Competence of Pneumococcal Isolates and Bacterial Transformations in Man

ELENA OTTOLENGHI-NIGHTINGALE

Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

A survey of pneumococci isolated from 19 healthy carriers and from 23 patients with pneumococcal disease showed that, for both groups, 25 to 30% of the isolates were competent for transformation by soluble deoxyribonucleic acid (DNA) in vitro. Untransformable type 3 and type 8 pneumococci, whose capsules had been hydrolyzed by the Bacillus palustris enzymes prior to exposure to DNA, remained untransformable. Thus, at least for these isolates, it was not the presence of capsule that prevented transformation. Type 9 pneumococci in a healthy human carrier were transformed by DNA released from living unencapsulated pneumococci sprayed onto the pharynx. The donor bacteria were resistant to 1,000 μg of streptomycin/ml. Two types of streptomycin-resistant bacteria were recovered from the carrier's pharynx: a type 9 pneumococcus and an alpha-hemolytic streptococcus. No streptomycin-resistant, gram-positive cocci were isolated from this individual prior to inoculation of the pharynx with the resistant organisms. It seems possible that transformations can occur in the natural environment of some gram-positive cocci.

In 1928, Griffith (14) proposed that, during convalescence from type 1 pneumococcal pneumonia, the serologically different pneumococci often found in sputum might arise by type transformation. Such transformations were sought by Webster and Hughes (34) but were not found. In the intervening 44 years, experimental demonstration of transformations in a human host has not been reported, but the possible occurrence and the significance of such transformations have remained interesting topics for discussion.

Following the classic paper of Avery, MacLeod, and McCarty (3) on the deoxyribonucleic acid (DNA) nature of the transforming principle, knowledge about transformations advanced rapidly. By making use of antibiotic resistance markers (17), it became possible to demonstrate the transfer of genetic information from one bacterium to another by freshly released DNA. Such cell-to-cell transformations occurred both in the test tube (22, 23) and in the mouse (8, 24, 25). Recently, "spontaneous" transformations have been observed in the mouse lung following aerosol infection (1). In view of these results, it was of interest to try to detect bacterial transformations in man.

MATERIALS AND METHODS

Bacterial strains. Pneumococcal strain R36NC (24), originally obtained from Colin M. MacLeod, was transformed to resistance to 1,000 μg of streptomycin/ml and 0.1 μg of erythromycin/ml by DNA extracted from spontaneous mutants resistant to these drugs. This culture, previously referred to as R36NCSm^{r}ery^r (25), is now designated R36NCStr-rEry-r. Resistance to 1,000 μg of streptomycin/ml will henceforth be abbreviated as Str-r, and resistance to 0.1 μg of erythromycin/ml will be abbreviated as Ery-r.

Streptococcal strain "D," a strain of *Streptococcus viridans* which is competent for transformation, was also obtained from Colin M. MacLeod. Its characteristics have been described (6). Streptococcus "D" was made resistant to 0.1 μg of erythromycin/ml by transformation with DNA from a spontaneous mutant of pneumococcus. The resistant form is designated Streptococcus "D" Ery-r.

Media. Fluid cultures were grown in freshly prepared beef infusion broth (BIB) as described previously (25). For in vitro transformation, human serum which had been heated to 60 C for 30 min was added to BIB to a concentration of 10% by volume and Trypticase soy agar to 0.2%. This is transformation medium (TM).

Transformation method in vitro. The isolate (see below) to be tested for competence was inoculated into BIB with 5% defibrinated rabbit blood and incubated for 18 hr at 37 C. For each test, 2 ml of TM was inoculated with 0.01 ml of the 18-hr culture. DNA extracted from nonencapsulated, streptomycin-resistant pneumococci was added to a concentration of 2 μg/ml, and the tube was incubated for 18 hr at 37 C. The same DNA preparation, kindly provided by

Muriel Roger of the Rockefeller University, was used throughout the study. It was prepared as described by Hotchkiss (18). The number of streptomycin-resistant, colony-forming units (CFU) was estimated by the double-layer technique (27), with a final concentration of 400 μg of streptomycin/ml of agar.

Fresh pneumococcal isolates: carriers. The method of Hodges, MacLeod, and Bernhard was used to identify pneumococcal carriers (16), except that the broths were incubated for only 2 hr before inoculation into mice.

The subjects were volunteers at the Maryland House of Correction at Jessup, Maryland, and staff members and medical students at The Johns Hopkins University School of Medicine.

Fresh pneumococcal isolates: patients. Twenty-three fresh isolates were obtained from the Bacteriology Laboratory of The Johns Hopkins Hospital.

Handling of isolates. Pneumococci obtained from mice in the case of the carriers and from plates in the case of the patients were grown in BIB for 4 hr at 37 C. Sterile glycerin was added to a concentration of 15%, and the cultures were stored at −50 C. All isolates were tested for bile solubility, optochin resistance, and transformability. Capsular type was determined by the Neufeld method by using rabbit antisera (Wyeth, Inc., Philadelphia) to cause capsular swelling (quellung) (21).

Test for transformation in the human pharynx. The subjects were healthy carriers of pneumococci of known type and sensitivity to streptomycin and erythromycin. The men had no history of rheumatic fever and were free from allergy to penicillin.

After routine throat culture, the pharynx was sprayed with 1 ml of a fresh, 18-hr culture of R36NCStr-rEry-r from a DeVilbiss glass nebulizer. At intervals after the inoculation, the subject gargled with 2 ml of BIB, after which the throat was swabbed. A 0.5-ml sample of throat washing and the swab were transferred to 7 ml of BIB containing 5% defibrinated rabbit blood, 0.2% dextrose, and 200 μg of pancreatic deoxyribonuclease (1× recrystallized, Worthington Biochemical Corp.). The deoxyribonuclease was added to prevent in vitro transformation. After 1.5 hr of incubation at 37 C, 0.5 ml of the culture was inoculated intraperitoneally into each of three mice. The culture was also streaked onto Trypticase soy agar containing 5% sheep blood and swabbed to cover the surface of blood agar plates containing 500 μg of streptomycin/ml or 0.1 μg of erythromycin/ml. Most of the mice died within 48 hr and were autopsied immediately or frozen at −20 C for 2 or 3 days prior to autopsy. About 0.1 ml of heart blood and peritoneal washing from each mouse was plated on Trypticase soy agar containing 5% sheep blood, with and without streptomycin (400 μg/ml), or erythromycin (ilotycin gluceptate, Lilly, 0.1 μg/ml). After 24 to 48 hr of incubation of plates at 37 C, all drug-resistant colonies were typed to ascertain that they corresponded to the pneumococcal type which the carrier had originally harbored.

Digestion of capsule by hydrolytic enzymes prior to transformation. An enzyme which hydrolyzes type 3 pneumococcal polysaccharide (depolymerase) was kindly provided by M. R. Smith. This enzyme was prepared on 15 February 1935, by R. Dubos from a soil bacterium of uncertain taxonomy later referred to as *Bacillus palustris* (4). The bacillus was grown with type 3 polysaccharide as the sole carbon source, and the enzyme was extracted by the method of Dubos (10, 12).

About 10^5 pneumococci from a fresh culture in BIB were added to 2 ml of TM without agar. The culture was incubated at 37 C for 4 hr, concentrated eightfold by centrifugation, and added to 0.25 ml of TM containing 2 mg of enzyme/ml. After 45 min at 37 C, the cultures were checked for capsule by the Neufeld method and stored at 0 C for 18 hr. By this time, the capsules had virtually disappeared. The cells were then washed in TM and resuspended in 1 ml of TM. DNA was added as before. After incubation at 30 C for 60 min, streptomycin-resistant transformants were estimated by the double-layer technique (27).

RESULTS

Pneumococcal isolates and their competence for transformation. To evaluate the possible role of pneumococcal transformation in man, the isolates from both healthy carriers and patients hospitalized because of pneumococcal disease were tested for transformability by exogenous DNA.

Carriers. Of 20 staff members and second year students at The Johns Hopkins University School of Medicine who were cultured in September and October of 1970, only two proved to be carriers of pneumococci. However, 17 carriers were found among 91 volunteers at the Maryland House of Correction between January and March of 1971. The carrier rate of 19% in the prison population is somewhat lower than other reports for this time of year (15, 16) when mice were used as the pneumococcal selective agents. Still lower rates are obtained if cultural methods alone are used (9, 16, 29). Of 36 students who were screened by using plates alone, none showed pneumococci.

Patients. Pneumococci were obtained from 23 patients at The Johns Hopkins Hospital. Nineteen of these specimens were from sputa of patients suffering from respiratory infections, one was from spinal fluid, and three were from blood.

The distribution of pneumococcal types is shown in Table 1. The isolates were tested for transformation to streptomycin resistance as described. Saturating amounts of DNA and a long incubation period were used; thus, even a low frequency of transformation would have been detected. The results are presented in Table 2.

Contrary to Griffith's original idea that a rough intermediate was necessary for type transformation (14), encapsulated pneumococci have been shown to be transformable (2, 26). The presence

TABLE 1. *Distribution of pneumococcal isolates by capsular type*

Capsular type[a]	Normal[b]	Patients[c]	Total no.
3	2	4	6
4	5	2	7
6	3	0	3
8	2	2[d]	4
9	2	1	3
13	1	0	1
14	1	1[d]	2
17	3	1	4
18	1	2	3
19	0	1[e]	1
20	0	1	1
23	0	4	4
24	0	1	1
33	0	1	1
NT[f]	0	2	2
Total no.	20	23	43

[a] Two types were isolated from one carrier and from one patient with pneumonia.

[b] Incidence of carriers: 17/91 (19%) of prison inmates, 2/20 (10%) of medical students and staff. Cultures taken between 10 January and 30 March 1971.

[c] Except where indicated, these pneumococci were isolated from sputa. Cultures taken between 16 March and 20 May 1971.

[d] Isolated from blood.

[e] Isolated from cerebrospinal fluid.

[f] Not typable with pools A-F and type 37 antisera.

of large amounts of capsular polysaccharide, however, reduced the frequency of transformation by DNA from bacteria which were isogenic to the recipient except for the capsule locus (26). Since most of the pneumococci found in man are encapsulated, it was of interest to determine whether the capsular material was responsible for the lack of detectable transformation in some of the isolates. The type 3 hydrolyzing enzyme of Dubos (10) was used. Taylor (31) and later Bernheimer (5) were able to increase the transformation frequency of type 3 pneumococci by as much as 50%, if the specific hydrolytic enzyme was used to digest the capsular polysaccharide prior to exposure of the cells to DNA. The effects of the hydrolytic enzyme on a type 3 isolate, which showed negligible transformation, and on an untransformable type 8 are presented in Table 3 and illustrated in Fig. 1. The capsular material of type 3 diminished perceptibly after a short incubation period at 37 C and continued to diminish in the cold until virtually no capsule was visible when specific antiserum was added to

the cells. Capsular hydrolysis of type 8 became evident after several hours at 4 C.

(The *Bacillus palustris* enzyme used in these studies was prepared by R. Dubos in 1935 with type 3 polysaccharide as the sole carbon source for the growth of the bacillus. The hydrolytic enzyme is inducible and extremely substrate-specific [11, 12]. Both types 3 and 8 pneumococci were tested here because the type 3 and type 8 polysaccharides both contain D-glucuronic acid and D-glucose and show immunological cross-reactivity [11].

The enzyme preparation did in fact hydrolyze both type 3 and type 8 polysaccharide probably because, even though only type 3 polysaccharide was used as the inducer, it is now known that a low background of anti-type 8 enzyme can be produced by these bacilli. The two enzymes are specific and different from each other: the type 8 hydrolyzing enzyme is an eliminase and the type 3, an endohydrolase [A. M. Pappenheimer, *personal communication*; references 4, 33]. In

TABLE 2. *Classification of transformable pneumococcal isolates*

Capsular type	Isolates which transformed/ total of type	Origin		No. of transformants to streptomycin resistance[a]	
		Normals (n)	Patients (p)	Low	High
3	1/6		1	1p[b]	
4	2/7	1	1	1n; 1p	1n
6	2/3	2		1n	1n
8	1/4	1			1n
9	2/3	1	1		1n; 1p
17	1/4	1			1n
23	3/4		3		3p
No. transformed/total no. isolates[c]	12/43	6/20[d]	6/23	4/43	8/43
% which transformed	28	30	26	9	19

[a] Low: <70 transformants/ml. High: 2,000 to 40,000 transformants/ml. Competent unencapsulated R36NC culture under same conditions: 4,000,000 transformants/ml. Total population at selection time: 10^8 to 10^9 colony-forming units/ml. In the absence of DNA, streptomycin-resistant pneumococci were never recovered.

[b] n, Isolate obtained from normal carrier; p, isolate obtained from patient.

[c] All types: see Table 1.

[d] One man carried a type 6 and a type 9, both of which were transformable.

TABLE 3. *Effect of depolymerase on capsule of pneumococci of types 3 and 8*

Culture (4-hr) of:	Size of quellung reaction[a]								
	Incubation (min)				Min at 30 C after removal of enzyme				
	0	15 at 37 C	45 at 37 C	45 at 37 C + 16 hr at 0 C	0	10	20	30	60
Type 3 isolate									
Plus enzyme, 1 mg/ml	++++	++	++	+	+	+	++	++	++++
No enzyme	++++	++++	++++	++++	++++	++++	++++	++++	++++
Type 8 isolate									
Plus enzyme, 1 mg/ml	++	++	++	±	±	±	+	+	++
No enzyme	++	++	++	++	++	++	++	++	++
R36NCStr-rEry-r									
Plus enzyme, 1 mg/ml	−	−	−	−	−	−	−	−	−
No enzyme	−	−	−	−	−	−	−	−	−

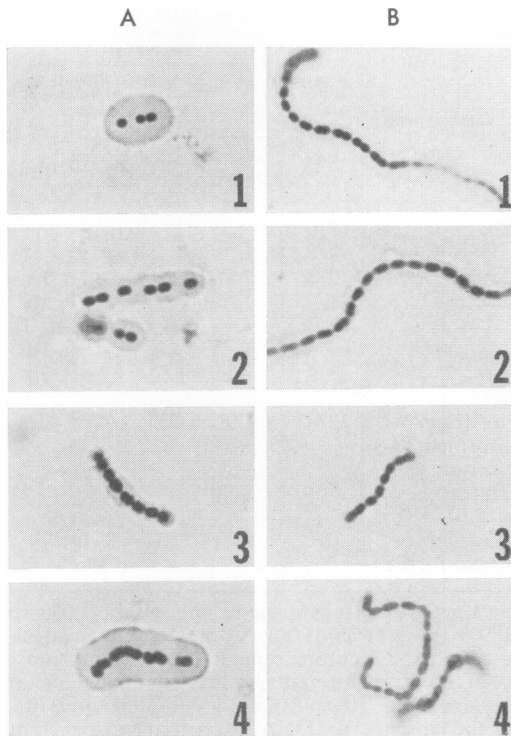[a] Fresh antiserum was added to the sample at the end of each incubation period.

FIG. 1. *Capsular swelling of type 3 (A, 1–4) and type 8 (B, 1–4) pneumococci with specific antisera following exposure of cells to polysaccharide hydrolyzing enzyme. Panel 1, 0 min at 37 C; 2, 45 min at 37 C; 3, 16 hr at 0 C after 45 min at 37 C; 4, 60 min at 30 C after enzyme removal.*

1935, the above information was not available, and the preparation which was thought to be uniform probably contained more than one enzyme.)

Slides of quellung reactions prepared after type 3 and type 8 pneumococci had been exposed to hydrolytic enzyme for 45 min at 37 C, were sealed with paraffin and stored at 0 C for 16 and 48 hr. The antiserum blocked enzyme activity since no further diminution in the size of the capsule was observed. In the absence of specific antiserum, capsular digestion continued in the cold.

After the enzyme was washed off, addition of antiserum again produced capsular swelling which returned to normal size after the cells were incubated for 1 hr at 30 C in the absence of enzyme. However, for at least the first 30 min of exposure to DNA, the cells were coated by very little capsular polysaccharide (Table 3). Since neither the manipulations nor the enzyme interfered with the growth or transformation of the control rough culture, it was concluded that, for these type 3 and type 8 isolates, the presence of capsular material was not a limiting factor in their lack of transformability by DNA from unrelated pneumococci (see Materials and Methods, Table 4). In addition, different isolates of the same capsule type differed in their ability to be transformed. Thus, it may well be that the percentage of fresh isolates which did transform (Table 2) reflects a variability in genetic potential of pneumococci in the wild (as in the ability to

TABLE 4. *Effect of depolymerase on transformation*

Culture	Total count (CFU/ml)	Streptomycin resistant (CFU/ml)
Type 3 isolate		
With enzyme	10^7	0
Without enzyme	10^7	0
Type 8 isolate		
With enzyme	5×10^7	0
Without enzyme	6×10^7	1
R36NCStr-rEry-r		
With enzyme	6.4×10^7	1.2×10^6
Without enzyme	6.6×10^7	1.3×10^6

produce competence factors [32]) and that the capsular polysaccharide per se does not preclude genetic exchange. As Ravin (26) showed, however, transformations among almost isogenic bacteria may be significantly reduced by the presence of capsular polysaccharide on the potential recipients.

Recombination among bacteria in the human pharynx. Fate of R36NCStr-rEry-r. The total number of R36NCStr-rEry-r present in 2 ml of throat washings was estimated at intervals after inoculation of the volunteer's pharynx with approximately 10^9 organisms (see Materials and Methods). Although these assays are approximate, it can be seen from Table 5 that, in all 11 trials, no R36NCStr-rEry-r survived for as long as 24 hr. In three cases, drug-resistant, rough pneumococci could not be recovered even after only 1 hr. In the remainder, more than 99% of the inoculum had disappeared by the end of 1 hr. These results are in agreement with those of Johanson et al. with type 3 pneumococci (19). Since it seems very difficult to colonize the pharynx with pneumococci, one would expect transformation of the carrier organisms by DNA released from exogenously introduced bacteria rather than the reverse.

Occurrence of recombinants. A total of five volunteers carried pneumococci known to be transformable by in vitro testing. Only three of these candidates were available for the study. In view of this small number, the experiment was also done with six carriers whose pneumococci had not transformed in vitro, since conditions in vivo might be more favorable to transformation (20).

No recombinants were recovered from 9 out of 10 trials (one person volunteered twice). Streptomycin-resistant, alpha-hemolytic, gram-positive cocci were isolated from subject number 8. This individual carried pneumococci of type 6

and type 9, and both types were transformable in vitro. In this case, the pharynx was inoculated with R36NCStr-rEry-r a second time, 3 hr after the first inoculation. Streptomycin-resistant type 9 pneumococci were recovered from a mouse inoculated with throat washings taken just prior to the second exposure of the volunteer to R36NCStr-rEry-r. In addition, this and later samples contained other streptomycin-resistant, alpha-hemolytic, gram-positive cocci (see below). The results of this experiment are summarized in Table 6. Since no gram-positive cocci resistant

TABLE 5. *Recovery of R36NCStr-rEry-r following inoculation of pharynx with 10^9 CFU*

Carrier subject no.	R36NCStr-rEry-r- in 2 ml of throat washing (CFU)		
	1^a	4^a	24^a
1	40	0	0
2	5.3×10^3	0	0
3	5.2×10^3	2×10^4	0
4	0	0	0
5	0	0	0
6	2×10^3	2×10^3	0
7	4.6×10^3	1.6×10^2	0
8 (Trial 1)	0	0	0
8 (Trial 2)[b]	1.2×10^4	0	0
9 (Trial 1)	7.2×10^3	40	0
9 (Trial 2)	1.6×10^4	80	0

[a] Hours postinoculation.

[b] Second inoculation 3 hr after the first.

Table 6. *Streptomycin resistance of pharyngeal flora after inoculation with R36NCStr-rEry-r*

Time after inoculation	No. alpha-hemolytic colonies from:			
	Throat smear on blood agar plus:		Material[a] from mice on blood agar plus:	
	Strepto-mycin	Erythro-mycin	Strepto-mycin	Erythro-mycin
1.5 hr	None	None	None	None
3 hr[b]	None	None	56[c] + 19[d]	1[e]
4 hr	300[e]	300[e]	12[c]	1[e]
24 hr	400[c]	None	450[c]	None
13 days	40[c]	None	7[c]	None
29 days	30[c]	None	100[e]	None
4.5 months	13[c]	None	None	None

[a] Blood and peritoneal washings.

[b] Followed by second inoculation.

[c] Small colonies all of same size, considerably smaller than R36NCStr-rEry-r, which proved to be streptococci.

[d] Type 9 pneumococci.

[e] R36NCStr-rEry-r.

to even 200 μg of streptomycin/ml were found in this subject's pharynx prior to inoculation with R36NCStr-rEry-r, it is probable that the emergence of two kinds of these cocci, both resistant to 1,000 μg of streptomycin/ml, was due to genetic exchange.

It is very unlikely that transformation took place during the incubation of the gargle material in vitro since deoxyribonuclease was present. Also, at the time the "transformants" were isolated, no R36NCStr-rEry-r were recovered from the pharynx (see Table 5) and thus were presumably absent from the incubation mixture. The possibility remains that the type 9 pneumococci mutated to high-level streptomycin resistance in the mouse. The drug-resistant organisms were found in equal but very small numbers both in the blood of the mouse and at the site of injection. One very recent mutational event is thus unlikely. In this author's experience with transformations in the mouse (1, 24, 25), no spontaneous mutations of pneumococci to high-level streptomycin resistance were found.

In conclusion, it seems strongly probable that a transformation of type 9 pneumococcus to streptomycin resistance took place in the human pharynx.

Nature of the nonpneumococcal, streptomycin-resistant cocci. Small alpha-hemolytic, erythromycin-sensitive colonies were found on streptomycin-containing blood agar plates inoculated directly with throat washings obtained at 24 hr after inoculation and at later times (Table 6). These bacteria (gram-positive cocci in short chains) were also isolated from mice injected with throat washings. The cocci had no demonstrable capsule by India ink or by pools A-F of pneumococcal antisera. They were resistant to optochin (Baltimore Biological Laboratories, 5 μg/disc), were not bile soluble, and did not grow at all in Difco SF broth. They were resistant to 1,000 μg of streptomycin/ml but sensitive to 0.1 μg of erythromycin/ml. They were somewhat resistant (2-mm zone) to tetracycline and novobiocin, but were sensitive to chloromycetin, penicillin (2 units), lincocyn, methicillin, and cephalin.

In view of the growth characteristics and of the antibiotic resistance pattern, it was felt that these bacteria were not enterococci and probably belonged to the viridans group of streptococci.

Because of the possible transformation of this alpha-hemolytic streptococcus by pneumococcal DNA in the pharynx, spontaneous transformation was looked for in vitro as described (23, 25). The results are presented in Table 7. Whereas both R36NCEry-r and *Streptococcus viridans* "D" were transformed to streptomycin resistance by DNA released from the new streptomycin-resist-

TABLE 7. *Transformations involving streptomycin-resistant, alpha-hemolytic streptococcus*

Materials added to streptomycin-resistant, alpha-hemolytic streptococcal cells[a]	Transformants/ml of incubation mixture after 18 hr at 37 C
R36NCEry-r DNA, 2 μg/ml	None
R$_6$Ery-r DNA[b], 2 μg/ml	None
R36NCEry-r cells	10 R36NCStr-rEry-r
R36NCEry-r cells + deoxyribonuclease, 20 μg/ml	None
Streptococcus "D" Ery-r cells . . .	7.4 × 10^4 streptococci Str-rEry-r[c]
Streptococcus "D" Ery-r cells + deoxyribonuclease, 20 μg/ml . .	None

[a] In each case, the inoculum consisted of 3 × 10^5 CFU of pneumococci or streptococci per ml of transformation medium.

[b] This DNA, extracted from rough, erythromycin-resistant pneumococci, was a gift from Muriel Roger.

[c] About 40% of the colonies were small like the streptococcal isolate, and 60% were large and more mucoid like streptococcus "D," but positive identification was not possible.

ant streptococcus, this organism was not itself transformed to erythromycin resistance by freshly released or by extracted pneumococcal DNA in vitro. Transformation to erythromycin resistance may have occurred with freshly released streptococcal DNA. Since the alpha-hemolytic streptococcus isolated from subject 8 interacted genetically with both pneumococci and streptococci in vitro, transformation probably took place in the pharynx. In fact, DNA from the streptomycin-resistant streptococci could have transformed the type 9 pneumococci in the pharynx or in the mouse to streptomycin resistance.

In the absence of selective pressure, the resistant pneumococci did not survive in the pharynx, whereas some resistant streptococci were still present after 4.5 months.

DISCUSSION

Several investigators have reported the release of genetically active DNA by bacteria during growth (7, 22, 30). This DNA is probably released from living cells (13, 23, 28). The studies presented in this communication lend support to the notion, previously discussed (1, 23, 24, 25), that transformation among bacteria may occur in man.

A survey of 19 healthy carriers of pneumococci and of 23 patients with pneumococcal disease revealed that about 25 to 30% of the fresh pneumococcal isolates were transformable by soluble DNA in vitro. This is a conservative estimate because the screening for competence was done under only one set of conditions. The transformability was not related to the amount or type of capsule. Three of the six transformable isolates obtained from patients were of type 23. Hospital

records revealed that two specimens were from patients on the same ward at the same time; one had pneumococcal pneumonia on admission and the second, an alcoholic admitted for gastrointestinal bleeding, developed pneumonia 3 days later. The third transformable type 23 was obtained from the daughter of the house officer who was taking care of one of the patients with pneumonia. When the relationship among the three isolates was elucidated some weeks later, a routine throat culture on the house officer in question produced type 4 transformable pneumococci. It seems that the likelihood of transformations in man can be increased by local spread of transformable pneumccocci from one individual to another.

Although it is difficult, in general, to colonize the human pharynx experimentally with exogenous pneumococci (19), certain individuals, such as the house officer above, provide a good environment for naturally acquired pneumococci. It is in these cases that genetic exchange would be likely to occur among the flora of the pharynx. In the presence of very large numbers of competent bacteria such as were found in some of the patients with pneumococcal disease, transformation might occur more frequently. Experimental proof of this hypothesis is, of course, impossible. The finding presented here, of streptococci resistant to streptomycin after temporary exposure in vivo to rough, drug-resistant pneumococci, supports the view that genetic exchange can occur among different components of the pharyngeal flora. When selective pressures arise, one species may survive by sharing the genetic past of another (23).

In this study, about one fourth of all persons, healthy and diseased, who were harboring pneumococci, were harboring transformable ones. In addition, conditions in the human pharynx do not seem to preclude transformation. Thus, these studies support the contention that bacterial transformations occur in man.

ACKNOWLEDGMENTS

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