ON THE SPECIFICITY OF THE DESOXYRIBONUCLEIC ACID WHICH INDUCES STREPTOMYCIN RESISTANCE IN HEMOPHILUS*

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If two microorganisms are to exchange genetic material which produces a heritable change, it is alleged that they must be closely related. This premise is supported by experimental work in bacterial viruses and in recombination in *Escherichia coli*. Heritable changes which have been induced in microorganisms by transformation have, prior to 1955, been reported only within a species of bacteria. In 1955, two short reports were published on interspecies induction of streptomycin (SM) resistance in *Hemophilus* (1 a, 1 b, 2). In each report, heterospecific transformation was of a much lower order of frequency than homospecific transformation.

The designations "species" and "genus" used in this paper follow the nomenclature of the American Society of Bacteriologists (3). The precise connotations inherent in the term "species" when applied to higher organisms are not implied here. With this reservation, "species" and "genus" will be used in this paper without further qualifications.

All evidence on transformation supports the premise that in pneumococci, *H. influenzae*, and meningococci the active component of the heredity determinant is desoxyribonucleic acid (DNA). The DNA which controls SM resistance in one serological type of *H. influenzae* has been shown capable of inducing resistance to SM in a heterologous type (4).

These results stimulated an exploration of the specificity of the SM resistance transforming agent in an attempt to answer the question: Is the DNA which controls resistance in homologous type or species different from that which controls SM resistance in heterologous species of the genus *Hemophilus*? The genetic marker of resistance to high concentrations of SM was selected because it may appear in a single mutational step in many species of bacteria and can be induced in transformation experiments in a single step; also, a highly selective environment is available so that transformed cells can be not only detected but also counted accurately.

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This paper presents the results of study of the specificity of DNA controlling SM resistance among types of *H. influenzae* and *H. parainfluenzae* and among the species *H. influenzae*, *H. parainfluenzae*, and *H. suis*. The data indicate that a transforming agent which induces SM resistance has greater activity for members of the species from which it is derived, but its activity is not limited to the homologous species. The results suggest that in a given sensitive population the ratio of the number of cells in which SM resistance is induced by a heterologous DNA, to the number induced by a homologous DNA, reflects the closeness of kinship between the recipient and donor heterologous populations. This ratio among types of a species may approach unity; between species it is of a low order.

Materials and Methods

Strains Used as Recipient Populations:

H. influenzae.—As recipient populations, two groups of non-iridescent, non-typable (R) strains of *H. influenzae* have been used. One is composed of strains which arose spontaneously during artificial cultivation of types *a*, *b*, *c*, *d*, *e*, and *f* *H. influenzae*. The other group comprises strains which were obtained from cultures of the human nasopharynx and blood and are of unknown type derivation.

H. parainfluenzae.—Four groups of *H. parainfluenzae* isolated from the human nasopharynx have been used. Three of the groups have been identified by immunological reactions with absorbed rabbit antisera (5). Group IV includes those strains which show no immunological reaction with antibody against Groups I, II, or III. After elimination of cross-reacting antibodies by the agglutination absorption technique, Groups I, II, and III may be identified by the agglutination, precipitation, or capsular swelling technique. It is possible that Group IV is a heterogeneous group. One or more strains of each group were used in the present study.

H. suis.—The *Hemophilus* strain of swine origin used as a recipient population was isolated from an epidemic in Iowa.[1] Four strains were examined and found to require "V" factor (di- or triphosphopyridine nucleotide) but not "X" factor (hemin) for growth. They might, therefore, more appropriately be termed *H. parasuis* if an analogy is made with the basic criterion used for distinguishing *H. parainfluenzae* from *H. influenzae*. However, since the category *H. parasuis* is not recognized at present, the term *H. suis* will be used.

The strains are iridescent, and immunological techniques have established that they are of one type and that they are identifiable by capsular swelling on exposure to type-specific antiserum. One strain of this type was used in the present study.

Streptomycin-Resistant Strains Used as Donors of Transforming Agents:

Crude DNA-containing extracts possessing transforming activity were made from the following SM-resistant strains (1000 μg. per ml.).

H. influenzae.—Types *a* through *f* and 3 R strains of unknown type of origin.

H. parainfluenzae.—One strain of each of the four groups.

H. suis.—One strain belonging to the type described above.

These strains were obtained by seeding large populations of the SM-sensitive parent strains into Levinthal agar containing 1000 μg. per ml. SM. Cells which form colonies under

[1] Sent to us through the courtesy of Dr. Richard Shope.

these conditions are resistant to 1000 μg. per ml. SM. Progeny of the colonies selected for use as source of the transforming agent were not dependent on SM for growth. The method of extraction of the DNA-containing fractions (transforming agents) has been reported (6).

The DNA-containing extracts from SM-resistant strains of type-specific *H. influenzae* were selected in order to permit the use of two genetic markers in some studies. Techniques used for the induction of type specificity and SM resistance follow those previously described (6, 4).

In experiments in which aliquots of a recipient population were simultaneously exposed to a number of transforming agents which induce SM resistance, the following procedure was used:—

Levinthal broth, 50 ml., in a 125 ml. Erlenmeyer flask was seeded with 0.5 ml. or 1.0 ml. of an 18 to 24 hour old culture of a strain to be used as recipient population and incubated for 5 to 6 hours at 37°C. The volume of the inoculum and the period of incubation depended on rapidity of growth of the strain. A population of approximately 5×10^8 to 1×10^9 cells per ml. was attained after the 5 to 6 hours' incubation period.

To each of 1.7 ml. aliquots of the incubated young population, 0.1 ml. of saline (0.85 per cent) dilution of one of the DNA-containing extracts (diluted to contain 20 to 21 μg. per ml. DNA) was added. This concentration of DNA is in excess of that needed for maximum expression of induced resistant cells. After 15 minutes exposure to the transforming agents, 0.2 ml. of 20 μg. per ml. desoxyribonuclease (DNAse) in 0.03 M $MgSO_4$ nutrient broth was added. Five minutes were allowed for the degradation of the DNA by the enzyme.

The treated populations, appropriately diluted, were then seeded in pour plate preparations of Levinthal agar; at least 2 samples of each dilution were examined. When it was necessary to use the undiluted suspension of treated cells for estimating the number of induced SM-resistant colonies, 2 to 5 pour plate preparations were made of 0.1 ml. quantities of the undiluted treated suspensions. After incubation for 2 hours at 37°C., the seeded pour plate preparations were layered with an approximately equal volume of Levinthal agar containing 2000 μg. per ml. SM and after hardening of the agar overlay, incubated for at least 48 hours at 37°C. The number of colonies formed in the presence of 1000 μg. per ml. SM was then recorded.

Controls were of two types—recipient cells not exposed to the DNA-containing extracts and cells exposed to the extracts treated with DNAse. For DNAse controls, the enzyme was added to the DNA-containing extracts 5 minutes before the addition of recipient cells.

EXPERIMENTAL RESULTS

The present diagnostic criterion of the *influenzae* bacillus group, the requirement for the growth factors X (hemin) and V (di- or triphosphopyridine nucleotide), reflects basic differences in enzyme equipment and therefore in heredity determinants. However, whether this characteristic is the best guide to degree of kinship is open to question.

Preliminary experience with intertype and intraspecies transformation suggested that reactivity of DNA's of recipient cells with those of the donor cells supplying the heredity determinant which controls SM resistance, might also serve as a guide to the degree of kinship between types and species.

The experiments to be reported have resulted in an examination of the specificity of the DNA which controls SM resistance—(*a*) among specific types of *H. influenzae*; (*b*) among strains of non-typable *H. influenzae*; (*c*) among

different serologic groups of *H. parainfluenzae*; and (*d*) among members of the genus *Hemophilus—H. influenzae, H. parainfluenzae,* and *H. suis.*

Intraspecies Induction of SM Resistance

H. influenzae:

The influence of type of origin of donor cells supplying the DNA which induces SM resistance was explored within two groups of organisms, typable and non-typable, classified as *H. influenzae* on the basis of the need for both

TABLE I

Intraspecies Transformation—Type-Specific H. influenzae

Proportion of populations of (R) *H. influenzae* of type-specific origin in which resistance to SM (1000 μg. per ml.) is induced by DNA's from homotype and heterotype SM-Resistant *H. influenzae.*

Recipient population	Experiment	Induced SM-resistant cells per 10^8 SM-sensitive cells					
		Transforming agents—source of DNA_{SM1000}*					
		S*a*	S*b*	S*c*	S*d*	S*e*	S*f*
R*a*	I	103	31	40	52	109	92
	II	11	4	2	3	10	4
R*b*	I	6,500	12,000	8,000	9,200	4,700	4,000
	II	1,200	5,600	1,700	3,400	1,500	1,400
R*d*	I	20,300	48,500	33,500	37,000	21,000	17,000
	II	20,200	55,500	30,600	71,800	25,900	18,300
R*e*	I	22	13	6	10	68	11
	II	16	17	10	27	90	36

S, Type-specific *H. influenzae.* Letters after S refer to type.
R, non-encapsulated *H. influenzae.* Letters after R refer to type of origin.
SM1000, resistance to 1000 μg. per ml. streptomycin.
* Crude DNA-containing extract.

hemin (X factor) and di- or triphosphopyridine nucleotide (V factor) for growth. The two groups were represented by: (*a*) the 6 specific types of *H. influenzae, a, b, c, d, e,* and *f,* and (*b*) R strains (of non-typable *H. influenzae*) isolated from the human blood or nasopharynx.

1. Transformation among specific types of H. influenzae.—Transforming agents were derived from the 6 specific types of *H. influenzae.*

Populations of non-type-specific cells (R) propagated from colonies arising spontaneously during artificial cultivation of types *a, b, d,* and *e H. influenzae* were used as the recipient cells and designated R*a*, R*b*, R*d*, and R*e* (Table I). As reported earlier (7), induction of SM resistance has not been demonstrated

in R cells derived from types *c* and *f*. In each case aliquots of a single population were simultaneously exposed to the 6 DNA-containing extracts derived from SM-resistant cells of each of the 6 types. The treated SM-sensitive cells were then examined for the number of colonies rendered resistant to 1000 μg. per ml. SM by the method described above.

The results listed in Table I are in support of previous observations that the type of origin of recipient cells plays an important role in determining the frequency of induced SM resistance in *H. influenzae* cells (7). Of less importance is the donor source of transforming agent. Homologous and heterologous type transforming agents (DNA's) do not appear to differ greatly in their transforming activity, although homologous type transformations, in general, insure the highest number of transformed cells. Some recipient populations show the same quantitative response to certain heterologous type DNA's as to homologous type DNA. For example, in R*a* populations the DNA's from types *e* and *f* induce resistance in the same number of cells as does the DNA from type *a*, and in R*b* and R*d* populations there is no significant difference between the action of DNA from type *b* and type *d*. On the other hand, in R*a* populations the DNA's from types *b*, *c*, and *d* differ from the action of the homologous type DNA; this is also true in R*b* and R*d* for the heterologous DNA's from types *a*, *e*, and *f*. The order of difference, although low, appears to be consistent and not due to any variables known to influence the proportion of cells in which resistance can be induced (7). The variability in the numbers of resistant cells formed in different experiments on the same recipient strain exposed to the same DNA preparation may be a result of differences in the cultural conditions used for the production of recipient populations. Although an attempt was made to control known variables, it was impossible to establish identical conditions in every experiment. However, the variability noted is of no significance when aliquots of a single population are treated simultaneously with each of the DNA's.

The importance of the specific type of origin of the recipient population for the demonstration of transformation to heterologous types of *H. influenzae* has been reported (7). Heterologous type transformation was not demonstrated in R*a* and R*e* populations. Transformation to types *ab*, *b*, *c*, and *d* only has been demonstrated in R*b* populations; all six specific types have been induced in R*d* populations. These limitations in intertype transformations refer to our experience with transforming agents derived from type-specific cells of natural origin (cerebrospinal fluid, nasopharynx, or blood). The type of origin of the DNA used for inducing SM resistance does not seem to limit the interaction of the DNA among heterologous types. No satisfactory explanation for this difference in behavior of the two varieties of transforming agents is offered at present. There is reason to believe that an inhibitory mechanism plays some role in limiting heterologous type-specific intertype transformations. For ex-

ample, the induction of type *a* cells in R*b* populations exposed to DNA derived from natural type *a* has not been demonstrated. However, when R*d* cells are transformed to type *a*, the DNA from the transformed type *a* can readily induce type *a* in R*b* populations (8). These results suggest that the inducing DNA may be changed by a cell as a result of incorporation as a heredity determinant and that the failure to demonstrate the induction of the above heterologous type specificities may be related to differences in structure of the DNA's which fail to interact.

TABLE II

Intraspecies Transformation—(R) H. influenzae

Proportion of populations of (R) *H. influenzae* of unknown type in which resistance to SM (1000 μg. per ml.) is induced by DNA's derived from homostrain and heterostrain SM-resistant (R) *H. influenzae.*

Recipient populations		Induced SM-resistant cells per 10^8 SM-sensitive cells			
		Transforming agents—source of DNA_{SM1000}*			
R strain	Source	Strain 4	Strain 5	Strain 6	S*d*
1	N.P.	6,900	12,500	2,490	2,230
2	N.P.	748	5,600	817	521
3	Blood	7,880	44,670	6,570	4,240
4	N.P.	13,910	7,520	8,260	4,460
5	N.P.	213	3,400	185	171
6	Trachea	0	0	0	0
R*d*	S*d*	9,800	20,900	21,000	24,000

N.P., nasopharynx.
S*d*, type *d H. influenzae.*
R*d*, non-encapsulated (R) strain derived from type *d*, included for comparison.
SM1000, resistance to 1000 μg. per ml. SM.
0, no evidence of transformation.
* DNA, crude DNA-containing extract.

2. Transformation among Non-Type-Specific (R) Strains of H. influenzae.—Strains of *H. influenzae* found in the majority of normal individuals during the respiratory infection season have been shown to be a heterologous group serologically, although cross-reactions demonstrated by agglutination suggest that they possess antigens in common. Whether these organisms represent R variants of the 6 specific types, *a*, *b*, *c*, *d*, *e*, or *f* or of types still unrecognized or whether they represent a different species which happens to require X and V is unknown. The comparison of the action of heterologous strain SM resistance DNA with the effect of homologous strain DNA which controls SM resistance has been used as a tool to explore the relationship of these *H. influenzae* populations. Aliquots of a single population of each recipient strain (Table II) were

exposed simultaneously to the DNA extracts of SM-resistant cells of each of the 3 donor strains, 4, 5, and 6. The DNA from SM-resistant type *d* cells was included for comparison. The treated populations were then compared for the number of cells which could form colonies in SM-containing agar (1000 μg. per ml.). The results of this comparison are expressed as the number of cells rendered SM-resistant in a population of 10^8 SM-sensitive cells.

The recipient populations were exposed to the transforming agents when they reached the late logarithmic or early stationary phase of growth and a population size which under these circumstances can be expected to contain significant numbers of cells susceptible to transformation. Susceptible cells appear to be absent in 10^8 cells of strain 6.

In strains 4 and 5 the homologous DNA's show a definite advantage over the heterologous DNA's. The action of SM resistance DNA from strains 5, 6, and type *d* on the R*d* population is quite similar. A close relationship is suggested.

The greater efficacy of the transforming agent extracted from strain 5 resistant cells is apparent from the results produced on recipient populations 1, 2, and 3, being more striking in the latter two. This raised the possibility that strains 1, 2, and 3 may be very similar to strain 5. To explore this point a DNA-containing extract was prepared from resistant cells of strain 3 and examined for its capacity to induce SM resistance in strain 3; it was demonstrated in 4 experiments that strain 5 DNA transformed approximately twice as many cells as did strain 3 DNA. Increase in the concentration of the homologous strain DNA did not increase its efficiency. The nature of this phenomenon has not been explored. However, since strain 5 DNA is no more than twofold more efficient than the homologous strain DNA, these findings are believed not to detract from the hypothesis that the degree of kinship between strains may be reflected in the degree of reactivity of their DNA's.

All 6 of the non-typable strains, 1, 2, 3, 4, 5, and 6, have been exposed to the action of DNA-containing extracts, controlling type specificity, from each specific type of *H. influenzae* (*a*, *b*, *c*, *d*, *e*, and *f*). It has not been possible to demonstrate the induction of type specificity in any of these strains. However, the relationship of these strains to each other and to type *d H. influenzae* is suggested by their capacity to transfer SM resistance to each other.

H. parainfluenzae:

Organisms of the *Hemophilus* genus which resemble *H. influenzae* but which require V factor and not X factor have been classified as *H. parainfluenzae*. Organisms which fulfill these criteria were isolated from the nasopharynx of the majority of young adults examined during the winter season (5). Some of these organisms produced iridescent colonies and capsular swelling occurred on exposure to homologous antibody. The serologic distinction among these

iridescent strains was demonstrable by precipitation with antiserum after adsorption of the antisera with heterologous groups; the iridescent strains fell into 3 groups, I, II, and III, and the others which could not be demonstrated to belong to any of these, were classified as Group IV.

Ten different strains of iridescent *H. parainfluenzae* were used as the recipient strains; the serologic grouping is indicated in each case (Table III). DNA-containing extracts were prepared from spontaneously occurring SM-resistant mutants (1000 μg. per ml.) selected from a representative of each

TABLE III

Intraspecies Transformation—Antigenic Groups of H. parainfluenzae

Proportion of populations of four antigenic groups of *H. parainfluenzae* in which SM resistance (1000 μg. per ml.) is induced by DNA's derived from homogroup and heterogroup SM-resistant *H. parainfluenzae.*

Recipient populations		Induced SM-resistant cells per 10^8 SM-sensitive cells			
		Transforming agents—source of DNA_{SM1000}*			
Strain	Serologic group	Group I	Group II	Group III	Group IV
		Strain 1	Strain 4	Strain 5	Strain 6
1	I	2,100	1,050	858	193
2	I	0	0	0	0
3	I	74	76	97	14
4	II	0	0	0	0
5	III	0	0	0	0
6	IV	0	0	0	0
7	IV	36,000	25,700	34,000	9,700
8	IV	418	603	535	220
9	IV	70	50	83	134
10	IV	2	4	4	23

0, no evidence of transformation.

* Crude DNA-containing extract from donor cells resistant to 1000 μg. per ml. Streptomycin (SM).

serologic group; strain 1 from Group I, strain 4 from Group II, strain 5 from Group III, and strain 6 from Group IV. The capacity of these SM resistance DNA's to induce SM resistance in the sensitive recipient population was used to study the specificity of SM resistance DNA's among strains of *H. parainfluenzae.* The results are shown in Table III. The data are obtained from experiments in which aliquots of the same recipient population were exposed simultaneously and individually to each of the 4 DNA's controlling resistance to SM. The treated aliquots were then examined for the number of colonies forming in SM-containing agar. Cells susceptible to transformation appear to be absent in a population of 10^8 cells of strains 2, 4, 5, and 6 since induction

of resistance was not demonstrated. Strains 4 and 5 are the only representatives available for Groups II and III. The number of cells in which SM resistance is induced by the DNA's of Groups I, II, or III origin show a striking similarity. On the other hand the number of SM-resistant cells induced by a DNA derived from a Group IV member is different. These results suggest the need for a re-examination of the criteria for dividing Groups I, II, and III, and further exploration of the group labelled as IV. Strains 7 and 8 bear reinvestigation of their classification as Group IV.

Interspecies Induction of Streptomycin Resistance

Three species of *Hemophilus*, *H. influenzae*, *H. parainfluenzae*, and *H. suis* were selected to explore the specificity of the DNA which controls SM resistance among different species of the genus *Hemophilus*. Table IV presents the results obtained when recipient populations of the three species were exposed to transforming agents which induce SM resistance, derived from homologous and heterologous species. The figures offer a comparison of the number of cells in which SM resistance is induced by these transforming agents.

Aliquots of a single recipient population were exposed simultaneously to DNA's which control SM resistance derived from 9 different sources: the 6 specific types of *H. influenzae*, a non-typable strain of *H. influenzae* (strain 4), a strain of *H. parainfluenzae* (strain 1) and a strain of *H. suis*. The data of Table I were selected from the data of Table IV.

From these results a measure of the proportion of cells in which SM resistance is induced would seem to reflect the kinship between the cells from which the transforming agent is derived and the recipient cells. There appears to be a close one between the type-specific strains and the non-typable strain of *H. influenzae*. While the data indicate that there are differences among types, they suggest that *H. influenzae* strains, typable or non-typable, are more closely related to one another than to *H. parainfluenzae*. These results therefore parallel the criterion of need for X and V growth factors.

The failure to demonstrate induction of SM resistance in R*a*, R*e*, and *H. suis* populations exposed to heterologous species DNA may be a reflection of the low proportion of susceptible cells as is shown on exposure to homologous type DNA, rather than the lack of properties which permit the interaction of DNA's of the host cell and those of the heterologous species. If the degree of reactivity with heterologous DNA's which induce SM resistance may be used as an index of kinship, then the strain of *H. parainfluenzae* used is not closely related to the strain of *H. suis* used even though both strains require V factor but not X factor for growth. Interaction of the DNA's of these two species has not been demonstrated.

We have been unable to induce the character of independence of X factor for growth in *H. influenzae* populations exposed to *H. parainfluenzae* DNA-

containing fractions. Repeated subculturing in the presence of the fractions has failed to reveal the presence of cells which parallel *H. parainfluenzae* in their requirement of V factor but not X factor for growth.

TABLE IV

Comparison of Homologous and Heterologous Species Transformation within Genus Hemophilus Induction of Resistance to 1000 μg. per ml. Streptomycin (SM)

Recipient populations	Experiment	Induced SM-resistant cells per 10^8 SM-sensitive cells								
		Transforming agents—source of DNA_{SM1000}*								
		H. influenzae							*H. parainfluenzae*	*H. suis*
		Sa	Sb	Sc	Sd	Se	Sf	Non-typable (Strain 4)	Strain I	
H. influenzae										
Ra	I	103	31	40	52	109	92	20	0	0
	II	11	4	2	3	10	4	1	0	0
Rb	I	6,500	12,000	8,000	9,200	4,700	4,000	2,100	92	3
	II	1,200	5,600	1,700	3,400	1,500	1,400	697	41	1
Rd	I	20,300	48,500	33,500	37,000	21,000	17,000	12,400	80	7
	II	20,200	55,500	30,600	71,800	25,900	18,300	17,500	174	9
Re	I	22	13	6	10	68	11	6	0	0
	II	16	17	10	27	90	36	17	0	0
Non-typable (strain 4)	I	9,900	7,200	4,900	6,900	7,500	6,400	14,000	304	5
	II	6,800	4,200	3,200	3,900	5,700	6,100	18,300	293	5
H. parainfluenzae strain 1	I	2	1	2	3	2	2	1	830	0
	II	31	21	22	27	19	20	25	5,720	0
H. suis	I	0	0	0	0	0	0	0	0	100
	II	0	0	0	0	0	0	0	0	379

S, encapsulated *H. influenzae*. Letter after S refers to type.
R, non-encapsulated *H. influenzae*. Letter after R refers to type of origin.
Non-typable *H. influenzae*, strain 4 listed in Table II.
Strain 1, *H. parainfluenzae*, strain 1 listed in Table III.
* DNA_{SM1000}, transforming agent derived from cells resistant to 1000 μg. per ml. SM.

The numbers of *H. parainfluenzae* cells transformed to SM-resistant by the 7 different types of *H. influenzae* DNA's listed in Table IV are strikingly uniform, the variability being 2 to 3 in Experiment I and 19 to 31 in Experiment II. These data suggest that the proportion of cells rendered SM-resistant in heterologous species transformations is independent of the type or strain source of the heterologous DNA.

To confirm this suggested independence of the type or strain source of the DNA which induces SM resistance in heterospecies transformation, recipient *H. influenzae* populations were exposed to the transforming agents derived from the 4 groups of *H. parainfluenzae*. Four strains of *H. influenzae* were used—R*b*, R*d*, and strains 3 and 4 of the non-typable recipient populations listed in Table II. The results are given in Table V. The transforming agents derived from each of the 4 groups of *H. parainfluenzae* induce SM resistance in each of these *H. influenzae* populations and the proportion of transformants appears to be independent of the antigenic group from which the heterologous species transforming agent is derived.

The ratio of the number of cells transformed by heterologous species DNA to the number transformed by homologous type DNA has been calculated from the data in Table IV. In Table VI this ratio is expressed as the per cent of the number of cells transformed by homologous type DNA that are transformed by heterologous species DNA. The ratios are seen to be relatively constant for a given recipient population in each of the two experiments. In a total of 11 experiments in which R*d* cells of *H. influenzae* were exposed to *H. parainfluenzae* (strain 1) DNA, the range of variability of the ratio, expressed as per cent, was 0.1 to 0.4 per cent, with an average of 0.2 per cent. The same range of variability (0.1 to 0.4 per cent) was demonstrated when populations of strain 1 of *H. parainfluenzae* were exposed to *H. influenzae* (R*d* or S*d*) DNA; in a total of 7 experiments only one gave a value outside this range (0.006 per cent).

The relative constancy of the heterospecies-homotype ratio from experiment to experiment suggests that there is a relatively fixed probability of a given heterospecies transformation, at least for SM resistance, for a given recipient population. The magnitude of the probability may be a reflection of the closeness of kinship between the recipient and donor species and thus might offer a criterion of relationship of bacteria. By this criterion, the non-typable strain of *H. influenzae* is more closely related to *H. parainfluenzae* than are strains R*b* and R*d*, and *H. influenzae* and *H. parainfluenzae* are genetically closer to each other than either is to *H. suis*.

We have failed to demonstrate the induction of SM resistance in *H. influenzae* and *H. parainfluenzae* populations exposed to the SM resistance DNA derived from pneumococcus type 29 or meningococcus type I SM-resistant cells.

Since the specificity of the SM resistance DNA of one species for cells of another species is of a low order, it was of interest to determine whether the specificity of the same DNA changed after its incorporation and replication in cells of a heterologous species.

For this purpose, DNA-containing extracts were made from the progeny of *H. influenzae* cells (R*d*) in which resistance was induced by *H. parainfluenzae* DNA (strain 1), and from the progeny of cells of *H. parainfluenzae* (strain 1) in which resistance was induced by *H. influenzae* DNA (S*d*).

TABLE V

Influence of Strain and Group Origin of DNA_{SM1000} from H. parainfluenzae on the Proportion of H. influenzae Cells in Which Streptomycin (SM) Resistance is Induced

Recipient populations of *H. influenzae**		Induced SM-resistant cells per 10^8 SM-sensitive cells			
		H. parainfluenzae DNA_{SM1000} ‡			
Strain	Source	Group I	Group II	Group III	Group IV
		Strain 1	Strain 4	Strain 5	Strain 6
R*d*	S*d*	97	77	79	87
R*b*	S*b*	13	13	13	16
4(R)	NP	41	44	52	36
3(R)	Blood	12	12	11	31

S, encapsulated *H. influenzae*. Letter after S refers to type.

R, non-encapsulated *H. influenzae*. Letter after R refers to type of origin.

3(R), 4(R), non-typable *H. influenzae* of unknown type of origin.

NP, nasopharynx.

* Strains used in data for Table I and II.

‡ Crude DNA-containing extract from donor cells resistant to 1000 μg. per ml. SM.

TABLE VI

Ratio (Expressed as Per Cent) of Number of Cells Transformed to SM-Resistant by Heterologous Species DNA_{SM} to Number Transformed by Homologous Type DNA_{SM}

Recipient populations	Experiment	Induced SM-resistant cells per 10^8 SM-sensitive cells		
		Transforming agent—Source of DNA_{SM1000}*		
		H. influenzae	*H. parainfluenzae*	*H. suis*
H. influenzae				
R*b*	I	12,000	92(0.8%)‡	3(0.02%)‡
	II	5,600	41(0.7%)	1(0.02%)
R*d*	I	37,000	80(0.2%)	7(0.02%)
	II	71,800	174(0.2%)	9(0.01%)
Non-typable (strain 4)	I	14,000	290(2.0%)	5(0.04%)
	II	18,300	293(1.6%)	5(0.03%)
H. parainfluenzae strain 1	I	2–3(0.3%)	830	0
	II	19–31(0.4%)	5720	0
H. suis	I	0	0	100
	II	0	0	302

Data are from Table IV.

R, non-encapsulated *H. influenzae*. Letter after R refers to type of origin.

Strain 4, non-typable strain of *H. influenzae* listed in Table II.

Strain 1, *H. parainfluenzae*, strain 1 listed in Table III.

* DNA_{SM1000}, transforming agent derived from cells resistant to 1000 μg. per ml. SM.

‡ $\%, \frac{\text{No. of cells induced by heterologous species DNA}}{\text{No. of cells induced by homologous type DNA}} \times 100.$

Aliquots of populations of *H. influenzae* (R*d*) and *H. parainfluenzae* (strain 1) were simultaneously exposed to these transforming agents as well as to those of natural heterologous species and of homologous strain origin. The results are given in Table VII. The two recipient populations were examined simultaneously for both experiments. The DNA's of heterospecific transformation origin in Experiments I and II differ for each recipient population in that they are derived from two independent clones.

TABLE VII

Comparison of the Transforming Efficacy of H. influenzae and H. parainfluenzae DNA_{SM1000} *before and after Their Replication in Cells of the Heterologous Species*

DNA_{SM1000}	Induced SM-resistant cells per 10^8 SM-sensitive cells			
	Recipient populations			
	H. influenzae (R*d*)		*H. parainfluenzae* (Strain 1)	
	Experiment			
	I*	II*	I*	II*
H. influenzae				
Rd_{SM}	11,700	40,000	5	8
$Rd_{SM(H.p.i.)}$	26,600	70,000	85	91
H. parainfluenzae				
Strain 1_{SM}	41	73	5,500	7,600
Strain $1_{SM(H.i.)}$	143	460	5,300	8,000

SM, streptomycin.

DNA_{SM1000}, transforming agent derived from cells resistant to 1000 μg. per ml. SM.

$Rd_{SM(H.p.i.)}$, strain R*d* rendered SM-resistant with exposure to *H. parainfluenzae* (strain 1) DNA_{SM1000}.

Strain $1_{SM(H.i.)}$, strain 1 rendered SM-resistant with exposure to *H. influenzae* DNA_{SM1000} (type *d* strain from which strain R*d* isolated).

* Experiments I and II differ in that progeny of two independently induced SM-resistant cells of heterospecific transformation origin were used.

Cells transformed by a heterologous species SM resistance DNA yield transforming agents that are approximately 4- to 15-fold more efficient in heterospecific transformation than are heterologous species DNA's of natural origin. However, the data indicate that in general, the DNA's of heterospecific transformation origin are similar in their specificity to the SM resistance transforming agent of the parental recipient population and not to that of the donor heterologous species.

Transforming agents were also derived from *H. influenzae* cells rendered SM-resistant with exposure to DNA *H. parainfluenzae* (strain $1)_{SM(H.i)}$ and from *H. parainfluenzae* cells in which resistance was induced by DNA *H. in-*

fluenzae $(Rd)_{SM(H \cdot p \cdot i)}$. (Table VII). Exposure of the two recipient populations, *H. influenzae* (R*d*) and *H. parainfluenzae* (strain 1) to these "second step" transforming agents did not increase further the proportion of heterospecific transformed cells.

DISCUSSION

The specificity of the desoxyribonucleic acid (DNA) which induces streptomycin (SM) resistance has been explored within and among species of *Hemophilus* by comparing the number of cells in 10^8 populations of SM-sensitive cells in which this trait can be induced when the recipient and donor cells vary in degree of kinship. The degree of kinship used as a standard is based on the criteria currently used for bacteriologic classification. The distinctive criterion of comparison between *H. influenzae* on the one hand and *H. parainfluenzae* on the other is the difference in enzyme equipment which makes X factor (hemin) a necessary growth factor for *H. influenzae* but not for *H. parainfluenzae*. Our unpublished observations have demonstrated that *Hemophilus* strains of swine origin vary in their growth requirements. Some require both X and V factors; others grow in the absence of X factor. The *H. suis* strain used in experiments reported in this paper exhibited the latter trait.

The data presented indicate that criteria currently used for distinguishing the three species investigated are of practical value, but that an even more sensitive index to the species identity or relationship of strains of *Hemophilus* may be the degree of reactivity of their respective DNA's. This latter method for examining relationships not only reveals degrees of kinship among species but also among groups or types within a species. Application of the method indicates that *H. influenzae*, *H. parainfluenzae*, and *H. suis* are distinct groups, since the proportion of transformants in which SM resistance is induced by a heterologous species DNA is low relative to the proportion induced by a homologous species DNA. Moreover, if it is true that the degree of reactivity of DNA's may be accepted as a criterion of relationship, then *H. influenzae* is more closely related to *H. parainfluenzae* than to *H. suis* and the latter two species are only distantly related since it has not been possible to demonstrate reaction between their DNA's.

Our observations on the specificity of the DNA which induces SM resistance permit the following generalizations:—

1. The proportion of cells in which SM resistance is induced is greatest when the recipient cells are of the same strain or type as that from which the transforming agent, presumably DNA, is derived. However, certain types or groups within a species yield a SM resistance DNA which does not differ in its reactivity from that of the type or group to which the recipient population belongs.

2. There is a low degree of reactivity between DNA's of heterologous species.

Even though the mechanisms responsible for these degrees of specificity or reactivity of the DNA unit (molecule or part) which induces SM resistance are unknown, the data justify certain speculations. These degrees may be a reflection of the degree of homology of the genomes of the recipient and donor populations. Closely related cells may possess many structurally similar or homologous DNA units. On the other hand, more distantly related cells presumably have fewer of these DNA units in common.

When cells are not closely related the DNA units which control other traits or those which may be inert as heredity determinants may play an inhibitory or interfering role in the process of transforming a cell to SM-resistant. If the DNA unit which induces SM resistance is similar in each of the species of *Hemophilus* studied, its incorporation or expression of function within the host cell might be inhibited by the presence of one or more incompatible or non-homologous DNA units within the same receptor cell.

Demonstration of the transfer of linked hereditary factors in the transformation of pneumococci (9) and possibly *H. influenzae* (8) raises the possibility that one or more incompatible DNA units linked to one or more compatible ones might inhibit the successful incorporation of the latter into the genome of the host cell. An intracellular break in the linkage, with subsequent genetic exchanges might, however, eliminate the inhibitory effect of incompatible DNA units; the incorporation of compatible units might thus depend upon breaks and exchanges at particular loci. The frequency of such exchanges would depend on the probability of breaks at these loci. Such a process may explain, for a given recipient population, the relatively fixed homo-heterospecies transformation ratio which has been demonstrated in interspecific studies with *H. influenzae* and *H. parainfluenzae.*

Alternatively, if the DNA unit controlling SM resistance differs among and within the species of *Hemophilus* studied, one of two possibilities would obtain. The receptor cell must alter the unit so that it conforms to that of the host cell, or the cell must accept or incorporate the heterologous DNA unit.

Evidence has been presented which indicates that SM resistance DNA's derived from cells transformed by heterologous species DNA's are approximately 4- to 15-fold more efficient in heterospecies transformation than are heterologous species DNA's of natural origin. Whether this increase in the proportion of transformed cells is a reflection of an alteration of the heterologous species DNA unit by the host cell in the process of being incorporated as a heredity determinant, or another change in the forces responsible for the low order of reactivity of heterologous species SM resistance DNA units, cannot be answered at present.

SUMMARY

Streptomycin resistance of a high degree has been induced in sensitive populations of *Hemophilus influenzae* and *Hemophilus parainfluenzae* by desoxy-

ribonucleic acids (DNA's) derived from streptomycin (SM)-resistant cells of at least one heterologous species[2] of *Hemophilus*.

The specificity of the DNA which controls SM resistance has been studied within and among species of *Hemophilus* by comparing, in a given population, the proportion of cells transformed to SM-resistant by DNA's derived from highly resistant cells of heterologous type or species with the proportion changed by the DNA derived from SM-resistant cells of the homologous type or species. The ratio resulting from this comparison correlates in general with the degree of kinship between recipient and donor cells suggested by accepted methods of bacteriologic classification. The numerical value of the ratio is much lower when the species of the recipient population and donor of the DNA differ than when they are of the same species. The data suggest that this ratio is of value as an index of degree of kinship of recipient and donor cells.

Comparison of the activity of heterologous and homologous DNA's shows differences within species and degrees of differences among species not brought out by other available methods. The data suggest that *H. influenzae* is more closely related to *H. parainfluenzae* than to *H. suis* and that the relationship between *H. parainfluenzae* and *H. suis* is remote.

Within the species *H. influenzae* and *H. parainfluenzae* the ratio of heterospecific transformants to homospecific transformants appears to be relatively constant for a given recipient population. This ratio also appears to be independent of the type or group source of the heterologous species SM resistance DNA.

The low proportion of cells in *H. influenzae* populations which are transformed to SM-resistant by DNA's derived from SM-resistant *H. parainfluenzae* and *vice versa* has been increased 4- to 15-fold by the replication of the heterologous species SM resistance DNA in the heterologous species. An alteration of the heterologous DNA by the host is suggested.

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[2] It is important to emphasize here that the word "species" as applied to bacteria does not have the same connotation as when applied to higher organisms.