TRANSFORMATION REACTIONS BETWEEN PNEUMOCOCCUS AND THREE STRAINS OF STREPTOCOCCI*

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Transformation reactions with streptococci either as donors or receptors of DNA (desoxyribonucleic acid) have not been reported previously. In the present study DNA extracts of streptomycin-resistant pneumococci were applied to 42 different strains of *Streptococcus*. Two strains of *viridans* streptococci, strains D and NBSI, incorporated pneumococcal DNA and became streptomycin-resistant.

Pneumococci were caused to become streptomycin-resistant by DNA extracts of resistant mutants of the 2 strains of streptococci. In addition, resistance to optochin, which occurs naturally in these streptococci, was conferred on pneumococci by exposure to streptococcal extracts.

It has further been found that a strain of *Streptococcus salivarius* does not become streptomycin-resistant when either autologous or heterologous[1] DNA extracts are applied to it. However, DNA extracts prepared from a streptomycin-resistant mutant of *Str. salivarius* caused pneumococcus and streptococcal strains D and NBSI to become streptomycin-resistant.

Materials and Methods

Strains of Pneumococcus.—II-D39S: an encapsulated strain of pneumococcus Type II.

II-R36NC: a colonial variant of the rough strain II-R36. II-R36 was derived originally from strain II-D39S.

Strains of Streptococcus.—42 different strains of streptococci were exposed to DNA extracts of streptomycin-resistant (St^r) pneumococci. Of these only 2 related strains of *viridans* streptococci, strains D and NBSI, were transformed to streptomycin resistance. The following streptococci were not transformed: *Streptococcus hemolyticus*, Group A, 12 strains; *Str.*

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[1] In this paper the term *homologous* designates reactions between strains of the same bacterial species (homotransformation); the term *heterologous* refers to reactions between different species (heterotransformation). A third expression, *autologous* (autotransformation), is used to indicate reactions taking place between representatives of the same strain of a bacterial species.

hemolyticus, Group B, 1 strain; *Str. hemolyticus*, Group C, 5 strains; *Str. hemolyticus*, Group D, 2 strains; *Str. hemolyticus*, Group F, 2 strains; *viridans* streptococci, 14 strains; *Str. salivarius*, 1 strain; streptococcus MG, 3 strains.

Five strains of streptomycin-resistant streptococci have been used as *donors* of DNA for transformation reactions in which pneumococcus II-R36NC and the streptococcal strains D and NBSI have acted as *receptors*. Three of these, *viridans* streptococcus strains D St^r and NBSI St^r, and *Str. salivarius* I-R14 St^r, yielded DNA extracts that transformed pneumococcus and streptococcal strains D and NBSI to streptomycin resistance. DNA extracts of a strain of *Str. hemolyticus* Group A St^r, and a strain of *Str. hemolyticus* Group C St^r did

TABLE I

Comparison of Properties of Pneumococcus and of Streptococcal Strains D and NBSI

	Pneumococcus II-R36NC	Streptococcus D	Streptococcus NBSI
Hemolysis in blood agar plates	alpha	alpha	alpha
Solubility in 0.1 per cent Na desoxycholate	+	−	−
Growth in bile—2 per cent	−	+	+
5 " "	−	+	+
10 " "	−	−	+
20 " "	−	−	+
40 " "	−	−	+
Growth in optochin—1:10,000	−	+	+
1:1,000,000	−	+	+
Conversion to Gram negativity by silicate	yes	yes	yes
Killed by silicate	yes	yes	yes
Fermentation reactions: trehalose	+	−	−
inulin	+	−	−
Presence of pneumococcal "C" carbohydrate	+	−	−
Agglutination by antiserum to:			
R pneumococcus II-R36NC	4000	8	8
Streptococcus D	8	2000	2000
Streptococcus NBSI	8	2000	4000

not transform the sensitive strains from which they were selected nor did they transform pneumococcus or the *viridans* streptococcal strains D and NBSI.

Properties of Viridans Streptococcal Strains D and NBSI and Their Distinction from Pneumococcus.—Streptococcal strain D was obtained originally from Dr. F. L. Horsfall, Jr., in lyophilized form in 1948. A second specimen of strain D in lyophilized form, identical in all respects tested to that received in 1948, was obtained from Dr. Horsfall in 1955. The ampoules from which strain D was cultured were thought to contain an "R" variant of streptococcus MG, strain 9 as described by Mirick, Thomas, Curnen, and Horsfall (1). However, the properties of strain D are different from those of streptococcus MG so that there is some question whether strain D is an R variant of streptococcus MG (2). Certain of the properties of strain D are shown in Table I.

Streptococcal strain NBSI has been carried in the culture collection of the Department of Microbiology, New York University College of Medicine, for more than 15 years. Its more remote origin is unknown. As shown in Table I it is similar to strain D in many of its properties, though not identical.

Table I shows that streptococcal strains D and NBSI are similar in most respects tested except that strain NBSI grows in the presence of 40 per cent bile, whereas strain D is inhibited at a concentration of 10 per cent.

Strains D and NBSI differ from pneumococcus in many properties as is shown in Table I. They are not soluble in 0.1 per cent sodium desoxycholate and both strains grow in 5 per cent bile. Both are naturally resistant to optochin (ethylhydrocupreine). Their growth in blood agar is not inhibited by optochin 1:10,000 whereas pneumococcus is inhibited by a concentration of 1:1,000,000. Pneumococcus ferments trehalose and inulin; strains D and NBSI ferment neither of these sugars.

The taxonomic significance of the effects of silicate on pneumococcus and strains D and NBSI is not known. As shown by MacLeod and Roe (3) silicate converts pneumococci, strains D and NBSI and certain other streptococci from the Gram-positive to a Gram-negative state. Restoration to Gram positivity can be accomplished by washing in alkaline solutions of various salts, though not by washing in water. Silicate also has a lethal effect on pneumococcus and on streptococcal strains D and NBSI.

Immunological reactions serve to further differentiate strains D and NBSI from pneumococcus. Formamide extracts of cultures of strains D and NBSI prepared by Fuller's method (4) did not react with an antiserum which precipitates in the presence of purified pneumococcal C carbohydrate or a formamide extract of pneumococci.

Marked differences in immunological composition are revealed also by agglutination reactions. Sera were prepared by repeated injections of normal rabbits with suspensions of the respective heat-killed cells over a period of 3 to 4 weeks. The animals were bled to obtain serum 1 week after the last injection. For agglutination reactions, 0.3 ml. of serum dilutions were mixed with an equal volume of heat-killed bacterial suspension. The mixtures were incubated at 37°C. for 1 to 2 hours and readings made after refrigeration for 12 hours. The figures in Table I indicate the reciprocal of the highest dilution of serum causing definite agglutination. Antiserum prepared against R pneumococcus, strain II-R36NC, showed slight cross-reactivity with suspensions of strains D and NBSI. Antiserum to strain D agglutinated both strain D and strain NBSI to the same titer. Strain NBSI was agglutinated by its autologous antiserum in a titer of 1:4000; the same antiserum agglutinated strain D in a titer of 1:2000. On the other hand, antisera to strains D and NBSI agglutinated pneumococcus strain II-R36NC in a titer of only 1:8 in each case.

In summary of the data shown in Table I, it can be said that strains D and NBSI of *viridans* streptococci show many biochemical and immunological differences that distinguish them from pneumococcus. However, strains D and NBSI, while they appear to be closely related to each other, are not identical.

Properties of Streptococcus salivarius Strain I-R14.—This strain of *Str. salivarius* was obtained from Dr. F. L. Horsfall, Jr., in 1948. It is an R variant that had been selected from *Str. salivarius* Type I after cultivation for 14 successive transfers in broth containing Type I antiserum. Except for its lack of type-specific surface antigen it corresponds in all respects tested to the description of *Str. salivarius* given by Sherman, Niven, and Smiley (5). It is readily identified by the large mucoid colonies it forms owing to synthesis of copious amounts of levan when cultivated on the surface of blood agar plates containing 5 per cent sucrose. This property is not shared by pneumococcus or the strains of streptococci listed in Table I.

Selection of Streptomycin-Resistant Mutants.—10^{10} to 10^{11} organisms from broth cultures of streptomycin-sensitive strains of pneumococcus and the streptococci were spread on the surface of blood agar plates containing 10 mg. of streptomycin per ml. Plates were incubated for 24 to 72 hours at 37°C. Resistant colonies were transplanted from these plates to media that did not contain streptomycin.

Preparation of Transforming Extracts.—DNA extracts of pneumococci were prepared as

previously described (6). Mass cultures were lysed by sodium desoxycholate in the presence of citrate ion, precipitated by the addition of 1 volume of 95 per cent ethanol, redissolved in saline, and deproteinized by repeated shaking with chloroform and octyl alcohol. The extracts were then precipitated by alcohol and stored in this form.

The strains of streptococci were grown in broth containing 1 per cent glucose. Acid produced during growth was neutralized by intermittent addition of NaOH with phenol red as inside indicator. At the end of the growth period sodium citrate was added to a final concentration of 0.1 M in order to inhibit bacterial desoxyribonuclease. Organisms from 100 ml. of neutralized broth culture were collected by centrifugation, suspended in 5 ml. of 0.1 M sodium citrate–saline and vibrated in a Mickle disintegrator at 4°C. for 30 minutes with 4 gm. of No. 112 glass beads[2] and a drop of octyl alcohol. After vibration the suspensions were centrifuged for 1 hour in a Sorvall centrifuge at 4000 to 4500 R.P.M. To the supernate was added 1 volume of 95 per cent ethanol. The resulting precipitates were stored in alcohol overnight to sterilize them. They were then centrifuged, the alcohol was poured off, and the precipitates were taken up in sterile saline. Transformation reactions were carried out with these extracts and extracts that had been deproteinized by repeated shaking with chloroform and octyl alcohol. Both the crude and deproteinized extracts transformed appropriate receptor strains.

Transformation Reactions.—0.1 ml. of a 10^{-3} dilution in broth of an overnight blood broth culture of streptomycin-sensitive cells was added to charcoal-absorbed broth containing 0.1 ml. of an appropriate dilution of DNA extract from streptomycin-resistant cells and human pleural fluid or human serum that had been heated at 60°C. for 30 minutes. The final volume was 2 ml. After incubating for 18 to 24 hours the cultures were streaked on blood agar containing 100 μg. of streptomycin per ml. to determine whether streptomycin-resistant cells were present. Control cultures from which DNA extract was omitted, and cultures containing DNA extract and crystalline pancreatic desoxyribonuclease (DNAase), were incubated and plated in the same way.

The number of cells that became resistant to streptomycin because of transformation were counted in broth cultures incubated with DNA for 8 hours. After this period DNAase was added, the cultures were then diluted in broth and the number of resistant organisms was determined by plate counts in blood agar containing 100 μg. of streptomycin per ml. At the same time enumeration of the total bacterial population was made in blood agar from which streptomycin had been omitted. Control cultures to which DNA extract and DNAase had been added at the beginning of the experiment were examined similarly.

EXPERIMENTAL

Reciprocal Transformation of Streptomycin Resistance in Pneumococcus and Strains of Streptococcus.—The experiments summarized in Table II, in which streptomycin resistance (*Sm*^{r}) was used as genetic marker, show that transformation reactions can occur reciprocally between pneumococcus and strains D and NBSI of *viridans* streptococci. As indicated in Table II, DNA extracts were prepared from spontaneous *Sm*^{r} mutants of 2 strains of pneumococcus (II-R36NC *Sm*^{r} and II-D39S *Sm*^{r}), and from streptococcus D *Sm*^{r}, streptococcus NBSI *Sm*^{r}, and *Str. salivarius Sm*^{r}. These DNA extracts caused pneumococcus and strains D and NBSI to become streptomycin-resistant but not *Str. salivarius*. *Str. salivarius Sm*^{r} provided DNA extracts which transformed heterolo-

[2] Superbrite glass beads, Minnesota Mining and Manufacturing Company, Saint Paul, Minnesota.

TABLE II

Transformation to Streptomycin Resistance of Streptococcal Strains by DNA Extracts of Pneumococcus and Vice Versa

Donor of DNA extract	Origin of Sm^r mutant gene in donor	Receptor strain			
		Pneumococcus II-R36NC	Streptococcus D	Streptococcus NBSI	*Streptococcus salivarius*
Pneumococcus II-R36NC Sm^r‡	Pneumococcus II-R36NC	+§	+	−	0
Pneumococcus II-D39S Sm^r	Pneumococcus II-D39S	+	+	+	−
Streptococcus NBSI Sm^{r*}	Pneumococcus II-D39S	+	+	+	−
Streptococcus D Sm^{r*}	Pneumococcus II-D39S	+	+	+	−
Pneumococcus II-R36NC Sm^{r*}	Pneumococcus II-D39S	+	−	+	−
Streptococcus D Sm^r	Streptococcus D	+	+	+	0
Pneumococcus II-R36NC Sm^{r*}	"	+	+	+	−
Streptococcus NBSI Sm^r	Streptococcus NBSI	+	+	+	0
Pneumococcus II-R36NC Sm^{r*}	Streptococcus NBSI	+	+	+	−
Streptococcus salivarius I-R14 Sm^r	*Streptococcus salivarius* I-R14	+	+	+	0
Pneumococcus II-R36NC Sm^{r*}	*Streptococcus salivarius* I-R14	+	+	+	−
Streptococcus NBSI Sm^{r*}	*Streptococcus salivarius* I-R14	+	+	+	0
Streptococcus D Sm^{r*}	*Streptococcus salivarius* I-R14	+	+	+	0

‡ Strains designated Sm^r became resistant to streptomycin through mutation; strains designated Sm^{r*} became streptomycin-resistant through transformation.

§ + indicates that transformation to Sm^r occurred; 0 indicates that transformation was not observed; − indicates that the experiment was not carried out.

gous cells to streptomycin resistance but it was unable to act as recipient for either autologous or heterologous DNA.

DNA extracts were prepared also from pneumococcus and streptococcal strains D and NBSI in which streptomycin resistance was engendered by DNA extracts from homologous and heterologous Sm^r strains. These extracts of Sm^{r*}[8] strains caused sensitive pneumococci and streptococcal strains D and NBSI to become streptomycin-resistant.

Addition of DNAase at the beginning of experiments destroyed the activity of all extracts. Furthermore, when DNA extracts were omitted, streptomycin-resistant cells were not observed in samples of culture of the same size as those examined from tubes which contained DNA extracts.

The level of streptomycin resistance observed in recipient cells was the same whether the DNA extract was prepared from a donor that had mutated spontaneously to streptomycin resistance (Sm^r) or from a donor that had been made resistant through transformation (Sm^{r*}). In addition, the level of resistance of transformed strains of pneumococcus II-R36NC was the same when pneumococcus, streptococcus D, streptococcus NBSI, or *Str. salivarius* was the original source of the Sm^r property. Similar results were observed when strains D and NBSI acted as receptors. This finding was not unexpected since streptomycin resistance was of a high level in each of the Sm^r donors and had arisen through single step mutation.

Efficiency of Pneumococcus, Streptococcus D, and Streptococcus NBSI as Receptors in Transformation Reactions.—As shown in Table III streptococcus strain D consistently showed a much higher proportion of streptomycin-resistant cells than pneumococcus II-R36NC when grown with DNA extracts of Sm^r cells. The receptor efficiency of strain NBSI was similar to that of strain D but at a somewhat lower level. It was also superior to pneumococcus as receptor whether the DNA extract was of autologous or heterologous origin.

To determine the proportion of the receptor population transformed to streptomycin resistance, DNA extracts were added to the transformation tubes at the beginning of the experiment. After 8 hours' incubation at 37°C. DNAase was added to destroy remaining free DNA. Samples were removed for colony counts. The total bacterial population was enumerated by incorporation of appropriate dilutions of culture into pour plates of blood agar. The number of streptomycin-resistant organisms was counted by means of blood agar pour plates containing 100 μg. streptomycin per ml. The figures in columns 3 and 4 of Table III express the proportion of cells becoming resistant as per cent of the total receptor population after 8 hours' incubation.

Control tubes containing DNA and DNAase added at the beginning of the experiment were similarly examined for resistant cells. In 8 of 10 control tubes of pneumococcus II-R36NC and 5 of 9 control tubes of streptococcus D, no streptomycin-resistant cells were observed when 1 ml. of culture containing 10^8 sensitive organisms was plated in agar containing 100 μg.

[8] Sm^{r*} is used as the notation for strains that became streptomycin-resistant through transformation.

streptomycin per ml. There was 1 resistant organism per 10^8 sensitive organisms in each of 2 control tubes of pneumococcus II-R36NC and 3, 2, 1, and 1 resistant organisms, respectively, per 10^8 sensitive cells in 4 control tubes of streptococcus D.

TABLE III

Incidence of Transformation to Streptomycin Resistance of Pneumococcus II-R36NC and Streptococcus D by DNA Extracts of Pneumococcus and Streptococci

	Column 1	Column 2	Per cent of receptor population transformed to streptomycin resistance	
			Column 3	Column 4
	Donor of DNA extract	Origin of *Sm*^{r} mutant gene in donor	Pneumococcus II-R36NC	Streptococcus D
Line 1	Pneumococcus II-R36NC *Sm*^{r}‡	Pneumococcus II-R36NC	0.04	1.0
2	Pneumococcus II-R36NC *Sm*^{r}*	Pneumococcus II-D39S	0.06	Not measured
3	Streptococcus D *Sm*^{r}*	" "	0.01	3.0
4	Streptococcus NBSI *Sm*^{r}*	" "	0.02	3.0
5	Streptococcus D *Sm*^{r}	Streptococcus D	0.00001	1.0
6	Pneumococcus II-R36NC *Sm*^{r}*	" "	0.0005	0.1
7	Streptococcus NBSI *Sm*^{r}	Streptococcus NBSI	0.00002	1.0
8	Pneumococcus II-R36NC *Sm*^{r}*	" "	0.0008	0.06
9	*Streptococcus salivarius* I-R14 *Sm*^{r}	*Streptococcus salivarius* I-R14	0.0001	0.1
10	Pneumococcus II-R36NC *Sm*^{r}*	" "	0.009	0.1

‡ Strains designated *Sm*^{r} became resistant to streptomycin through mutation; strains designated *Sm*^{r}* became streptomycin resistant through transformation.

The reasons for the greater receptor efficiency of the streptococcal strains as contrasted with pneumococcus may be multiple. Only one possibility has been explored in the present study, namely the period of bacterial growth over which transformation takes place.

Parallel sets of transformation tubes containing approximately the same inoculum respectively of pneumococcus and strain D were incubated at 37°C. DNA was added to a tube

of each set at 0 time, and hourly thereafter to each successive tube through 9 hours. After exposure to DNA for 1 hour, DNAase was added to each tube. All tubes were kept in the incubator for 9 hours and samples then withdrawn to determine when transformation had taken place.

In the case of pneumococcus streptomycin-resistant cells were not observed at the end of the 1st and 2nd hours of exposure to DNA but began to appear during the 3rd hour and continued through the 8th hour. With streptococcus D, on the other hand, transformation began during the 1st hour and continued through the 8th hour. Neither receptor strain showed transformation during the 9th hour. It is apparent, therefore, that the greater receptor efficiency of streptococcus D is related in part to the fact that transformation of a growing culture of this strain begins at least 2 hours earlier than in the case of pneumococcus and, in common with pneumococcus, persists through the 8th hour of incubation.

Non-Identity of Transforming Principles from the 4 Different Strains That Produced the Same Degree of Streptomycin Resistance in the Various Recipients.— Although the phenotypic expression (level of resistance to streptomycin) of the Sm^r transforming principles (TP) derived from the 4 donor strains was the same, evidence was obtained that these TP's are not identical.

Table III shows the proportions of the total receptor populations of pneumococcus and streptococcus D that became streptomycin-resistant when grown with DNA extracts of the 4 Sm^r donor strains.

When pneumococcus II-R36NC Sm^r was the donor of DNA, 0.04 per cent of strain II-R36NC was transformed to streptomycin resistance. When pneumococcus II-R36NC Sm^{r*} was the donor, the Sm^r mutation having occurred in pneumococcus II-D39S, the proportion of strain II-R36NC transformed was approximately the same, 0.06 per cent (lines 1 and 2, Table III). Pneumococcal Sm^{r*} TP which had been reduplicated in streptococcal strains D and NBSI following transformation, yielded a slightly lower proportion of transformed cells (0.01 and 0.02 per cent respectively) when applied to pneumococcus as receptor (lines 3 and 4).

Lines 5 and 6 show that there was a much lower incidence of transformation of pneumococcus by Sm^r TP from streptococcus D whether the Sm^r TP had been replicated only in streptococcus D or after it had been reduplicated in pneumococcus (0.00001 and 0.0005 per cent respectively). Similar results for Sm^r TP's from streptococcus NBSI and *Str. salivarius* are listed in lines 7, 8, 9, and 10.

When streptococcus D was used as the receptor the differences observed in the proportion of cells that became resistant to streptomycin on exposure to various Sm^r TP's were less than when pneumococcus was used as a receptor. With all of the TP's streptococcus D was a much better receptor than pneumo-

coccus and this may have obscured differences between the activity of different TP's that were apparent with pneumococcus.

Following reduplication in heterologous strains the transforming activities of pneumococcal and streptococcal TP's were found to be changed, as measured by the proportion of cells of receptor strains that were transformed to streptomycin resistance (Table III). When TP from Sm^r pneumococci was introduced into strains D and NBSI, the proportion of pneumococcus II-R36NC transformed by extracts of these streptococcal strains was slightly less than when pneumococcal TP came directly from pneumococcus. At the same time the proportion of strain D transformed by these extracts was slightly increased (lines 1, 3, and 4). Further experiments should be done to determine whether the observed differences are significant.

The ability of the TP's from the 3 streptococcal strains to transform pneumococcus increased following reduplication in pneumococcus. For the TP from streptococcus D, the proportion of pneumococci transformed to streptomycin resistance increased by 50-fold (compare lines 5 and 6, column 3). There was an increase of 40-fold for the TP from strain NBSI (column 3, lines 7 and 8), and of 90-fold for the TP from *Str. salivarius* (column 3, lines 9 and 10). However, these streptococcal TP's still transform pneumococcus less efficiently than pneumococcal TP.

After TP from strain D had been replicated in pneumococcus its efficiency in transforming strain D decreased 10-fold (column 4, lines 5 and 6, Table III) and that of the TP from the related strain NBSI decreased 15-fold (column 4, lines 7 and 8). The efficiency of the TP from *Str. salivarius* to transform strain D was not changed by reduplication in pneumococcus.

Transformation of Pneumococcus to Optochin Resistance by DNA Extracts from Naturally Resistant Streptococci.—Pneumococcus in the wild state is highly susceptible to the antibacterial effect of optochin (ethylhydrocupreine). Streptococci in general are naturally resistant. This difference in susceptibility is so uniform that it has been suggested as a criterion to differentiate pneumococcus from streptococci (7). Morgenroth and Kaufmann (8) originally described the appearance of optochin-resistant pneumococci in mice infected with pneumococci and treated with optochin. Similarly, optochin-resistant pneumococci can be selected readily *in vitro* by cultivation in optochin-containing media, as shown by Tugendreich and Russo (9). Resistance to optochin can be conferred on susceptible pneumococci by transforming extracts prepared from spontaneously occurring, resistant mutants of pneumococcus. DNA extracts of streptococcal strain NBSI, which is naturally resistant to optochin, caused pneumococci to become optochin-resistant. The proportion of pneumococci transformed by pneumococcal TP to optochin resistance is much greater than with streptococcal TP.

DISCUSSION

The experiments reported in this paper demonstrate that transformation to streptomycin resistance can occur reciprocally between pneumococcus and 2 strains of *viridans* streptococci, strain D and the related strain NBSI.

The single strain of *Str. salivarius* studied, on the other hand, yielded DNA extracts that caused transformation of pneumococcus and streptococcal strains D and NBSI, but did not induce autologous transformation to streptomycin resistance. DNA extracts of streptomycin-resistant pneumococcus and strains D and NBSI likewise did not transform the single strain of *Str. salivarius* studied. It is known in the case of pneumococcus, a "transformable" species, that some strains can be transformed with the greatest difficulty or not at all. Since individual strains of pneumococcus may vary greatly in their ability to act as receptors of DNA, the single example of *Str. salivarius* which proved unreceptive may not be representative of this species.

It has also been observed that 2 strains of *viridans* streptococcus, D and NBSI, incorporate pneumococcal TP more efficiently than a strain of pneumococcus itself. This may be caused by a peculiarity of the strain of pneumococcus used throughout these studies, and does not prove that pneumococcus as a species is a poorer receptor of pneumococcal TP than certain strains of distantly related streptococci.

Based on present knowledge we do not believe that the relative efficiency or inefficiency of transformation reactions can serve as the basis for determining the relatedness of bacteria, as has been suggested by Leidy, Hahn, and Alexander in their studies of *Hemophilus* "species" (10). On the basis of transformation to streptomycin resistance, these authors suggest that *H. influenzae* and *H. parainfluenzae* are more closely related to each other than they are to *H. suis*, and that *H. parainfluenzae* and *H. suis* are distantly related because of failure of reciprocal transformation reactions to occur. Leidy and coworkers propose, therefore, that transformation reactions may be more sensitive indicators to define the relationship between species than the properties providing current criteria of classification. Our observations on transformation reactions between a pneumococcus and 3 strains of streptococci show that with these microorganisms closeness of relationship is not indicated by the efficiency of reciprocal transformation reactions.

Efficiency of transformation may depend upon factors that are unrelated to the ability of a receptor strain to fit a given piece of genetic material into its genome. For example a gene system distinct from that involved in a particular transformation reaction may affect the uptake of DNA by the cell, its incorporation into the genome or its expression following incorporation. This is not to deny that closely related species are able to cross because of genetic compatibility whereas distantly related species cannot cross or cross with

difficulty because of genetic incompatibility, but to point out that transformation reactions as thus far perceived cannot be relied upon to demonstrate whether bacteria are related.

Evidence has been presented that a TP may be changed when it is reduplicated in a foreign host. It retains, however, certain properties of the strain from which it was derived originally. Reduplication of TP from streptococcal strain D in pneumococcus appears to increase its efficiency in transforming pneumococcus and at the same time to decrease its efficiency to transform strain D. This change in the activity of the streptococcal TP upon reduplication in pneumococcus indicates that it has a degree of plasticity. Nonetheless, this heterologously reduplicated TP maintains to a considerable extent the properties of its streptococcal progenitor since its efficiency in transforming pneumococcus is still much lower than that of *Sm*^{r} TP originating in pneumococcus.

It is not possible on the basis of our experiments to determine whether the TP's responsible for streptomycin resistance in pneumococcus and the 3 strains of streptococcus consist of single, highly polymerized molecules of DNA unique for each strain. Although they induce the same degree of streptomycin resistance in both homologous and heterologous receptors, they may not be identical and this may account for the large differences in the transforming ability of various TP's when applied to different receptor strains. The increased efficiency of TP's derived originally from 3 strains of streptococci to transform pneumococcus, following their reduplication in pneumococcus, may mean that DNA characteristic of these streptococci has been modified in such a way that it now more nearly resembles pneumococcal DNA. Similarly, the decreased efficiency of a TP to transform the strain in which it arose after it has been reduplicated in an heterologous host may indicate that the DNA has become changed to resemble, in some way, that of the heterologous host.

Alternatively, the TP's responsible for streptomycin resistance in the various strains may or may not be unique, chemically different molecules of DNA that become changed in an heterologous host. It is reasonable to think that transforming extracts contain DNA molecules responsible for streptomycin resistance linked in chromosomal fragments to other DNA molecules. This might influence their incorporation into the genome of an heterologous receptor. An hypothesis of this sort has been advanced by Schaeffer (11) and by Leidy and coworkers (10). If this is the case, in the process of duplication in the heterologous host, one or more of the DNA molecules associated with the TP for streptomycin resistance may be replaced by DNA molecules of the receptor. This might result in less hindrance to incorporation of the *Sm*^{r} TP from such transformed strains into the genome of the heterologous receptor and would also explain the decreased efficiency of heterologously reduplicated *Sm*^{r} TP to transform the sensitive strain from which it was obtained originally.

SUMMARY

Reciprocal transformation reactions to streptomycin resistance have been demonstrated between pneumococcus and 2 strains of *viridans* streptococci, which by the usual bacteriological criteria are distantly related to pneumococcus.

Desoxyribonucleic acid (DNA) extracts of a strain of streptomycin-resistant *Streptococcus salivarius* have been found capable of transforming pneumococcus and two strains of *viridans* streptococci but the strain itself was not transformed by autologous or heterologous DNA extracts.

The two strains of *viridans* streptococci were more efficient than pneumococcus as receptors of both autologous and heterologous transforming principles. It is inferred, therefore, that efficiency of transformation does not necessarily indicate closeness of relationship among bacteria.

The efficiency of transforming principles (TP's) from three streptococcal strains to induce streptomycin resistance in pneumococcus increased following their reduplication in pneumococcus. At the same time the efficiency of two of these TP's to transform streptococcus was reduced.

Resistance to optochin (ethylhydrocupreine), a characteristic of most streptococci, can be transferred to pneumococcus, a naturally susceptible species, by transformation reactions.

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