal outcome is increased by antibiotic treatment because the already drug-resistant nonpathogen was transformed to capsule synthesis and thereby to virulence.

Transformation after injection of pneumococcal strains at different sites. In 1959, Hall and Gale (7) achieved pneumococcal transformation in mice by injecting a lysate of a type III *Pneumococcus* subcutaneously and a live rough *Pneu-*

TABLE 2. *Effect of streptomycin treatment on pneumococcal transformations in the mouse*

Determination	No. of mice from which transformant pneumococci (III-R36NCSm^r) were isolated	
	Streptomycin treatment	No streptomycin treatment
Sacrificed at 8 hr.....	17/39 (44)[a]	4/27 (15)
Sacrificed at 7 days...	0/12 (0)	0/14 (0)
Died.....	30/30 (100)	17/23 (74)
Total	47/81 (58)	21/64 (33)

[a] Numbers in parentheses represent percentages.

TABLE 1. *Characteristics of pneumococcal strains*

Strain designation	Colony type on agar	Streptomycin resistance	Average ip lethal dose for mice
IIIS-IR6[a]	Small, smooth type III	Sensitive	2×10^7
R36NCSm^r	Rough	1 mg/ml	2×10^8
IIIS-R36NCSm^r	Large, smooth type III	1 mg/ml	2×10^2
CD-VII-Sm^r	Small, smooth type VII	1 mg/ml	5×10^4
R36NCery^r [b]	Rough	Sensitive	2×10^8
R6	Rough	Sensitive	2×10^8

[a] When this strain acquires streptomycin resistance by mutation or transformation, the colonies remain small and smooth.

[b] Resistant to 0.1 μg of erythromycin (Ilotycin gluceptate, Lilly) per ml.

mococcus ip. In view of the above and of our present results, we tested whether live pneumococci injected subcutaneously in the neck region could bring about transformation of live pneumococci injected ip and vice versa.

Eight experiments were performed. In five of the experiments, a total of 61 mice was injected subcutaneously with 10^7 to 2×10^7 CFU of IIIS-IR6 and ip with 10^8 CFU of R36NCSm^r. The animals were sacrificed at 24, 48, and 72 hr, and no transformants were found. In two experiments involving 20 mice, the rough pneumococci were injected into the neck, and the smooth forms were injected into the peritoneal cavity. Again, no transformants were found. Streptomycin treatment had no effect. (With this type of mixed infection, about 80% of mice do not die.)

In one experiment, identical in all respects to those described above and in which the rough culture was injected ip, transformants were isolated from each of the 10 mice injected. Two of the 10 mice died within 48 hr, and the others were sacrificed and autopsied during the next 5 days. Transformants were isolated from the heart blood of 4 animals, the peritoneal cavity of 1 animal, and the neck region of all 10 animals. The transformants were type III, streptomycin-resistant, but had a small capsule. Evidently, in this case, hematogenous spread of either intact R36NCSm^r or genetically active R36-NCSm^r nucleoprotein occurred. Deoxyribonuclease controls were negative.

Transformation can occur among bacteria originating in different sites but this phenomenon s not easily reproduced. Conant and Sawyer (4) reported the frequent occurrence of such transformations but were never able to isolate transformants from the subcutaneous site of infection.

If the right conditions are met (as in the above experiment), transformations can occur in 100% of infected animals and the transformants can persist for several days in the subcutaneous region.

Effect of chronic pneumococcal infection on spontaneous transformation in mice. Pneumococci introduced into mice by various routes either cause death of the animal within 24 to 72 hr or are completely destroyed by host defense mechanisms within that time. However, under certain conditions and with certain pneumococcal strains, we observed infections lasting more than 21 days. These experiments will be reported in a separate publication.

The reason for trying to produce chronic pneumococcal disease in mice was that prolonged infection could offer continued opportunities for genetic exchange among the bacteria.

Spontaneous transformation experiments were also carried out with a type VII *Pneumococcus* (CD-VII) isolated by T. Salzman from a patient at Bellevue Hospital who had a protracted atypical bacterial pneumonia. This strain caused prolonged infection in mice and proved to be transformable in vitro. Results of transformation experiments with CD-VII are summarized in Tables 3 and 4.

It is evident that after mixed infection with CD-VII-Sm^r and R36NCery^r strains, transformations did occur in mice. The three types

i

TABLE 3. *Types of spontaneous bacterial transformations during chronic infection in mice*

Bacteria injected			Transformants found	
No.	Strain	Markers	No.	Type
P_1	CD-VII-Sm^r	VII capsule; streptomycin resistance	T_1	CD-VII-Sm^r ery^r (transformation of P_1 to ery^r)
P_2	R36NCery^r	Erythromycin resistance	T_2	R36NCSm^r ery^r (transformation of P_2 to Sm^r
			T_3	VII-R36NCery^r (transformation of P_2 to capsule type VII)[a]

[a] It was sometimes difficult to distinguish rough forms from these atypical type VII pneumococci by colonial morphology alone. To insure proper identification and to avoid doing a capsular swelling reaction with cells from each colony, blood was omitted from the plates and an overlay was poured on each plate. The overlay consisted of 5 ml of 1% Brain Heart Infusion Agar and 0.2% rabbit anti-VII serum. After 4 days at 37 C, colonies of type VII had a precipitation band around them. (Fig. 1). To distinguish T_1 from T_3 on initial isolation from mice, a loop of heart blood and ip fluid from each mouse was placed in broth containing (i) anti-VII serum and erythromycin and (ii) anti-VII serum, erythromycin, and streptomycin. If growth and agglutination occurred in tube (i) but not in tube (ii) transformation of R36NCery^r to capsule type VII must have occurred in that animal.

TABLE 4. *Frequency of spontaneous transformations in mice*[a]

Injecting dose		No. of mice injected	No. of mice with transformants	Per cent
CD-VII-Sm^r	R36NCery^r			
1.5×10^4	1.5×10^7	6	1	17
1.5×10^5	1.5×10^7	7	4	57
2×10^6 to 3×10^6	2×10^8	27	25	93
10^7	2×10^8	10	10	100
2×10^7 to 3×10^7	2×10^8	8	8	100

[a] Total number of mice injected, 58; total number of mice with transformants, 48; per cent with transformants, 83.

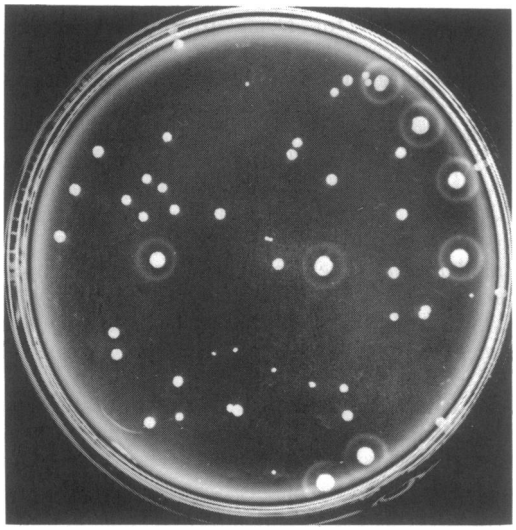

FIG. 1. *Agar-antiserum plate showing precipitation ring around colonies of CD-VII pneumococcus and no ring around the rough pneumococcal colonies. Magnification, 1×.*

of transformants which were isolated indicated that transformation occurred in both directions; that is, either strain could act as a donor or as a recipient of genetic material, and all of the markers were transferred.

Infection of mice with unencapsulated pneumococci speeded up the usual course of CD-VII infection so that most of the animals died after 1 week instead of over a period of 3 weeks. Transformants usually were detectable within 24 hr and persisted in fairly large numbers (10^2/ml of heart blood) for the life of the animal. The number of mice in which at least one detectable transformation event took place approached 100% (Table 4). With inocula of 1.5×10^7 or 2×10^8 rough pneumococci and from 2×10^6 to 2×10^7 IIIS-IR6, the percentage of mice in which transformation can be detected remains below 40% (12). Therefore, the high incidence of mice with transformations in the experiments summarized in Table 4 is due to the presence of the CD-VII-Sm^r.

In every mouse from which transformants were isolated, type T_1 (Table 3) was recovered in large numbers. Thus, strain CD-VII seems to be a highly competent strain for transformation in vivo in spite of the polysaccharide capsule which might act as a physical barrier to DNA penetration (14). The least frequently encountered transformant was T_8, although it also was encapsulated. R36NCSm^r was a good recipient for the erythromycin resistance marker, but it was less able to accept the sequence of nucleotides directing polysaccharide type VII synthesis.

This series of experiments demonstrates that a strain of *Pneumococcus* which can produce subacute infection in the mouse may be highly competent either as a donor or as a recipient of transforming DNA in vivo. Thus, pneumococcal infection lasting up to 7 days instead of the usual 24 to 48 hr does seem to provide increased opportunities for transformation to take place, and the polysaccharide capsule which only partially prevents DNA penetration in vitro does not impede transformation of virulent pneumococci to antibiotic resistance in vivo (14).

Spontaneous transformations between streptococci and pneumococci. Interspecific transformations mediated by purified DNA have been reported and described (9, 16). Because pneumococci and streptococci both normally reside in the human nasopharynx, it was of interest to determine whether transformation between these species could occur spontaneously.

Therefore several pneumococcal and streptococcal strains were tested both in vitro and in vivo. For the in vitro tests, 0.5 ml of cell-free 3-hr culture filtrates (Millipore Corp., Bedford, Mass.; 0.45 μm) were employed as a source of transforming DNA (Table 5).

Transformations of pneumococci by streptococcal filtrates and of streptococci by pneumococcal filtrates did occur regularly but infrequently. *Streptococcus* NBS-1 was a poor recipient but a good donor. The culture filtrates of NBS-1 did not markedly reduce the transforming activity of a known amount of purified streptococcal or pneumococcal DNA when the DNA was incubated in filtrate for 30 min at 37 C before use in a standard transformation experiment. The filtrates from *S. salivarius* behaved in a similar fashion, but those from strain D destroyed

transforming activity almost completely. The conclusion from these data and from previous transformation inhibition studies (11) is that NBS-1 and *S. salivarius* produce very little active deoxyribonuclease with growth but are, nevertheless, poor recipients.

Since interspecific transformations mediated by DNA in culture filtrates occurred at low frequency, it was hoped that, by allowing pneumococci and streptococci to grow in each other's presence, the transformation yield might improve.

One tube containing 7 ml of beef heart infusion broth was inoculated with 10^5 CFU of each bacterial strain. After an incubation period of 7 hr at 37 C, the mixed culture was diluted and plated on Brain Heart Infusion Agar containing the appropriate antibiotics. The results are summarized in Table 6 and are due to DNA-mediated transformations for the following reasons. (i) The number of cells which acquired streptomycin resistance from exposure to cell-free filtrates of resistant organisms varied from about 10 to more than 1,000 per ml out of a total recipient population of 10^7; the spontaneous mutation rate to high-level streptomycin resistance is only 0.5×10^{-8} to 4×10^{-8} for pneumococci and 2×10^{-10} to 8.4×10^{-10} for streptococci (15). (ii) Deoxyribonuclease (10 μg/ml) completely prevented such transformation; these controls were done at least once with each combination of bacteria and many times with the pneumococcal filtrates (11). (iii) The genetic activity of cell-free filtrates precludes a conjugational mechanism (which, to date, has never been found in pneumococci or streptococci).

In these experiments, interspecific transformations occurred at a much greater frequency than in the filtrate experiments probably because cells reaching the competent state could adsorb transforming DNA before competence disappeared; some of the transformants would also have divided.

TABLE 5. *Frequency of interspecific transformations mediated by culture filtrates in vitro*[a]

Recipient strain (10^7 CFU/ml)	Source of filtrate			
	Streptococcus NBS-ISm^r	*Streptococcus* DSm^r	*S. salivarius* Sm^r	*Pneumococcus* R6Sm^r
Streptococci				
NBS-1	±[b]	ND[c]	ND	ND
D	+	+	++	+
Pneumococci				
R36NC	+	ND	ND	++++
R6	+++	+	+	++++

[a] Symbols: ±, <10 transformants per ml of recipient culture; +, 10 to 20 transformants per ml of recipient culture; ++, 20 to 100 transformants per ml of recipient culture; +++, 100 to 1,000 transformants per ml of recipient culture; ++++, >1,000 transformants per ml of recipient culture.

[b] Observed once.

[c] Not done.

In view of the encouraging results from the in vitro experiments, we looked for spontaneously occurring in vivo interspecific transformations in the mouse.

The experiments were carried out as previously described. The LD_{50} for streptococcal strain D injected ip was found to be 3×10^9 CFU per mouse. Therefore, each mouse was injected with 10^8 to 3×10^8 CFU of streptococci and 2×10^6 to 5×10^6 CFU of pneumococci. Because of the rapid clearance of streptococci from heart blood and peritoneal cavity (Fig. 2), animals were sacrificed over a 2-hr period after the last injection and transformants were looked for as described. These streptococci can be easily distinguished from pneumococci by colonial size and morphology. Species identification was confirmed by bile solubility and by agglutination of rough pneumococci with specific rabbit antiserum. The data are summarized in Table 7.

TABLE 6. *Interspecific mixed culture transformations in vitro*[a]

Parental strains (pneumococci + streptococci)	Transformants	
	Type	No. per ml
R36NCSm^r + NBS-1ery^r	R36NCSm^r ery^r	130–175
R36NCery^r + NBS-1Sm^r	NBS-1Sm^r ery^r	70–100
R36NCSm^r + Doery^r		0
R36NCery^r + DSm^r	DSm^r ery^r	1,000–2,000
R36NCSm^r + DSm^s	DSm^r	10,000–100,000

[a] The rate of spontaneous mutation to streptomycin resistance was 0.5×10^{-8} to 4×10^{-8} for pneumococcus and 2×10^{-10} to 8.4×10^{-10} for streptococcus. The rate of spontaneous mutation to erythromycin resistance is in the range of 10^{-8} to 2×10^{-8} for both species (15).

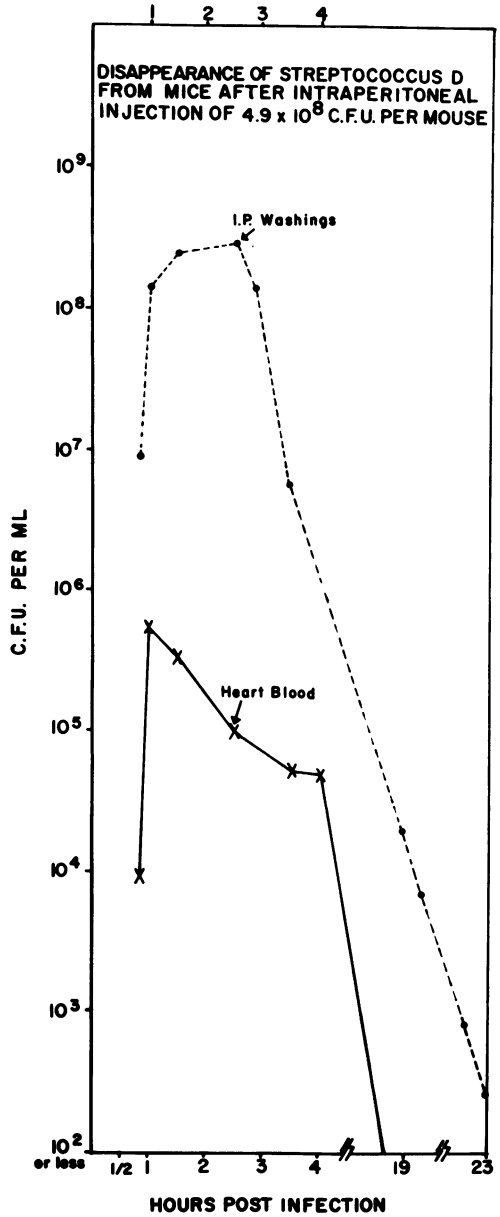

FIG. 2. *Disappearance of streptococcus D from mice after intraperitoneal injection of 4.9 × 10⁸ CFU per mouse.*

As can be seen from Table 7, spontaneous transformations between pneumococci and streptococci did occur in the mouse. Most of the transformants were streptococci which had picked up pneumococcal DNA. These results are not unexpected since competence lasts longer in streptococcal than in pneumococcal cultures (1, 9). Both streptomycin and erythromycin resistance were transferred. The frequency of transformation was quite high in that 75% of the animals had at least one transformant. Although the number of controls was small, in each experiment in the absence of deoxyribonuclease at least two of six and in three experiments five of six animals had a detectable transformant in the peritoneal fluid; therefore, the total absence of detectable transformants in the deoxyribonuclease controls is meaningful.

When the mice were sacrificed at 24 hr postinfection or later, very few bacteria and very few transformants were recovered since neither rough pneumococci nor these streptococci are virulent for mice. In conclusion, spontaneously occurring DNA-mediated transformation does occur between two species of bacteria in vivo.

DISCUSSION

The experiments described demonstrated that DNA-mediated transformations of certain bacteria may occur spontaneously in a living host (mouse) under a variety of conditions. Several antibiotic resistance markers and pneumococcal capsular markers (and therefore virulence markers) could be transferred reproducibly and with a high degree of frequency. When one of the two bacterial strains participating in the mixed infection was resistant to streptomycin, treatment of the animal with streptomycin increased the incidence of transformation, probably because of the increased release of genetic material from the dying, sensitive cells.

The infecting bacteria were found to participate in transformations when inoculated in different sites, although not as well as when inoculated into the same site. These results were to be expected in view of the presence of nucleases in the blood and body fluids of mice which would inactivate much of the released nucleoprotein so that transformation would depend primarily on hematogenous spread of intact bacteria. What is of interest, however, is that such transformations did occur at all.

When infections were prolonged, transformations occurred with greater frequency than in short-term, acute infections probably because of the increased opportunities for cell-to-cell interactions.

Interspecific spontaneous transformations were observed both in vitro and in mice suffering from a mixed pneumococcal and streptococcal infection. In fact, streptococci were transformed in vivo by pneumococcal DNA with great facility, as also occurs with purified DNA in vitro (9).

TABLE 7. *Interspecific transformations occurring in vivo*

Parental strains (streptococci plus pneumococci)	No. of mice with transformants[a]		Total no. of mice with transformants	Type of transformant
	ip Fluid	Heart blood		
DSm^s + $R36NCSm^r$	5/6	2/6	5/6	DSm^r
DSm^r + $R36NCery^r$	2/6	0/6	2/6	DSm^r ery^r
DSm^r + $R36NCery^r$	5/6	1/6	6/6	DSm^r ery^r + $R36NCery^r$ Sm^r
DSm^r + $R36NCery^r$	5/6	1/6	5/6	DSm^r ery^r
DSm^r + $R36NCery^r$ + deoxyribonuclease (control)	0/6	0/6	0/6	

[a] In total, 17 of 24 (71%) mice had transformants in ip fluid; 4 of 24 (17%) had transformants in heart blood. The total number of mice with transformants was 18 of 24 (75%).

Since the discovery of cell-to-cell transformations in *Pneumococcus*, other investigators have found such transformations in *B. subtilis* (5, 18) and *H. influenza* in vitro and have confirmed our work with pneumococci in vivo (4). This paper extends these observations to streptococci.

There is a growing body of evidence which indicates that release of DNA during growth of several bacteria in vitro (2, 3, 16) and during an infectious process may be a fairly widespread phenomenon. The chances for successful in vivo transformation may be greatly increased when the infection is prolonged. Since it is now clear that transformations can also occur between cells of different species, the role of such processes in nature may be of greater significance than supposed previously. The data reported here support the idea that spontaneous transformations may occur in nature and that they may play a role in bacterial infections. For example, bacteria such as the Viridans group of streptococci, which are part of the normal flora of the human nasopharynx, may be resistant to an antibiotic such as erythromycin because of mutation and selection from previous antibiotic therapy of the individual involved. If the patient now develops a pneumococcal infection, the pneumococci may be transformed to erythromycin resistance and may no longer be amenable to therapy by this drug. Thus, the rising incidence of antibiotic-resistant pneumococci in man (13) may be due to transformations as well as to mutations. The genes of one strain or species might, by transformation, become available to another. These possibilities for genetic mixing were discussed previously by the author and others (4, 8, 11).

Exchange of genetically active DNA by bacteria invading a living host has been demonstrated to occur frequently and reproducibly. These experiments indicate that DNA-mediated transformations may occur in nature.

ACKNOWLEDGMENTS

This investigation was supported by grant E-391 from the American Cancer Society and by Public Health Service grant AI 03713-05 and graduate training grant TO1-AI-00281-05 from the National Institute of Allergy and Infectious Diseases.

I thank Elizabeth O. Balkite for excellent assistance in all phases of this work and Charles Ellsworth for assistance with the photographs. I also thank John Y. Sugg for discussing this manuscript with me and for his many valued suggestions.

LITERATURE CITED

1. Bracco, R. M., M. R. Krauss, A. S. Roe, and C. M. MacLeod. 1957. Transformation reactions between pneumococcus and three strains of streptococci. J. Exptl. Med. 106:247–258.
2. Campbell, J. N., J. B. Evans, J. J. Perry, and C. F. Niven, Jr. 1961. An extracellular material elaborated by *Micrococcus sodonensis*. J. Bacteriol. 82:828–837.
3. Catlin, B. W. 1960. Transformation of *Neisseria meningitidis* by deoxyribonucleates from cells and from culture slime. J. Bacteriol. 79:579–589.
4. Conant, J. E., and W. D. Sawyer. 1967. Transformation during mixed pneumococcal infection of mice. J. Bacteriol. 93:1869–1875.
5. Ephrati-Elizur, E. 1968. Spontaneous transformation in *Bacillus subtilis*. Genet. Res. 11:83–96.
6. Griffith, F. 1928. The significance of pneumococcal types. J. Hyg. 27:113–159. (Reprinted, J. Hyg. 64:129–175, 1966).
7. Hall, R. H., and G. O. Gale. 1959. New observations on pneumococcal transformation *in vivo*. Proc. Soc. Expl. Biol. Med. 101:487–491.
8. Hotchkiss, R. D. 1951. Transfer of penicillin resistance in pneumococci by the deoxyribonucleate derived from resistant cultures. Cold Spring Harbor Symp. Quant. Biol. 16:457–460.
9. Krauss, M. R., and C. M. MacLeod. 1963. Intraspecies and interspecies transformation reactions in pneumococcus and streptococcus. J. Gen. Physiol. 46:1141–1150.
10. Ottolenghi, E., and R. D. Hotchkiss. 1960. Appearance of genetic transforming activity in pneumococcal cultures. Science 132:1257–1258.
11. Ottolenghi, E., and R. D. Hotchkiss. 1962. Release of genetic transforming agent from pneumococcal cultures during growth and disintegration. J. Expl. Med. 116:491–519.
12. Ottolenghi, E., and C. M. MacLeod. 1963. Genetic transformation among living pneumococci in the mouse. Proc. Nat. Acad. Sci. U.S.A. 50:417–419.
13. Percival, A., E. C. Armstrong, and G. C. Turner. 1969.

Increased incidence of tetracycline-resistant pneumococci in Liverpool in 1968. Lancet **1**:998–1000.

14. Ravin, A. W. 1959. Reciprocal capsular transformations of pneumococci. J. Bacteriol. **77**:296–309.
15. Ravin, A. W., and A. K. Mishra. 1965. Relative frequencies of different kinds of spontaneous and induced mutants of pneumococci and streptococci capable of growth in the presence of streptomycin. J. Bacteriol. **90**:1161–1173.
16. Schaeffer, P. 1958. Interspecific reactions in bacterial transformation. Symp. Soc. Exp. Biol. **12**:60–74.
17. Smithies, W. R., and N. E. Gibbons. 1955. The deoxyribose nucleic acid slime layer of some halophilic bacteria. Can. J. Microbiol. **1**:614–621.
18. Takahashi, I. 1962. Genetic transformation of *Bacillus subtilis* by extracellular DNA. Biochem. Biophys. Res. Commun. **7**:467–470.