Interspecific Transformation in Hemophilus; A Possible Index of Relationship Between *H. Influenzae* and *H. aegyptius.** (25151)

GRACE LEIDY, EROS HAHN AND HATTIE E. ALEXANDER
Babies Hospital (Presbyterian Hospital) and Dept. of Pediatrics, Columbia University, College of Physicians and Surgeons, N. Y.

Induction of streptomycin (SM) resistance by desoxyribonucleic acid (DNA) from one SM-resistant *Hemophilus* "species" in a sensitive population of a heterologous species of this genus has been reported by 2 groups of investigators(1,2). The proportion of recipient population transformed by SM-resistance transforming factor (DNA) from donor cells of a heterologous species was only a small fraction of those in which resistance was induced by DNA from donor cells of a homologous species. Schaeffer(3) suggested that the lower frequency of heterospecific transformation is a reflection of imperfect pairing of DNA units due to structural differences in parts of the DNA molecule. A basically similar but less precise explanation has been offered for our results; the degree of reactivity of the DNA's of recipient and donor cells reflects their genetic homology, and therefore might be used as a taxonomic guide(4). When this criterion was applied to *H. influenzae*, *H. parainfluenzae*, and *H. suis*, members of each species appear to belong to distinct groups; *H. influenzae* and *H. parainfluenzae* are more closely related than either is to *H. suis*. This report extends these observations to strains of *H. aegyptius*(5), originally classified as Koch-Weeks bacillus; the results suggest that this organism is closely related to *H. influenzae*. The degree of reactivity of SM-resistance transforming factor, derived from *H. aegyptius* strains, with *H. influenzae* recipient populations and *vice versa* is comparable to the reactivity between types of *H. influenzae*. Pittman and Davis(5) report that the Koch-Weeks bacillus differs sufficiently from *H. influenzae* morphologically, serologically, and by its capacity to agglutinate red blood cells, to warrant separation into a new species, *H. aegyptius*. Previous views on classification of Koch-Weeks bacillus have been reviewed by Pittman.

Results. It has previously been suggested (4) that in a given recipient population of Hemophilus the ratio of number of SM-resistant cells induced by DNA from heterologous type or species to the number transformed by a homologous type or species DNA provides a numerical index of the degree of relationship of recipient and donor cells. When the donor of the DNA is of the same strain or species as the recipient population, for example, different types of *H. influenzae*, the value of this ratio approaches 1 for some types, and may be as low as 0.1 for other types. However, when the SM-resistance transforming factor is derived from a heterologous *Hemophilus* species, the value of this ratio is of a different order of magnitude, varying from 0.01 to 0.0002 depending upon recipient population used and the species under study. This influence of species source of DNA is independent of DNA concentration and competence of the recipient population, and is not due to different rates of expression of transformants or DNA extraction procedure (review, 3).

Experiments similar to those reported earlier(4) have been designed to examine the relationship of *H. aegyptius* to *H. influenzae* and *H. parainfluenzae* by determining the value of this ratio. For this purpose, DNA's containing the SM-resistance factor were prepared from 2 different strains of *H. influenzae*, three different strains of *H. aegyptius*, and 1 strain of *H. parainfluenzae*. We are indebted to Dr. Pittman for the strains classified as *H. aegyptius*. Four strains of *H. influenzae*, three of *H. aegyptius*, and 2 of *H. parainfluenzae* were used as receptor strains. In any experiment, aliquots of the same recipient population were used for comparing the proportion of transformants induced by the SM-resistance transforming factor of different origins.

* This work was supported by grants from NIH.

TABLE I. Comparison of Number of Streptomycin (SM) Resistant Cells in Which Resistance Is Induced by Transforming Factor Derived from 3 "Species" of Hemophilus—*H. influenzae*, *H. aegyptius* and *H. parainfluenzae*.

			Induced SM resistant cells/10^8 SM sensitive cells					
			Species and strain source of transforming factor (DNA_{SM})					
			H. influenzae		*H. aegyptius*			*H. parainfluenzae*
Receptor species and strain*		Exp.	Rd	3	46	181a	15	1
H. influenzae	Rd	1	28,000		17,000			
		2	48,000		48,000	33,000	47,000	120
	Rb	1	780		510			
		2	510		180	190	260	
		3	1,880		1,030	660	1,240	23
	3	1	1,600		7,300	7,100	9,700	15
		2	3,200	11,800	13,300	8,800	13,000	25
	4	1	650		410	280	360	11
H. aegyptius	46	1	63		110			
	181a	1	780		1,300	1,200	2,500	8
		2	85	64	240	67	260	1
	15	1	250	190			830	22
		2	130	98	260	94	160	
		3	85		300	100	250	7
H. parainfluenzae	1	4			7	15	1	2,100
	7	26			15	43	23	4,500

* *H. influenzae* — Rb and Rd = Non-encapsulated variants selected from populations of Types b and d; 3 and 4 = Non-typable strains from blood and nasopharynx respectively. *H. parainfluenzae* — Isolated from nasopharynx.

The technics used for the conference of SM resistance in sensitive populations have been reported(4). For the present study, DNA extracts from cells resistant to at least 1,000 mcg/ml of streptomycin were impure preparations except for one purified DNA fraction of a strain of *H. aegyptius* (181a).† Recipient cells were exposed to DNA-containing extracts (approximately 1-2 mcg/ml of DNA) for 15 minutes and desoxyribonuclease (DNAase) then added. The treated cells, appropriately diluted, were seeded in pour plate preparations of Levinthal agar and incubated 2 hours to permit expression of the induced resistance trait before exposure to streptomycin to test for presence of resistance. Levinthal agar containing 2,000 mcg/ml of streptomycin was then layered over the pour plate preparations, and after hardening of the overlay the plates were incubated for 48 hours. Appropriate controls (desoxyribonuclease and untreated cells) were included.

Data in Table I indicate that in a given recipient population of *H. influenzae* or *H. aegyptius*, the number of cells transformed by either *H. influenzae* or *H. aegyptius* SM-resistance transforming factor is of the same order of magnitude. As shown in Table II, the hetero-homospecific transformation ratio for members of these 2 species may approach 1; the lowest value obtained is 0.2. Data from Table I and those published(4) were used to determine the ratios listed in Table II. The results obtained are therefore comparable to ratios found when comparisons are made of the number of transformants induced in a given recipient population of *H. influenzae* by DNA's derived from different type specific or non-type specific strains of *H. influenzae*((4) and Table II). On the other hand, the proportion of cells of either *H. influenzae* or *H. aegyptius* transformed to SM-resistant by DNA from *H. parainfluenzae* is of a much lower order of magnitude (Tables I and II); it is in the range expected in interspecific transformation(3,4). This is true also for strains of *H. parainfluenzae* exposed to the SM-resistance transforming factor of either *H. influenzae* or *H. aegyptius*.

Discussion. When aliquots of the same re-

† The purified product was prepared by Dr. Sheldon Greer.

TABLE II. Ratio of Number of Cells Transformed to Streptomycin (SM) Resistant by Heterologous "Species" DNA_{SM} to Number Transformed by Homologous Species DNA_{SM}. Range of ratio for homologous species transformation included for comparison.

Recipient species and strain		Hemophilus species source of transforming factor (DNA_{SM})		
		H. influenzae	*H. aegyptius*	*H. parainfluenzae*
H. influenzae	Rd	(.3 to 1)*	.6 to 1	.003 (.002 to .004)*
	Rb	(.2 to .8)*	.4 to .7	.012 (.007 to .008)*
	3	(.1 to >1)*	.7 to >1	.002 to .009
	4	(.3 to .6)*	.4 to .6	.017 (.016 to .02)*
H. aegyptius	46	.6		
	181a	.3 to 1	.3 to 1	.003 to .006
	15	.2 to 1	.3 to .8	.02 to .07
H. parainfluenzae	1	.002 (.003–.004)*	.003 to .007	(.1 to .5)*
	7	.006	.003 to .009	(.3 to 1)*

* Figures in parentheses obtained from published data(4).

cipient population of *H. influenzae* or *H. aegyptius* are exposed simultaneously to DNA's derived from streptomycin (SM)-resistant cells of the same strain or from different *H. influenzae* and *H. aegyptius* strains, the proportion of cells in which SM-resistance is induced is of the same order of magnitude. The results obtained are comparable to those found in intraspecific transformation(4). On the other hand, the proportion of cells of either *H. influenzae* or *H. aegyptius* transformed to SM-resistant by the DNA from *H. parainfluenzae* is in the range expected in interspecific transformation (4).

Summary. On the premise that the degree of reactivity of donor DNA with the heredity determinants of the recipient cell is a reflection of the degree of homology of the genome of donor and recipient cells, it has been proposed that transformation may serve as a valid criterion for bacterial taxonomy. If the use of a single genetic marker, SM-resistance, provides a valid basis for comparison, *H. aegyptius* may be a member of the *H. influenzae* group.

1. Schaeffer, P., Ritz, E., *C. R. Acad. Sci.*, Paris, 1955, v240, 1491.

2. Alexander, H. E., Leidy, G., *Am. J. Dis. Child.*, 1955, v90, 560.

3. Schaeffer, P., *Symp. Soc. Exp. Biol.*, 1958, v12, 60.

4. Leidy, G., Hahn, E., Alexander, H. E., *J. Exp. Med.*, 1956, v104, 305.

5. Pittman, M., Davis, D. J., *J. Bact.*, 1950, v59, 413.

Received June 23, 1959. P.S.E.B.M., 1959, v102.

Effect of Certain Tranquilizers on The Reticulo Endothelial System. (25152)

R. VINEGAR AND F. M. BERGER

Wallace Labs., Division of Carter Products, New Brunswick, N. J.

Several investigators observed that administration of reserpine or chlorpromazine lowered the resistance of patients to infectious diseases(7,9). Grosz and Norton(4) reported that chlorpromazine increased the susceptibility of mice to infection with *Salmonella enteritidis*. Meier *et al.*(8) showed that chlorpromazine depressed the phagocytic action of the reticulo endothelial system (RES) whereas mepazine (N-methyl-piperidyl-1,3-methyl phenothiazine) did not have this action. It was of interest to study the effect of several tranquilizers on the RES in greater detail and to carry out experiments relating to their mode of action.

Methods. Measurement of RES activity was accomplished by determining blood clearance of carbon particles (india ink, Gunther-Wagner, Hanover, Germany C11/1431A) as outlined by Heller *et al*(5). Average particle