

## Induction of Heritable New Type in Type Specific Strains of *H. influenzae*.<sup>\*</sup> (19161)

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Change in the inherited characteristics of pneumococci(1) and *H. influenzae*(2) induced by highly specific desoxyribonucleic acid containing extracts is now well established. Thus far all reports have been concerned with transformation in R cell populations, with one exception: Taylor(3) has presented data on the induction, by fractions containing desoxyribonucleic acid, of changes in mutants of type 3 pneumococcus which exhibited varying degrees of abnormality of the type 3 factor; the changes involved only homologous type. The present report offers evidence that a change in type specificity can be induced in a fully equipped type specific population of *H. influenzae* derived directly from a single clone.

**Methods.** The types b and d strains in which new types were induced included one from which R colonies had been used for earlier reports(4). The type b strains had been isolated from the spinal fluid of patients with influenzal meningitis, and the type d strains from the nasopharyngeal mucus of patients with respiratory infections. Soon after growth from these sources, the cultures were desiccated and sealed under vacuum by a method (5) which preserves their original status. The method of preparation of desoxyribonucleic acid extracts, the concentrations used in the transforming environment, and the procedure used were not different from those reported previously(4). The environment used for transformation differed in only one

respect, the presence of type specific antibody corresponding to the recipient cell type in place of anti R antibody; a 1:15 or 1:20 dilution of specific antiserum, produced in rabbits during one month of immunization, was employed. In order to aim for a genetically homogeneous culture, the suspension used for transformation was derived from a single S clone (colony).

**Results.** Three of the 5 type b populations tested were transformed into types a, c and d. Types a, b and c were induced in both of the type d strains used. All changes occurred with predictable regularity and were inherited by the progeny. In order to compare the frequency, in R and S populations, of cells susceptible of transformation, induction of type c from type d populations was used as a tool. Two technics described previously(4) were used for this comparison: 1) Study of the influence of population size when the transformation proceeds during growth for 48 hours and 2) Examination of populations of different sizes for the presence of initially susceptible cells; the transforming principle is destroyed by desoxyribonuclease after an exposure period of 5 minutes which is adequate for transformation.

In Table I are listed the results of three experiments which show the population sizes of type specific d cells in which transformation

TABLE I. Influence of Size of Population of Type d *H. influenzae* on Induction of Type c During 48 Hr Growth Period in Levinthal Broth Plus Type d Antibody.

| Exp. | Approximate population sizes |                   |                   |
|------|------------------------------|-------------------|-------------------|
|      | $1.0 \times 10^6$            | $1.0 \times 10^4$ | $1.0 \times 10^2$ |
| 1    | + (2)                        | + (2)             | + (2)             |
| 2    | + (3)                        | + (3)             | — (2)             |
| 3    | + (3)                        | + (3)             | + (3)             |

+ = Type c present.

— = No type c found.

No. in parentheses of either + or — symbols indicates No. of samples examined in each experiment.

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2. Alexander, H. E., and Leidy, G., *Proc. Soc. Exp. Biol. and Med.*, 1950, v73, 485.

3. Ephrussi-Taylor, H., in press.

4. Alexander, H. E., and Leidy, G., *J. Exp. Med.*, 1951, v93, 345.

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TABLE II. Influence of Population Size of Type d *H. influenzae* on Presence of Cells Initially Susceptible of Transformation to Type c.

| Transforming principle | —Approximate population sizes— |                   |                   |
|------------------------|--------------------------------|-------------------|-------------------|
|                        | $1.0 \times 10^6$              | $1.0 \times 10^5$ | $1.0 \times 10^4$ |
| TPC                    | — (1)                          | — (1)             | — (5)             |
|                        | + (4)                          | + (4)             |                   |
| TPC                    | + (3)                          | + (3)             | — (5)             |
|                        | — (2)                          | — (2)             |                   |

TPC = Transforming principle isolated from type c.

+ = Type c present.

— = No type c found.

No. in parentheses of either + or — symbols indicates No. of samples examined in each experiment.

occurs during a 48-hour growth period. They do not differ significantly from the results obtained with R cells derived from type d under comparable circumstances.

In Table II are listed data which determine the population sizes of type d which must be used in order to ensure the initial presence of susceptible cells. Here again, no significant difference is demonstrated between R and S cell population sizes which contain susceptible cells prior to growth.

**Discussion.** The slightly greater degree of irregularity seen in these experiments on S cells, as compared with previously published observations on R cells, may be explained by the fact that the identification of presence of new types is much less efficient because of the large number of recipient cells; the specific antibody concentration in the transforming environment is too low to separate completely the recipient cells from the induced new type by agglutination and sedimentation.

A stronger concentration can be expected to increase greatly the efficiency of detection of induced new type specific cells.

Since the type d populations used for induction of type c were derived directly from a single clone (colony), the populations can be viewed as homogeneous with respect to the type specific trait. Moreover it was very difficult to demonstrate the presence of an R colony before exposure to the transforming environment. Therefore, if susceptible cells arise by mutation, they are unlikely to be derived solely as mutants of R cells. The factor responsible for susceptibility must arise independently of the R and S genetic traits, for it has not been possible to demonstrate a significant difference in the frequency of susceptible cells in R and S populations.

**Summary.** 1. Normal strains of types b and d *H. influenzae* have been directly transformed to new types of *H. influenzae*; type b was changed to types a, c and d, and type d into types a, b and c. 2. The rate of occurrence in type d populations of cells susceptible of transformation has been shown to be not significantly different from that found earlier in R cell populations derived from type d. Comparisons in the presence of more potent anti d antibody have been made subsequently between populations of Rd and type d for frequency of cells susceptible (initially or during growth for 24 hours) to transformation to type c. No real differences could be demonstrated when this more efficient selective agent was used.

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### Hemodilution as a Result of Estrogen Therapy. Estrogenic Effects in the Human Female. (19162)

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Many articles concerning the effects of estrogenic substances on the so-called "blood picture" may be found in the literature. There are numerous publications concerning the effects of estrogens on the blood composition of

laboratory animals. Vollmer and Gordon(1), Tyslowitz *et al.*(2,3), Castrodale(4), and

1. Vollmer, E. P. and Gordon, A. S., *Endocrinology*, 1941, v29, 828.