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EFFECT OF INTERSPECIFIC TRANSFORMATION ON LINKAGE RELATIONSHIPS OF MARKERS IN *HAEMOPHILUS INFLUENZAE* AND *HAEMOPHILUS PARAINFLUENZAE*

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ABSTRACT

NICKEL, LOIS (University of Pennsylvania, Philadelphia), AND SOL H. GOODGAL. Effect of interspecific transformation on linkage relationships of markers in *Haemophilus influenzae* and *Haemophilus parainfluenzae*. J. Bacteriol. **88**:1538–1544. 1964.—Highly competent cells of *Haemophilus parainfluenzae* were obtained by a modification of the aerobic-"anaerobic" method of Goodgal and Herriott. The mutant markers for streptomycin and novobiocin resistance in *H. parainfluenzae* were shown to be unlinked. These same markers were transferred into *H. influenzae* in two transformation steps, and the deoxyribonucleic acid (DNA) was extracted. Transformation of recipient *H. influenzae* with DNA from the latter showed the markers to be linked, and transformation of recipient *H. parainfluenzae* with this DNA showed that the markers were not cotransferred. The linked streptomycin- and novobiocin-resistance mutations of *H. influenzae* were transformed into *H. parainfluenzae* in two stages, and the DNA was extracted. Transformation of recipient *H. parainfluenzae* with this DNA demonstrated the markers to be unlinked in *H. parainfluenzae*. When these markers, originally from *H. influenzae* and present as unlinked factors in *H. parainfluenzae*, are transferred back into *H. influenzae*, the markers showed their original linkage. The novobiocin- and erythromycin-resistance markers of *H. influenzae* were unlinked in *H. influenzae*, but were linked when transferred into *H. parainfluenzae*. The erythromycin-resistance marker of *H. influenzae*, when transferred into a novobiocin-resistant mutant of *H. parainfluenzae*, showed linkage with it. Evidence is presented to show that *H. parainfluenzae* streptomycin and novobiocin markers are homologous to streptomycin and novobiocin markers in *H. influenzae*.

Interspecific transformation between *Haemophilus influenzae* and *H. parainfluenzae* was reported by Shaeffer and Ritz (1955) and by Alexander and Leidy (1955). In addition, Leidy et al. (1956) demonstrated interspecific transformation among a number of species of *Haemophilus*.

This paper deals with the effects of interspecific transformations on the linkage of various antibiotic-resistance markers of *H. influenzae* and of *H. parainfluenzae*. The experiments were also designed to gain evidence for the extent of homology between *H. influenzae* and *H. parainfluenzae* chromosomes, to compare the linkage of various markers in *H. parainfluenzae* with those of *H. influenzae*, and to determine whether linkage relationships of the markers change depending upon the species of recipient cell in which they are present. Linked markers are defined as markers that are transferred together on the same deoxyribonucleic acid (DNA) particle in the process of transformation. Two markers in a double transformation are defined as nonlinked when two integrated markers come from different DNA particles.

Linkage of markers between species has been investigated by Ravin and De Sa (1964), using *Diplococcus pneumoniae* and *Streptococcus*. When streptomycin (S) or erythromycin (E) mutant markers of *D. pneumoniae* were transformed into a strain of *Streptococcus*, they showed linkage with the *Streptococcus* mutant E or S markers, and high-level S mutations of *D. pneumoniae* were allelic with low-level S mutations of two *Streptococcus* strains.

MATERIALS AND METHODS

Microorganisms. The strains used were *H. parainfluenzae* "colony 14" (kindly provided by G. Leidy) wild type (abbreviated P^+) and mutants and transformants obtained from it, and *H. influenzae* rough strain type d (abbreviated

Rd) wild type (obtained originally from G. Leidy and H. Alexander) and mutants and transformants obtained from it.

Media. Bacteria were grown and transformed in BBL Brain Heart Infusion (BHI). *H. influenzae* strains require the addition of hemin and nicotinamide adenine dinucleotide (NAD); *H. parainfluenzae* strains require the addition of NAD. Plating was done in 1.2% BBL BHI Agar.

Transforming DNA. DNA was prepared by the method of Goodgal and Herriott (1961); in some instances, however, only one ethanol precipitation was done. Cells were initially lysed with 1% (w/v) sodium dodecyl sulfate.

Preparation of competent cells. Highly competent cultures of *H. influenzae* were grown with the use of the Cameron variation of the aerobic-"anaerobic" method of Goodgal and Herriott (1961). Highly competent cultures of *H. parainfluenzae* were obtained by growing the cells at 36 to 37 C aerobically to a turbidity of about 0.32 in a Coleman Junior spectrophotometer or 160 in a Klett-Summerson colorimeter, then by setting the flasks without agitation at 37 C for 8 hr. Then 15% glycerol was added, and the cultures were stored at −70 C.

Transformation procedure. To 1.8 ml of BHI (containing 2 μg/ml of NAD with or without 10 μg/ml of hemin) was added 0.1 ml of the desired dilution of DNA; 0.1 ml of competent cells was added to the tube. Controls without DNA and without cells were also run. A viable count of the initial inoculum was also made. The tubes were shaken in a water bath (35 to 37 C) for 30 min, and then 1 μg/ml of deoxyribonuclease was added to each tube. *H. influenzae* recipient cells were shaken for an additional 1.5 hr, *H. parainfluenzae* recipients for an additional 2.5 hr. At the end of the shaking period, appropriate dilutions of cells were made into petri dishes, and agar containing the desired amount of antibiotic was added to the plates. The plates were incubated at 37 C for 24 to 48 hr.

When the DNA was used at various concentrations, it was diluted in citrate-saline (0.15 M NaCl and 0.014 M citrate), and then 0.1 ml of each dilution was added to a separate test tube containing 1.8 ml of BHI plus 0.1 ml of competent cells.

Calculation of random doubles. Theoretical random doubles (the number of double transformants due to random incorporation of singly marked DNA particles) were calculated by the method of Goodgal and Herriott (1961). This method was applicable to an overlay procedure, i.e., where the cells were plated and allowed to express *in situ* and were then challenged with a selective agent. In the pour-plate method, the transformants are permitted to express before plating with the selective agent. Empirically, it has been found that the same number of competent cells may be obtained by dividing by 8 the viable count at 2 hr for *H. influenzae* or at 3 hr for *H. parainfluenzae.*

Antibiotics. The antibiotics used in this study were streptomycin, novobiocin, erythromycin, and kanamycin. Organisms resistant to these materials are termed mutants to S, N, E, or K resistance, respectively.

Nomenclature for interspecific transformation. *H. influenzae* strains are indicated by Rd; *H. parainfluenzae* by P. Interspecific transformations are indicated by the superscript "t" after the mutant marker transformed. Intraspecific transformations are indicated by the subscript "t" after the mutant marker transformed. Mutants are indicated by the subscript "m". The transformation procedure is indicated by a multiplication sign between cells and the DNA (DNA is in parentheses).

RESULTS

Linkage relationship of mutant S and N markers in H. parainfluenzae. It was necessary to know the linkage relationship of mutant S and N resistance markers in *H. parainfluenzae* before the effects, if any, of interspecific transformation on linkage could be ascertained. Wild-type cells of *H. parainfluenzae* (P^+) were transformed with various concentrations of PS_mN_t DNA. (PS_mN_t = an *H. parainfluenzae* streptomycin-resistant mutant also resistant to novobiocin, the N marker having come from DNA extracted from an *H. parainfluenzae* novobiocin-resistant mutant via transformation.) The data (Fig. 1) are in accord with the nonlinkage of the S and N markers. The ratio of SN/S transformants varies in the linear portion of the DNA concentration curve, and the number of SN transformants is within the range of that expected from random interaction of singly marked molecules. The SN transformants follow the shape of the curve for random SN transformants rather than for single S transformants. This finding is what would be expected for two unlinked markers. The experiment was repeated with similar results

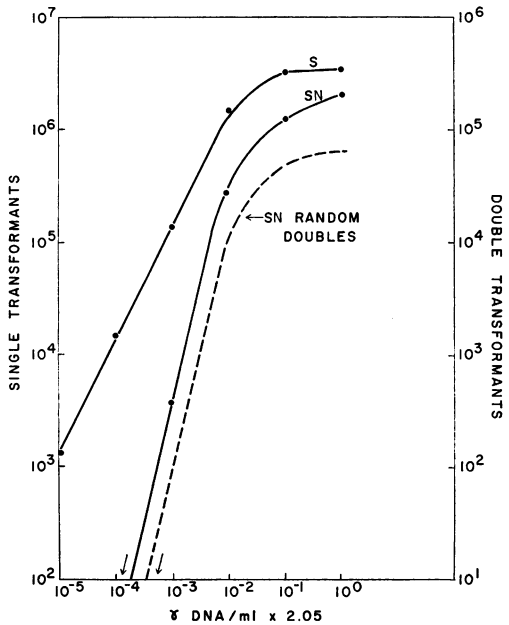

FIG. 1. *Number of S and SN transformants of Haemophilus parainfluenzae as a function of H. parainfluenzae DNA concentration. The recipient cells were H. parainfluenzae wild type and the donor was* PS_mN_{t2}. *S = streptomycin-resistant transformants; SN = transformants resistant to both streptomycin and novobiocin; SN random doubles = theoretical streptomycin and novobiocin resistance transformants. DNA was diluted in 0.15 M NaCl and 0.014 M citrate, and cells were plated with 200 μg/ml of streptomycin and 10 μg/ml of novobiocin.*

with a different PS_mN_t DNA preparation. It was concluded from these data that the S and N resistance mutant markers transferred to *H. parainfluenzae* are unlinked in *H. parainfluenzae*.

Transfer of H. parainfluenzae S and N mutant markers to H. influenzae and effects on linkage. The transformant RdS^tN^t was acquired by two transformations: (i) $RD^+ \times (PS_m)$ yielding RdS^t, and (ii) $RdS^t \times (PN_m)$ yielding RdS^tN^t. DNA was extracted from RdS^tN^t cells, purified, and used to transform cells of *H. influenzae* and *H. parainfluenzae*. When 3 μg/ml of DNA were used, the markers showed approximately 30% linkage in recipient cells of *H. influenzae;* in recipient cells of *H. parainfluenzae*, the level of SN double transformants was less than 0.6%.

When RdS^tN^t DNA was used in the linear region of the DNA concentration curve to transform cells of *H. influenzae*, the S and N markers exhibited linkage as expected (Fig. 2). The ratios of SN/S transformants give an index of co-transfer of about 33%, which is close to that found for SN/S ratios for intraspecific transformation in *H. influenzae*. The number of SN transformants greatly exceeded the expected number of random doubles. It was concluded from these data that, when *H. parainfluenzae* mutant S and N resistance markers which are unlinked in *H. parainfluenzae* are transferred into *H. influenzae* via transformation, and when DNA is extracted from these cells (RdS^tN^t) and is used to transform *H. influenzae* cells, the markers showed linkage. When used to transform *H. parainfluenzae* cells, the markers did not exhibit linkage.

Transfer of H. parainfluenzae mutant S and N resistance markers from H. influenzae to H. influenzae and to H. parainfluenzae. When the *H.*

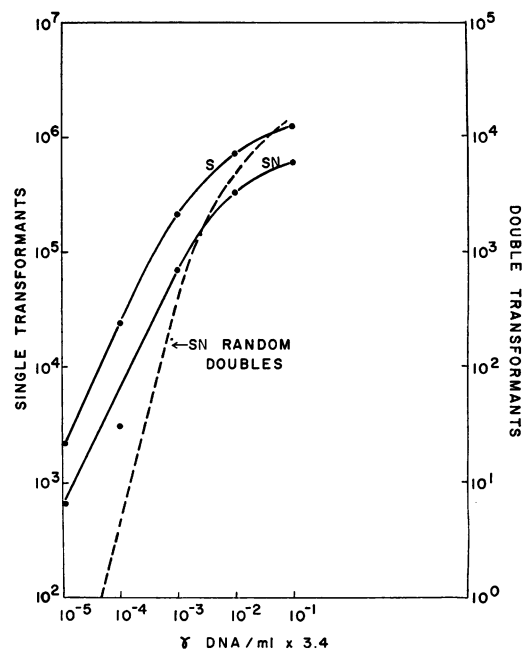

FIG. 2. *Number of S and SN transformants of Haemophilus influenzae as a function of H. influenzae DNA concentration. The recipient cells were H. influenzae wild type and the donor DNA was* RdS^tN^t. *S = streptomycin-resistant transformants; SN = transformants resistant to both streptomycin and novobiocin; SN random doubles = theoretical streptomycin and novobiocin-resistant transformants. DNA was diluted in 0.15 M NaCl and 0.014 M citrate, and cells were plated with 200 μg/ml of streptomycin and 2 μg/ml of novobiocin.*

TABLE 1. *Relation between the number of S and SN transformants in the transformation of Haemophilus influenzae with various concentrations of PS^tN^{25} DNA*

Plate*	Dilution factor	Cells/plate	Cells/ml in transformation mixture	Calculated no. of random doubles
Viable count	5×10^5	143	7.3×10^7	
Viable count at 3 hr	1×10^7	103	1.0×10^9	
Cells + DNA and:				
S	5×10^0	1,412	7.1×10^3	
N	5×10^0	1,848, 1,952	9.5×10^3	
SN	1×10^0	1, 0	0	5.2×10^{-1}
Controls:				
Cells without DNA				
S	5×10^0	0, 1		
N	5×10^0	1, 2		
SN	1×10^0	0, 0		
DNA without cells	1×10^0	0		

* Concentrations of antibiotics used: 2 μg/ml of novobiocin; 200 μg/ml of streptomycin.

parainfluenzae S and N mutant markers in *H. influenzae* were again transferred to *H. influenzae*, the linkage relationship did not change. When the markers were transferred into *H. parainfluenzae*, linkage was not maintained. For both series of experiments, the relationship between DNA concentration and number of transformants was similar to those shown in Fig. 1.

Transfer of H. influenzae mutant S and N resistance markers to H. parainfluenzae and effects on linkage of S and N markers. H. influenzae S and N resistance mutant markers were transferred into *H. parainfluenzae* by means of two transformations: (i) $P^+ \times (RdS_m)$ DNA yielding PS^t, and (ii) $PS^t \times (RdN_{25})$ yielding PS^tN^{25}. When DNA was extracted from the PS^tN^{25} transformant, purified, and used to transform *H. influenzae* cells, there were essentially no SN transformants, indicating nonlinkage of the S and N markers (Table 1).

When cells of *H. parainfluenzae* were transformed with various concentrations of PS^tN^{25} DNA in the linear region of the DNA concentration curve, the data substantiated a nonlinkage of the S and N markers (Table 2). It was concluded that *H. influenzae* S and N resistance mutant markers in *H. parainfluenzae* are shown not to exhibit linkage when either *H. influenzae* or *H. parainfluenzae* is transformed.

Linkage relationship of one H. influenzae marker (S or N) and one H. parainfluenzae marker (S or N) in H. parainfluenzae and H. influenzae. To establish whether there is homology of genetic regions between *H. influenzae* and *H. parainfluenzae*, it was necessary to determine whether mutant *H. influenzae* and *H. parainfluenzae* markers, when put together in one species, established the same linkage relationships as two homologous markers put in that species. If the linkage relationships are the same, the genetic regions can be considered homologous. Linkage relationships of the following strains were tested: PS_mN^t and PS^tN_m (tested in *H. parainfluenzae*), RdS_mN^t and RdS^tN_m (tested in *H. influenzae*). DNA was extracted from these cells and was used to transform either *H. influenzae* or *H. parainfluenzae*. Various concentrations of DNA in the linear region of the DNA concentration curve were used. The results showed that the linkage relationships of the mutant genes in these heterologously marked strains, when transformed into *H. parainfluenzae* or *H. influenzae*, were the same as in the homologously marked strains.

Linkage relationship between H. influenzae mutant E resistance marker and H. parainfluenzae mutant N resistance marker in H. parainfluenzae. When testing for linkage of S and N resistance markers in *H. parainfluenzae*, it was thought advisable to compare the linkage of the S and N markers with that of an outside unlinked marker. The E marker was chosen as a possible unlinked marker in *H. parainfluenzae*, since it had been shown to be unlinked in *H. influenzae*. Linkages of S and N, N and E, and S and E, therefore, were compared. The transformant $PS_mN_tE^t$ was obtained by transforming PS_mN_t cells with *H. influenzae* E_{10} DNA. DNA was extracted from

TABLE 2. *Relation between the number of S and SN transformants in the transformation of Haemophilus parainfluenzae with various concentrations of PS^tN^{25} DNA**

Concn of DNA	Plate	Cells/plate	Cells/ml in transformation mixture	Calculated no. of random doubles	SN/S ratio
μg/ml					
	Viable count	107	1.0×10^9		
	Viable count at 3 hr	133	1.3×10^9		
0.33	S	124, 117	6.0×10^5		
	N	144, 118	6.6×10^5		
	SN	96, 110	2.6×10^4	2.4×10^3	0.04
0.033	S	79, 54	1.7×10^5		
	N	60, 69	1.6×10^5		
	SN	93, 70	2.0×10^3	1.7×10^2	0.01
3.3×10^{-3}	S	66, 58	1.6×10^4		
	N	75, 59	1.7×10^4		
	SN	7, 14	2.6×10^1	1.6×10^0	0.002
3.3×10^{-4}	S	56, 76	1.7×10^3		
	N	58, 70	1.6×10^3		
	SN	2	5.0×10^{-1}	1.6×10^{-2}	0.0003
3.3×10^{-5}	S	44, 37	1.0×10^2		
	N	39, 36	9.4×10^1		
	SN	0		5.7×10^{-4}	

* Concentrations of antibiotics used: 10 μg/ml of novobiocin; 200 μg/ml of streptomycin. All control plates were negative, except cells with novobiocin which had 12 and 11 colonies at a 2.5×10^0 cell dilution.

the $PS_mN_tE^t$ cells, purified, and used to transform *H. parainfluenzae* wild-type cells (Table 3). SN and SE transformants follow the numbers expected for the calculated random doubles for SN, SE, and NE. The NE transformants, however, are directly proportional to the number of S, N, and E single transformants, as in Fig. 3, indicating linkage of *H. parainfluenzae* C and *H. influenzae* E markers. It was concluded from these data that the *H. influenzae* mutant E resistance marker is linked to the *H. parainfluenzae* mutant N resistance marker in *H. parainfluenzae*.

Linkage relationship of H. influenzae N and E mutant resistance markers in H. parainfluenzae. A transformant PN^tE^t was obtained by two transformations: $P^+ \times (RdN_m)$ yielding PN^t, and $PN^t \times (RdE_{10})$ yielding PN^tE^t. DNA was extracted from the PN^tE^t cells, purified, and used to transform *H. parainfluenzae* wild-type cells (Fig. 3). The number of NE transformants above that were expected from random interactions greatly increased with decreasing DNA concentration. The NE transformant curve follows the shape of those for single N or E transformants and not that for NE transformants due to random doubles. It was therefore concluded that *H. influenzae* mutant N and E resistance markers when in *H. parainfluenzae*, exhibit linkage of approximately 7%.

DISCUSSION

These experiments were performed to investigate the effects of interspecific transformation on linkage of S, N, and E resistance markers in *H. influenzae* and *H. parainfluenzae*. It was hoped that these data would offer some information on the organization of the *H. parainfluenzae* chromosome and on the homology between *H. influenzae* and *H. parainfluenzae*.

It was shown that markers for S, N, and E are able to be transferred from *H. influenzae* to *H. parainfluenzae*, indicating homology between these two species for at least some part of the DNA molecules on which the markers are transferred.

Mutant *H. influenzae* S and N resistance markers have previously been shown to be linked (Goodgal, 1961), and the mutant E resistance marker not to be linked to either S or N. In *H. parainfluenzae*, mutant S and N markers are shown not to be linked, but N and E markers are linked. Two possibilities can be postulated for the organization of these markers in the *H.*

parainfluenzae chromosome. Either the N and E loci are in close proximity to each other on the chromosome and S is at some distance from both of them, or there is a specific breakage point between S and N or S and E but not between N and E. It has been shown, however, that if *H. influenzae* S and N markers which are linked (i.e., transferred on the same particle of DNA) are transformed into *H. parainfluenzae* and DNA extracted from such transformed cells is used to transform *H. parainfluenzae*, S and N are shown not to be linked. This finding supports the view that the S and N loci in *H. parainfluenzae* are actually some distance apart on the chromosome.

TABLE 3. *Relation between the number of S and SN, E and SE, N and NE transformants in the transformation of Haemophilus parainfluenzae cells with various concentrations of H. parainfluenzae DNA*

Concn of DNA	Plate plus antibiotic	Cells/ml in transformation mixture	Calculated no. of random doubles	Ratio of doubles to singles
μg/ml				
0.41	S	2.9×10^6		
	N	2.9×10^6		
	E	2.7×10^6		
	SN	1.3×10^5	4.8×10^4	0.044
	SE	1.0×10^5	4.5×10^4	0.04
	NE	3.3×10^5	4.4×10^4	0.12
4.1×10^{-2}	S	1.9×10^6		
	N	1.7×10^6		
	E	1.9×10^6		
	SN	5.7×10^4	1.8×10^4	0.03
	SE	4.1×10^4	2.0×10^4	0.02
	NE	2.6×10^5	1.8×10^4	0.14
4.1×10^{-3}	S	4.5×10^5		
	N	6.3×10^5		
	E	3.8×10^5		
	SN	5.0×10^3	1.6×10^3	0.01
	SE	3.7×10^3	9.8×10^2	0.01
	NE	3.1×10^4	1.4×10^3	0.08
4.1×10^{-4}	S	3.7×10^4		
	N	4.0×10^4		
	E	4.6×10^4		
	SN	3.3×10^1	8.6×10^0	0.001
	SE	3.0×10^1	1.0×10^1	0.001
	NE	1.0×10^3	1.1×10^1	0.02
4.1×10^{-5}	S	2.9×10^3		
	N	4.0×10^3		
	E	5.0×10^3		
	SN	2.5×10^{-1}	6.8×10^{-2}	0.0001
	SE	5.0×10^{-1}	8.3×10^{-2}	0.0002
	NE	1.3×10^2	1.2×10^{-1}	0.03

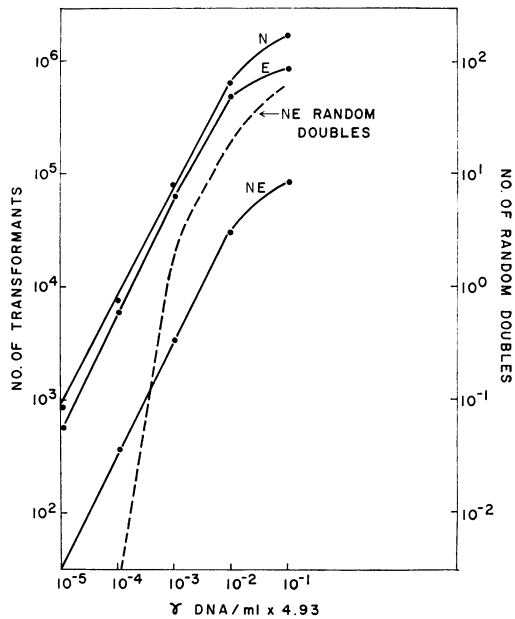

FIG. 3. *Number of N, E, and NE transformants of Haemophilus parainfluenzae as a function of H. parainfluenzae DNA concentration. The recipient cells were H. parainfluenzae wild type and the donor DNA was PN^tE^t. N = novobiocin-resistant transformants; E = erythromycin-resistant transformants; NE = transformants resistant to both novobiocin and erythromycin; and NE random doubles = theoretical novobiocin- and erythromycin-resistant transformants. DNA was diluted in 0.15 M NaCl and 0.014 M citrate, and the cells were plated with 8 μg/ml of erythromycin and 6 μg/ml of novobiocin.*

An alternative, but less simple explanation, would be that the *H. influenzae* DNA particle carrying the S and N markers undergoes a specific breakage between S and N during the process of integration. If several markers not linked to S and N could be found between S and N, this circumstance might at least permit resolution of the question as to whether there is a substantial distance between these two marker. It would not, however, exclude the possibility that there was also a specific breakage point between *H. parainfluenzae* S and N markers or that the *H. influenzae* S and N markers showed a specific breakage during integration.

H. influenzae DNA as prepared in solution has the linkage group SKN and E as an unlinked marker (Goodgal, 1961); *H. parainfluenzae* has two linkage groups, SK (*unpublished data*) and NE. In *H. parainfluenzae*, SK transformants

show linkage but NK does not. If a specific breakage point exists, therefore, it must be between K and N. Whether this breakage occurs during the process of DNA extraction or after the DNA has entered the cell has not yet been established.

Escherichia coli and *Salmonella* have been shown to be related in terms of similar DNA guanosine plus cytosine (GC) content and similar ordering of various genes in their chromosomes as shown by conjugation experiments. Evidence for genetic homology of *H. influenzae* and *H. parainfluenzae* includes their similar nutritional requirements (both require complex media and NAD), similar GC content of DNA, and similar antibiotic-resistance markers (S, K, N, and E). These markers may also have a common order in both species, even though they comprise different linkage groups in the two organisms. This arrangement would be the case if different breakage points existed between the markers in the two strains, or if the map distances between the markers in the two strains varied considerably.

The linkage of two markers in *H. influenzae* and *H. parainfluenzae* is shown by transformation reactions to depend upon the last host in which the markers were located and not upon the linkage relationship of the markers in the original strain; e.g., $Rd^+ \times (PN^tS^t)$ does not show linkage, although S and N were originally linked in *H. influenzae*.

It has been shown that the frequency of transformation of a strain with homologous DNA containing two heterologous markers is of the same order as the frequency of transformation with a completely homologous DNA; e.g., $P^+ \times (PS^tN^t)$ is of the same frequency as $P^+ \times (PS_mN_t)$. This observation is in accord with Shaeffer's (1958) imperfect pairing hypothesis; i.e., once an interspecific transformation has occurred, most of the obstacles to pairing must have been eliminated. Other mechanisms are, of course, not excluded.

The finding by Ravin and De Sa (1964) that a mutation of pneumococcus (S or E resistance marker) showed linkage with a mutation in a streptococcal strain (S or E resistance marker) led to the conclusion that interspecific transformation involved genetic recombination between the host genome and the heterospecific determinant. A similar relationship can also be said to exist in *H. influenzae* and *H. parainfluenzae*, since the *H. influenzae* E mutant marker has been shown to link with the *H. parainfluenzae* N mutant marker in *H. parainfluenzae*, and *H. parainfluenzae* N or S mutant markers show linkage with *H. influenzae* S or N mutant markers, respectively, in *H. influenzae*. As Ravin stated, therefore, loci which control similar functions may pair with each other.

By using the differences in linkage groups between *H. influenzae* and *H. parainfluenzae*, it should be possible to investigate problems dealing with the integration and recombination of genetic markers in the two species.

ACKNOWLEDGMENTS

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LITERATURE CITED

ALEXANDER, H. E., AND G. LEIDY. 1955. Origin of specie in microorganisms: use of transformation as a tool. Am. J. Diseases Children **90**:860.

GOODGAL, S. H. 1961. Studies on transformation in *Hemophilus influenzae*. IV. Linked and unlinked transformations. J. Gen. Physiol. **44**:205-228.

GOODGAL, S. H., AND R. M. HERRIOTT. 1961. Studies on transformation of *Hemophilus influenzae*. I. Competence. J. Gen. Physiol. **45**:1201-1227.

LEIDY, G., E. HAHN, AND H. E. ALEXANDER. 1956. On the specificity of the DNA which induces streptomycin resistance in Hemophilus. J. Exptl. Med. **104**:305.

RAVIN, A., AND J. D. H. DE SA. 1964. Genetic linkage of mutational sites affecting similar characters in pneumococcus and streptococcus. J. Bacteriol. **87**:86-96.

SHAEFFER, P. 1958. Interspecific reactions in bacterial transformation. Symp. Soc. Exptl. Biol. **12**:60-74.

SHAEFFER, P., AND E. RITZ. 1955. Transfert interspecifique d'un caractre hereditaire chez des bacteries du genre Hemophilus. Compt. Rend. **240**:1491.