rabbit cornea may be grafted onto the chorio-allantoic membrane of the chick embryo and kept viable.

2. When the graft is successful, it presents a bluish appearance and is firmly adherent to the membrane. Histologically, such grafts are seen to be invaded by chicken blood vessels into the normally avascular stroma, and show preservation and proliferation of the corneal epithelium.

3. The viruses of vaccinia and herpes simplex were found to give rise to inclusion bodies within 24-72 hours in the epithelial cells of the corneal graft.

4. Serial passages of corneal grafts were complicated by the frequent loss of the epithelium and in only one case were inclusion bodies found after the inoculation during the second grafting.

Received January 6, 1950, P.S.E.B.M., 1950, v73.

Transformation Type Specificity of *H. influenzae*.* (17721)

HATTIE E. ALEXANDER AND GRACE LEIDY.

From the Babies Hospital and the Department of Pediatrics, Columbia University, College of Physicians and Surgeons.

Avery, MacLeod and McCarty(1) isolated from type III *D. pneumoniae* a desoxyribonucleic acid which in a suitable environment endowed R cells, derived from type II *D. pneumoniae,* with characteristics of S cells of type III. The transformed cells became encapsulated, elaborated the specific carbohydrate of type III and exhibited colony traits of this type. McCarty and Avery later reported an improved method for isolating specific pneumococcus desoxyribonucleic acids which they applied to types II, III and VI(2). Boivin and others(3) and Weil and Binder(4) reported the very irregular occurrence of transformations of *E. coli* and *S. dysenteriae* in the presence of crude desoxyribonucleic acid containing extracts made from the specific type into which the R variant was changed. This paper reports 1) the isolation from type b *H. influenzae* of a desoxyribonucleic acid which transforms an R strain Rbl, derived from type b *H. influenzae,* to type b cells during an 18 to 24 hour period of growth and 2) the isolation from type c *H. influenzae* of an unpurified transforming principle which endows the same R strain, Rbl, with properties of type c *H. influenzae.*

Material and Methods. Isolation of transforming principles. The desoxyribonucleic acid of type b *H. influenzae* was isolated and purified by the method which McCarty and Avery(2) applied to pneumococcus. Type b *H. influenzae* grown for 6 to 7 hours on Levinthal agar plates was harvested by washing each plate with 4 cc of a solution containing 0.1 M sodium chloride and 0.1 M sodium citrate. After 2 washings in this fluid the yield from 300 plates was reduced by centrifugation to a volume of 40 to 50 cc and frozen in carbon dioxide ice. After thawing at 4°C the volume was made up to 500 cc with the citrate saline solution and 5 cc of 10% sodium desoxycholate was then added; marked dissolution of the organisms occurs within 30 minutes at room temperature, the creamy appearing suspension clearing almost completely. Three extractions with chloroform and amyl alcohol were carried out to remove protein. The addition

* The work in this communication was supported by a Commonwealth Fund grant.

1. Avery, O. T., MacLeod, C. M., and McCarty, M., *J. Exp. Med.,* 1944, v79, 137.

2. McCarty, M., and Avery, O. T., *J. Exp. Med.,* 1946, v83, 89.

3. Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia,* 1945, v1, 334; Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., *Experientia,* 1946, v2, 139; Boivin, A., and Vendrely, R., *Helvet. chim, acta,* 1946, v5, 1938.

4. Weil, A. J., and Binder, M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 349.

of 2 volumes of absolute ethyl alcohol to the partially deproteinized solution yielded a fibrous precipitate which was allowed to remain in the alcohol at 4°C overnight. The fibrous precipitate drained of alcohol and redissolved in 0.85% sodium chloride pH 7.0 was extracted twice more with chloroform and amyl alcohol, again precipitated with 2 volumes of alcohol, washed in absolute alcohol and redissolved in physiologic saline. Ribonucleic acid was eliminated by digestion with ribonuclease, and the desoxyribonucleic acid was separated from the specific polysaccharide of type b by precipitation in the presence of 0.1 volume of 10% calcium chloride and 0.2 volume of absolute alcohol. The final precipitate was dissolved in 20 cc of 0.85% saline. This solution formed no precipitate with type b specific rabbit antiserum, but showed positive reactions for desoxyribonucleic acid when examined by the diphenylamine(5) and Stumpf (6) tests. We are indebted to Dr. Zacharias Dische for these last determinations. The isolation of unpurified type c *H. influenzae* transforming substance has been carried out by a process identical with that used for type b up to the point of digestion with ribonuclease. The type c substance used in the experiments to be reported is therefore a crude product and contains the specific polysaccharide of type c. The final fibrous precipitate obtained from the growth on 100 plates was dissolved in 25 cc of physiologic saline, pH 7.0.

R strain used for transformation: The R strain of *H. influenzae,* Rbl, used in these experiments was derived from a strain of type b *H. influenzae* by selection of a spontaneously appearing, non-iridescent, non-opaque colony from among the iridescent, opaque ones characteristic of S cells after growth on Levinthal agar for 18 hours; subculture and isolation of characteristic R colonies have been repeated many times. Cultures of this Rbl strain have been used for various purposes in our laboratory for 6 years, and at no time has evidence of type specificity been detectable by study of colony type, capsular swelling tests, or precipitin reactions on supernatants of broth cultures. Twenty passages of this strain through mice (in mucin suspensions) failed to demonstrate presence of type specific organisms. Anti-R *H. influenzae* antiserum: Rabbits were injected intravenously with gradually increasing volumes of a suspension of Rbl cells of *H. influenzae* (approximately 1.5 to 2.0 billion cells per cc) in 0.2% formalinized saline. An initial dose of 0.1 cc was increased daily by 0.1 cc for 4 days of each week until 1.0 cc daily was reached. Serum of rabbits injected for a period of at least 2 weeks has provided a suitable environment when used in a 1:50 dilution in the presence of desoxyribonucleic acid under the circumstances of the experiments described. The antiserum was heated at 60°C for 30 minutes.

Experimental. Transformation of nontypable, R *H. influenzae* strain, Rbl, to type specific cells: The environmental constituents found to be essential for the transformation of pneumococci by their specific desoxyribonucleic acids(7) proved adequate for transformation of *H. influenzae.* These constituents could be supplied by the addition of whole anti-Rbl rabbit antiserum or by the addition of the 3 separate factors found to be responsible for its transforming activity —1) R agglutinins, furnished by the gamma globulin fraction of anti-Rbl serum prepared by method of Taylor(8), 2) a protein substance, furnished by bovine serum albumin fraction V† and 3) sodium pyrophosphate, which restores a function lost by dialysis of rabbit anti-R antiserum. These 2 varieties of environmental influence—1) whole rabbit anti-R *H. influenzae* antiserum or 2) the 3 separate components—were examined for their effect in a system containing a transforming principle of *H. influenzae,* either

5. Dische, Z., *Mikrochemie,* 1930, v8, 4.

6. Stumpf, P. K., *J. Biol. Chem.,* 1947, v169, 367.

7. McCarty, M., Taylor, H. E., and Avery, O. T., Symposia on Quantitative Biology, 1946, XI, 174. Cold Spring Harbor, New York.

8. Taylor, H. E., *J. Exp. Med.,* 1949, v89, 399.

† Prepared by Armour and Co. according to techniques developed in the laboratory of Dr. E. J. Cohn.

TABLE I.
Transformation of Rbl Cells of *H. influenzae* into Specific Type b and Type c *H. influenzae.*

Environment		Transformation Quintuplicate tests 1 through 5
Levinthal broth plus	Transforming principle	
Whole rabbit anti-Rbl *H. influenzae* antiserum dilution 1:50	0	R only
	DNAb	S^b + R
	type c unpurified	S^c + R
Gamma globulin from anti-Rbl *H. influenzae* antiserum + bovine albumen fraction V + Na pyrophosphate	0	R only
	DNAb	S^b + R
	type c unpurified	S^c + R

DNAb—Desoxyribonucleic acid of type b *H. influenzae.*
S^b—Type b *H. influenzae.*
S^c—Type c *H. influenzae.*

type b or type c, and a representative population of young Rbl *H. inflenzae* cells. The nutrient medium, Levinthal broth, which provided R cell growth during which transformation occurred, had not been adsorbed with charcoal.

Transformation of R strain Rbl to type b *H. influenzae*: The circumstances under which transformation of R cells to S cells of type b *H. influenzae* occurred are shown in Table I. It is seen that when type b desoxyribonucleic acid (0.1 cc of 1:10 dilution) is present in an environment containing approximately 1,000,000 cells (a 2 mm loopful of a 6 hour culture) of R *H. influenzae* in 2 cc of Levinthal broth containing 1:50 dilution of anti-R *H. influenzae* rabbit serum, 5 samples after incubation for 18 to 24 hours showed presence of S cells of type b *H. influenzae.* The change to encapsulated type specific cells was evidenced by capsular swelling with type specific antiserum and formation of iridescent, opaque colonies on Levinthal agar. This transformation occurred also in the presence of the 3 separate constituents of anti-R serum, chosen by analogy with comparable experiments in conversion of R to S pneumococcocci.

Transformation of R strain, Rbl, to type c *H. influenzae*: When an unpurified transforming principle isolated from type c *H. influenzae* was added in 0.1 cc quantities of a 1:10 dilution to environments identical with those described above, the R cells Rbl were transformed into type c *H. influenzae;* the transformed cells showed capsular swelling when exposed to type c specific antiserum, and opaque colonies exhibiting iridescence appeared after growth for 18 hours on Levinthal agar. The transformation in all of the quintuplicate tests are also shown in Table I.

Summary. The data provide another example of the control of the genetic constitution of microorganisms by specifically oriented desoxyribonucleic acids. An R strain, Rbl, derived from type b *H. influenzae* was endowed with properties of type b under the influence of a desoxyribonucleic acid isolated from type b *H. influenzae.* This same R strain, Rbl, was transformed to type c *H. influenzae* when grown in an appropriate environment in the presence of an unpurified transforming principle isolated from type c *H. influenzae.*

Rbl has also been transformed to Sa and Sd. The transforming activity of desoxyribonucleic acid has been shown to be destroyed by a crystalline desoxyribonuclease supplied by Dr. Maclyn McCarty.

The authors acknowledge with gratitude the helpful suggestions of Dr. Maclyn McCarty.

Received January 24, 1950, P.S.E.B.M., 1950, v73.