BACTERIAL TRANSFORMATION REACTIONS

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In reviewing previously the chemical nature and biologic specificity of the substance inducing transformation of pneumococcal types (31), McCarty suggested that the principles involved and the results observed in the study of this reaction have implications beyond those referable to the biology of pneumococcus. The correctness of this point of view has been borne out by the investigations of the ensuing five years. In this period the variety of cellular characters which can be controlled by transformation reactions and the number of bacterial species susceptible to transformation have been expanded; also the conditions requisite for the carrying out of such alterations in the hereditary structure of microorganisms have been defined more precisely. To correlate these more recent observations with each other and with earlier work concerned with bacterial transformations is the purpose of the present review.

A transformation reaction may be defined as an hereditary alteration in a susceptible cell resulting from the acquisition from its environment, by other than sexual means, of a genetically active unit directing the inheritable change. An additional feature of such reactions is that the genetically active material should be demonstrable in the progeny of the transformed cell and recoverable from such populations in quantities in excess of the amount requisite to induce the initial alteration. In other words, within the cell, the transforming principle should behave as a self-reproducing substance. From the definition just stated, it is apparent that certain conditions must be fulfilled in order that a transformation reaction take place. First a competent cell, one capable of undergoing transformation, must be available; second, genetically active material must be provided; and third, environmental conditions must be suitable for the union of the two reacting components of the system. An analysis of these factors is, therefore, pertinent.

FACTORS CONCERNED WITH BACTERIAL TRANSFORMATION

1. *Cellular Competence in Bacteria.* In addition to pneumococcus, transformation reactions have now been described employing strains of *Hemophilus influenzae* (1, 2), *Escherichia coli* (8), and *Shigella paradysenteriae* (42). In all four species, transformation has been restricted to certain strains, other seemingly similar strains failing to participate in the reaction under comparable conditions. In each instance, the transforming principle has been derived from cells

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The author expresses his appreciation to Dr. Harriet Ephrussi-Taylor and to Dr. R. D. Hotchkiss for permitting him access to the manuscripts of references 40 and 21 prior to their publication.

of an heterologous type within the same species. Little is known concerning the bases in bacterial cells upon which competence in transformation reactions rests, though a few data relevant to this subject are available.

The possibility that the structure of the cell surface may be a modifying factor in such reactions in pneumococcus is suggested by the observations of Neufeld and Levinthal (37) relating the bile solubility of two unencapsulated strains to their ability to undergo transformation to encapsulated forms *in vivo*. The strain more susceptible to lysis participated readily in the reaction, whereas the more difficultly soluble one did not. Similar relations to competence in *in vitro* reactions have been noted by others (28), but a more careful study of this problem will be necessary before its significance can be evaluated.

A second factor related to the competence of an organism with regard to a specific transformation reaction may reside in its genetic constitution. It has been suggested that an organism producing one of a series of analogous cellular components such as a capsular polysaccharide cannot undergo transformation with production of an heterologous member of the series without loss of the genetic factor responsible for the production of the member of that series already manifest (4). This concept derives from the study of the transformation of surface carbohydrates. In this reaction, an unencapsulated variant of one capsular type within a species which lacks presumably the genetic factor for the production of surface carbohydrate of any type is first selected. Upon exposure to transforming principle from an homologous or heterologous cell type of the same species, the genetically deficient cell acquires again the ability to produce capsular polysaccharide, the type being dependent upon the source of the transforming principle. Within certain limits, this concept appears to be valid. In pneumococcus, however, there exist variants within a given capsular type which differ in the quantity of capsular carbohydrate which they produce. The amount of capsular carbohydrate made by some strains may be so small that their cells fail to give a quellung reaction, and, when grown on solid media, they give rise to colonies indistinguishable in appearance from completely unencapsulated variants. Such strains may prove incapable of transformation to an heterologous capsular type (27) but may be competent in transformation reactions involving alterations in the quantity of homologous capsular polysaccharide produced (27, 38). Hence, a cell may be incompetent in one reaction and competent in another.

Whether or not a cell must be genetically deficient to be capable of being transformed must be questioned in view of other experimental data. There exist two unencapsulated variants of pneumococcus, one described by Griffith (18), the other by Dawson (12). The Griffith variant (R) is the well recognized one which produces small hemispherical colonies with well defined margins on solid media and grows diffusely in broth. The Dawson variant (ER) has been the subject of little study, can be derived from the Griffith R variant by selective cultural techniques, produces very rough colonies with irregular margins on solid media, and grows in an agglutinated state in liquid media. It has been shown that the transformation, ER $\rightarrow$ R, may be effected with transforming extracts derived

either from R variants or from encapsulated variants of pneumococcus (4, 38). More recently, Taylor (39) has reported that transformation in the reverse direction, R → ER, may be carried out with a transforming extract of the ER variant and has suggested that the reversible nature of these transformations may result from an exchange of genetically active material between the cell and its environment. In the light of these findings, the earlier observations of Dawson and Warbasse (13) on the transformation of pneumococci of one capsular type directly to a second capsular type are of interest. When an encapsulated strain of pneumococcus type II was grown in a medium containing pneumococcal type I antiserum in the presence of a transforming vaccine of pneumococcus type III, occasional colonies of pneumococcus type III were recovered. The development of competent unencapsulated (R) variants of the type II strain during the course of the experiment cannot be excluded, but the possibility of an exchange reaction resulting in the appearance of type III organisms must be borne in mind in view of the more recent observations.

Another factor which may have bearing upon the ability of a bacterial cell to undergo transformation is its production of extracellular desoxyribonuclease. Although pneumococcus produces an active desoxyribonuclease (6), little or none of the depolymerase activity is found in the media of unautolysed cultures (41). Similarly, the strains of *E. coli* used in transformation experiments are reported to have little enzymatic activity of this type (8). It is not unreasonable to anticipate that difficulty may be encountered in effecting transformation within such bacterial species as group A β-hemolytic streptococci, which introduce into their environment such large amounts of desoxyribonuclease.

2. *The Nature of Transforming Principle.* Because of the broad biological implications of transformation reactions, interest in the precise chemical nature of transforming principle has continued to be manifested. In 1944, Avery, MacLeod, and McCarty (6) showed the activity of the transforming principle of pneumococcus to be associated intimately with a desoxyribonucleic acid fraction of that organism. Studies of their preparation revealed it to be homogeneous both in electrical and ultracentrifugal fields and to be composed of molecules of an estimated molecular weight of 500,000. A more recent examination of the transforming principle of pneumococcus type III with the techniques of deutron and electron bombardment has indicated that its molecular weight may be appreciably greater than this value (17). Subsequent studies by McCarty (32) and by McCarty and Avery (33) on the purification and action of desoxyribonuclease upon pneumococcal transforming principle gave added support to the idea that the biologic specificity of transforming principle resided in nucleic acids of the desoxyribose type. Despite these findings, potential objections to this concept continued to be raised on several grounds by those believing that a small amount of protein associated with the transforming principle might be responsible for the specificity of its activity (36). It was argued that tests for the purity of nucleic acids were inadequate, permitting a protein residue of 2 to 3% to remain undetected, that failure of proteolytic enzymes to destroy activity did not assure the absence of protein as all proteins are not susceptible to such proteolytic ac-

tivity, that despite the small weight of material used to effect transformation, the number of protein molecules might not be unduly small, and that desoxyribonuclease might alter the activity of a nucleoprotein complex the specificity of which resided in the protein moiety. The following experiments by Hotchkiss (20) have in large measure answered these objections. Use of desoxyribonucleases devoid of proteolytic activity both of bovine pancreatic and of streptococcal origin in concentrations as low as 0.001 μg/ml has destroyed transforming activity of highly purified material. Progressive purification of the transforming principle has been accompanied by the progressive approach of its properties to those of bovine thymus nucleic acid, and analyses of hydrolysates of the transforming principle have shown the amount of thymine present in highly purified material to be the same as that present in the crude transforming principle. It has been shown further, by use of the gasometric ninhydrin reaction, that with purification there is a steady reduction in the *alpha* amino acid content of the transforming principle detectable after acid hydrolysis. All the *alpha* amino acid in such preparations was found to be glycine, and the quantity recovered was equal to the amount anticipated from the breakdown of adenine which yields glycine on hydrolysis (22). Supporting evidence for this latter fact has been derived from a comparative study of the kinetics of hydrolysis of adenine, protein, and transforming principle. From these observations, it appears that the purified transforming principle studied could not contain more than 0.2% protein. In the light of these findings, Hotchkiss has observed that if the transforming principle possessed the hypothetical, biologically active protein, such a protein would have to be endowed with the unusual properties of being concentrated by nonspecific procedures which at the same time eliminated other proteins. These elegant experiments leave little doubt that the biologic activity of transforming principle resides in desoxyribonucleic acid.

Studies concerning the nature of the transforming principle of two other bacterial species are in agreement with those concerning that of pneumococcus, though with neither species has analysis been carried to a comparable degree of refinement. Boivin and his collaborators (8) found the transforming principle of *E. coli* to be resistant to the action of pepsin and of ribonuclease but highly susceptible to the depolymerizing activity of desoxyribonuclease. Similarly, Alexander and Leidy (1, 2) have shown the transforming principle of *H. influenzae*, obtained by techniques resembling those employed for its extraction from pneumococcus, to give positive Dische and Stumpf reactions for desoxyribonucleic acids and to be inactivated readily by desoxyribonuclease. More recently, Zamenhof and his co-workers (43) have reported further purification of desoxypentose nucleic acid from *H. influenzae*, type c. Using electrophoretic techniques, they obtained a preparation possessing transforming activity in amounts as small as 0.01 μg and containing less than 1% pentose nucleic acid and 0.2% serologically active polysaccharide. These findings lend added support to the importance of the role of desoxyribonucleic acids in determining bacterial inheritance. The nucleic acid functions not only as a self-reproducing unit but also as the determinant for the production of a cellular component of a different chemical class.

To date, however, no evidence has appeared to relate transforming principle

to the geographical concepts of genes and chromosomes. Recent cytologic and genetic studies of certain species of bacteria indicate strongly the presence of nuclear structures (14, 29) and of sexual forms within one, *E. coli* (25). If it were possible to transform a strain of *E. coli* which, like strain K-12, was capable thereafter of participating in sexual union, it might be possible from a study of the offspring of such matings to link the genetically active transforming principle to a discrete cellular structure of chromosomal nature. Such a study would be of unusual interest.

3. *Environmental Conditions for Bacterial Transformation.* The environmental conditions in which transformation reactions will take place vary among bacterial species. Those permitting transformation of pneumococci are complex. Reactions carried out *in vitro* require, in addition to a nutrient medium, certain factors present in serum or in serous fluids (6), upon both the nature and function of which recent analyses (23, 35, 38) have shed some light. Three environmental components of the pneumococcal transforming system in addition to a nutrient medium have now been recognized: agglutinating antibody, a dialyzable component replaceable by pyrophosphate ion, and a serum factor present in fraction V of bovine serum albumin. Agglutinating antibody appears to be concerned with the creation of a suitable local environment for transformation of pneumococcal variants which grow diffusely in liquid media in its absence. Need for agglutinating antibody in the transformation of such strains can be obviated by the use of a semisolid medium which contains the other factors essential to the reaction. It has been shown, moreover, that ER variants of pneumococcus which are autoagglutinable may be transformed to the R variant in liquid medium in the absence of agglutinating antibody (38). Additional observations suggest that the maintenance of local reducing conditions may be the essential role of agglutinating antibody. When pneumococci were grown in very shallow layers of the complete transforming system containing agglutinating antibody, transformation did not take place. In one instance, however, the addition of glutathione to the medium permitted the occurrence of transformation. Establishment of proper oxidation-reduction potential is doubtless of importance to completion of the reaction, for McCarty (30) has shown that pneumococcal transforming principle may undergo reversible oxidative inactivation. The more recent observation of Hotchkiss (21) that the reaction in which diffusely growing, penicillin sensitive pneumococci are transformed to penicillin resistant forms does not require agglutinating antibody may be explained by the unusually high selectivity of the method employed for the detection of transformed cells.

A second factor required by most strains of pneumococci for their successful transformation is a dialyzable component of serum or serous fluids (35). When such fluids are dialyzed, their ability to support transformation is lost, but they can be reactivated promptly by the addition of pyrophosphate ions. The role of such ions has not been determined but is thought to be concerned, perhaps, with an enzymatic reaction. It is of interest that one strain of pneumococcus has been found which does not require the dialyzable component of serous fluids or pyrophosphate ions to undergo transformation.

A third component of serum or serous fluids which has proved essential to all

pneumococcal transformations can be supplied by fraction V of bovine serum albumin (23, 38). The specific role of this factor is unknown.

Study of pneumococcal growth in the complete transforming system has yielded further pertinent data (35). If desoxyribonuclease is added to such a system at varying intervals following its inoculation with competent pneumococci, transformation of capsular type will not take place if the enzyme is introduced within four hours of inoculation. Thereafter, desoxyribonuclease has no effect upon the reaction. If the pneumococcal cells are grown in the serum medium for four hours in the absence of transforming principle at which time the latter is then introduced, addition of desoxyribonuclease fifteen minutes later will fail to inhibit transformation. These observations indicate that an initial period of growth in the transforming system is required for the pneumococcal cells to become "sensitized". Once "sensitization" has occurred, the union of cell and transforming principle can take place very rapidly. That the selection of a mutant is not involved in these reactions is indicated by the fact that the change in population size during sensitization is not very great and by the fact that sensitization is a transitory state, being lost on incubation for an additional two to four hours or after washing the sensitized cells in nutrient broth. These relationships pertaining to the sensitization of pneumococci and to the time required for the union of cell and transforming principle have been shown to hold in transformations of ER variants to R variants and of an intermediate capsular variant of pneumococcus type III to a fully encapsulated variant of the same type (38).

Environmental conditions for the transformation of other bacterial species are less complex than those required by pneumococcus which, of the organisms now known to be susceptible to transformation, is the only one lacking the cytochrome and catalase enzyme systems. It is possible that absence of the latter enzyme may result in the accumulation of hydrogen peroxide and oxidative inactivation of transforming principle under certain conditions of growth. *E. coli* (8) and *S. paradysenteriae* (42) will undergo transformation in nutrient media which support growth. It has been shown further that the transformation of *H. influenzae* (2) will take place when competent cells are placed in contact with transforming principle for 15 minutes in an environment which fails to support multiplication. The environmental requirements for transformation of certain bacterial species, therefore, may be much simpler than those demanded by pneumococcus.

BACTERIAL CHARACTERS SUSCEPTIBLE TO TRANSFORMATION

1. *Type-Specific Surface Polysaccharides.* Bacterial transformation was noted first by Griffith (19) in a study of the conditions responsible for acquisition of a capsule by unencapsulated strains of pneumococci. By inoculating mice subcutaneously with a mixture of living unencapsulated R variants derived from a strain of one capsular type and of heat-killed encapsulated S variants of a different capsular type, he was able to recover from mice succumbing to pneumococcal sepsis living encapsulated organisms of the same capsular type as the heat-killed S variants apparently inducing the change. The validity of this ex-

periment rested upon the demonstrable absence of viable organisms from the vaccine of S pneumococci. The subsequent finding of type-specific somatic protein antigens in pneumococcus which vary independently of capsular antigens (3) has simplified the demonstration of capsular type transformation *in vivo*. The combination of a capsular polysaccharide of one type with the somatic protein of a viable organism of an heterologous type in an experiment of the variety performed by Griffith provides conclusive evidence that transformation *in vivo* has taken place. Analogous experiments with pneumococcus employing drug resistance as a somatic marker have also been reported (24).

Transformation of pneumococcal capsular types *in vitro* has been performed with unencapsulated organisms derived from a variety of capsular types and with transforming principle obtained from a comparable diversity of strains. At present, there is no evidence to indicate that a competent unencapsulated pneumococcal cell derived from an organism of one capsular type cannot be transformed to any of the other known capsular types under the influence of the appropriate transforming principle. When studied, the agent responsible for the synthesis of the new capsular material has been shown to be desoxyribonucleic acid (34).

More recently, observations relating transformation reactions to the quantitative aspects of polysaccharide production within a given capsular type of pneumococcus have appeared. MacLeod and Krauss (27) studied an intermediate capsular variant of pneumococcus type II which produced very small amounts of type II polysaccharide indistinguishable immunologically from that produced by fully encapsulated strains. In many respects this variant resembled completely unencapsulated forms, producing rough colonies on solid media, failing to give a quellung reaction with homologous antiserum, and lacking virulence for mice. It differed from the R variant, however, by growing diffusely in anti-R serum and in its ability to absorb completely the antibodies to type II capsular polysaccharide from antiserum prepared with a fully encapsulated strain of pneumococcus type II. From this intermediate variant was obtained a transforming principle capable of transforming a completely unencapsulated R variant of pneumococcus type II to a similar intermediate capsular variant of the same type. In addition, with the aid of heat-killed vaccine of a fully encapsulated strain of pneumococcus type II, the original intermediate variant of pneumococcus type II could be transformed *in vivo* in the subcutaneous tissues of the mouse to a fully encapsulated variant of the same type. An attempt to achieve transformation under comparable conditions with a vaccine of heat-killed pneumococcus type III was unsuccessful. The authors suggested that the genetic factor for the production of polysaccharide by the intermediate variant might differ from that for the production of polysaccharide by the fully encapsulated variant as do genes of an allelic series in higher organisms. They suggested also that transformation of an R strain of pneumococcus to a fully encapsulated S variant might involve more than a single character or that variation in a separate genetic factor controlling permeability of the cell to capsular polysaccharide might be responsible for phenotypic differences.

Amplification of these observations is to be found in the detailed studies of

Taylor (38, 40) of a series of capsular variants of pneumococcus type III. Three distinct capsular phenotypes were examined: intermediate mutants resembling the capsular variant of pneumococcus type II described by MacLeod and Krauss and designated SIII-1, intermediate mutants resembling fully encapsulated strains of other pneumococcal capsular types designated SIII-2, and fully encapsulated, typically mucoid strains of pneumococcus type III designated SIII-N. Serologic and enzymatic studies with the three varieties of pneumococcus type III indicated that the polysaccharide produced by each was similar. Transforming principle derived from any one of the three capsular variants gave rise in transformation reactions with competent R cells to transformed cells resembling the variant from which the transforming principle was obtained. Transformation of R derivatives from the two intermediate types of capsular variant showed that each could be transformed to the SIII-N variant under the influence of the transforming principle of an SIII-N strain, indicating that the intermediate variants were capable of synthesizing normal quantities of type III polysaccharide when possessing the transforming principle of the SIII-N strain. That the difference among the type III pneumococcal variants was not due to quantitative differences in the type III transforming principle was evidenced by failure to detect, with rare exception, the intermediate variants in populations transformed with the SIII-N principle. The results are interpreted to indicate that each of the SIII transforming principles is a distinct entity.

Experiments with an SIII-1 variant showed that it was capable of being transformed under the influence of certain transforming principles when grown in a suitable environment. Agglutination of the cells of this variant in the transforming system was achieved by growing them either in the presence of the enzyme hydrolyzing type III polysaccharide plus R agglutinins or in the presence of small amounts of SIII agglutinin. When exposed to transforming principle from an SIII-N strain, the SIII-1 strain was transformed regularly to the fully encapsulated variant of type III. That the reaction was in fact a transformation of the SIII-1 mutant was indicated by the dependence of the reaction upon the quantity of SIII-N transforming principle, the extreme rarity of spontaneous mutation of SIII-1 cells to SIII-N, the necessity for the presence of serum factor for the reaction to occur with regularity, and the requirement of a sensitizing period during which the reaction could be blocked by the introduction of desoxyribonuclease. Transforming principle obtained from SIII-N cells arising from transformation of SIII-1 mutants, when applied to an R variant of pneumococcus, gave rise only to SIII-N variants. No SIII-1 cells were formed to indicate that cells from which the transforming principle was obtained had ever existed in the form of the SIII-1 variant. There had been, therefore, either an exchange of SIII-1 transforming principle within the cells for the SIII-N principle in the environment or the former had in some manner been modified by the latter.

When analogous transformations were carried out with cells of the SIII-1 variant and the transforming principle of an SIII-2 strain, three types of cells were detected at the end of the reaction: residual untransformed SIII-1 variants, large numbers of SIII-2 variants, and a small number of SIII-N variants. Inas-

much as the action of SIII-2 transforming principle upon R pneumococci led always to the appearance of SIII-2 variants, some form of interaction between the transforming principle of the SIII-1 strain within the cell and that of the SIII-2 mutant introduced into the transforming system must be postulated to account for the appearance of the SIII-N cells. In the reaction of the SIII-2 transforming principle with the SIII-1 cell, two types of activity could, therefore, be distinguished, one leading to the appearance of cells of the phenotype from which the transforming principle was obtained and the other resulting in the appearance of cells differing in phenotype both from the cell transformed and that providing the transforming principle. Taylor (40) has designated the former type of transforming activity, "autogenic", the latter, leading to the appearance of a new phenotype, "allogenic".

A study of a variety of SIII-1 mutants has revealed that while such strains may be phenotypically similar, they may differ in their genetic constitution. A given SIII-1 strain, grown in the presence of the transforming principle of an heterologous SIII-1 strain, may undergo allogenic transformation to one of the capsular variants, SIII-2 or SIII-N. The type of allogenic transformation is determined by the source of the transforming principle and the strain to be transformed; and the relations are reciprocal, the same allogenic transformation resulting if the strain yielding transforming principle and the one undergoing transformation are interchanged. In no instance has the simultaneous occurrence of two allogenic transformations resulted from exposure of an SIII-1 mutant to the transforming principle of a single heterologous strain. Neither has allogenic transformation been observed when a given SIII-1 strain was grown in the presence of its own transforming principle. When SIII-1 strains have been transformed by extracts of SIII-2 organisms, both autogenic transformation to capsular type SIII-2 and allogenic transformation to capsular type SIII-N have been noted. Transforming principles from different SIII-2 strains have differed in their ability to induce allogenic transformation in the same SIII-1 strain indicating that phenotypically similar strains of this capsular variant also may differ in their genetic structure. Study of the transforming principles obtained from the allogenic strains of pneumococci has failed to reveal any indication that they contained more than one factor concerned with the production of capsular polysaccharide. These results are compatible with the earlier suggestion (27) that, in certain strains of pneumococci, the factors concerned with the quantitative control of capsular polysaccharide production may be related in a fashion analogous to genes in an allelic series. They do not exclude, however, the possibility that, in other strains, the production of capsular polysaccharide may prove to be influenced by more than a single factor.

To explain these variations in the behavior of the transforming principle responsible for the production of pneumococcal capsular polysaccharide, Taylor has advanced the following argument and hypothesis. In view of the fact that most recent knowledge concerning the structure of chromosomes indicates that they are composed of proteins, ribonucleic acids, and desoxyribonucleic acids and that the former two classes of compounds are not detectable in the highly

purified preparations of transforming principle, it appears improbable that the activity of transforming principle resides in chromosomal fragments. The experimental results, however, can be accounted for most simply by envisioning the factor responsible for the normal production of capsular polysaccharide as a linear structure, any alteration of which results in a quantitative reduction in polysaccharide production. When the transforming principles of two mutant strains are altered in such fashion that their defects overlap partially, interaction between them results only in partial reconstitution of the normal transforming principle and in partial restoration of quantitatively normal polysaccharide production. When the two mutational defects are so placed that interaction between the two transforming principles results in reconstitution of the normal linear structure, then normal production of polysaccharide ensues. It is recognized that, at present, there is no knowledge available to enable physicochemical characterization of the hypothetical linear structure and that if, as is thought, the capsular principle is a molecule of desoxyribonucleic acid, it is unlikely that a simple geometric image describes the true relation of the mutant capsular principles. However that may be, it has been demonstrated that the agent responsible for the production of capsular polysaccharide is a unit composed of subunits, even though their nature at present cannot be defined. That spontaneous mutation of these "subunits" may result either in increasing or in decreasing polysaccharide synthesis is noteworthy.

Capsular type transformation has been carried out with *H. influenzae* (1, 2) as well as with pneumococcus. Transformation of cells with acquisition of capsular polysaccharide has been accomplished with R variants from capsular types *a*, *b*, and *d*, and transformation to each of the six known capsular types has been effected with transforming principle derived from homologous strains. The R strain derived from a culture of capsular type *a* could be transformed in controlled experiments only to the parent type, a finding suggestive of the possibility that intratype transformation may occur in *H. influenzae* as well as in pneumococcus. As noted earlier, transformation of influenzal capsular types may take place within fifteen minutes following exposure of a competent strain to transforming principle without the "sensitizing" period required by pneumococcus and in an environment lacking not only in serum factors but failing also to support multiplication.

Transformation of a strain of *E. coli* with acquisition of the ability to produce a new surface polysaccharide has also been reported (8). From the parent S culture of one strain, a series of R variants was obtained, one of which, when grown in the presence of either culture filtrates or the desoxyribonucleate fraction of an heterologous, polysaccharide producing strain, acquired the ability to produce the polysaccharide of the heterologous strain. As with *H. influenzae*, the reaction took place in the absence of serum factors. Transformation was accompanied by loss of the ability to attack sucrose, but the significance of this accompanying alteration was difficult to assess in view of the spontaneous mutability of sucrose fermenting activity.

Using culture filtrates, Weil and Binder (42) were able to obtain alteration

of cells of three antigenic types of *S. paradysenteriae* to two heterologous types, the reactions taking place in ordinary bacterial media without the development of detectable R variants. In one instance, the antigenic change was accompanied by loss of the ability to form indole. The chemical nature of neither the active material in the culture filtrates nor of the transformed antigen was ascertained, and it is difficult to be certain that the reactions were comparable to those more precisely defined in other bacterial species.

At the present time it is doubtful that any interspecific transformation of surface polysaccharides has been achieved. Though Coleman and Reid (11) have reported the conversion of strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the "S" phase to the "M" phase of the heterologous species, it is unlikely that taxonomic separation of these two groups of organisms rests on very solid grounds. By growing an unencapsulated "S" strain of *P. tumefaciens* in the presence of culture filtrates of an encapsulated "M" strain of *A. radiobacter*, they were able to demonstrate agglutination of the strain of *Phytomonas* by antisera against the "M" phase of *A. radiobacter* following such treatment. Alteration of "S" variants of *A. radiobacter* under comparable conditions was not achieved. Whether or not a true transformation occurred in these experiments cannot be established from the published data. Further experiments in which tomato plants were inoculated with an unencapsulated variant of *A. radiobacter* resulted in tumor formation and recovery of cells agglutinable by antisera against the "M" phase of *P. tumefaciens*. The latter observation suggests the possibility that spontaneous mutation to either "M" phase may occur and raises a question as to whether or not transformation, in the absence of more adequate controls, has actually been demonstrated in the *in vitro* experiments.

The possibility of carrying out interspecific transformation with bacteria producing immunologically related antigens has been considered at times, but evidence for the successful completion of such a reaction is still wanting. It is perhaps unlikely that such a reaction can be effected between any but closely related "species". A report describing the temporary agglutinability of an unencapsulated variant of *Bacillus friedländeri* (*Klebsiella pneumoniae*) by type II antipneumococcal serum following its exposure to an autolysate of type II pneumococci has appeared (10), but the data presented are inadequate to establish the results as an example of bacterial transformation.

2. *Bacterial Proteins.* Transformation of bacteria with the acquisition of a somatic protein has been demonstrated in pneumococcus. Like group A β-hemolytic streptococci, pneumococci produce somatic type-specific proteins similar in their properties to the M proteins of the former species (3). When an unencapsulated variant of pneumococcus is selected from an encapsulated strain possessing both type-specific polysaccharide and protein antigens, the protein antigen persists as a property of the unencapsulated cells. Transformation of such cells with the transforming principle of a pneumococcal strain producing heterologous types of both capsular polysaccharide and M protein results usually in the acquisition of ability to produce the heterologous polysaccharide by the

strain undergoing transformation without alteration of its original M protein (4). It appears, therefore, that production of type-specific capsular carbohydrate and of somatic M protein varies independently of one another. These findings are of interest in view of the natural occurrence of pneumococcal strains of the same capsular type associated with different M proteins and of different capsular types associated with the same protein.

When an unencapsulated R variant of pneumococcus is grown in the presence of homologous anti-M protein serum or when an ER variant is selected from it by appropriate cultural techniques, strains are obtained which produce apparently less M protein than those from which they were derived. When such strains are transformed *in vitro* in the presence of the transforming principle of an heterologous encapsulated strain, two types of clones may be obtained, one producing the M protein of the parent culture and the capsular polysaccharide of the heterologous strain and the other, having undergone a double transformation, producing both the capsular polysaccharide and M protein of the heterologous strain. Unencapsulated variants selected from a culture having undergone originally double transformation are capable of being transformed to a third capsular type. Thus, inheritable characters of two heterologous strains may be combined within the cells of a third strain. Transformation of pneumococci with the acquisition of type specific M protein may also be accomplished *in vivo* with the technique of Griffith. Study of transformation reactions concerned with M protein has been made difficult by lack of a simple technique for the recognition of such transformations and by want of a strain with a genetic deficit for the production of M protein which might undergo regularly transformation with acquisition of this character. The double transformations effected with transforming extracts derived from encapsulated strains of pneumococci suggest that these extracts contain a multiplicity of transforming principles. Although the extracts employed in these experiments were not devoid of ribonucleic acid and pneumococcal polysaccharides, it is highly probable that the active agent was a desoxyribonucleic acid. Similar experiments with other bacterial species have not been reported.

3. *Bacterial Colonial Forms.* Many bacterial species are known to exist in a variety of morphologic forms. Inheritable change from one morphologic type to another is believed at present to arise through the process of mutation, selective factors in the environment favoring the predominance of one or another of such mutants under any given set of conditions. Morphologic variation within a given species may give rise to recognizable differences in the appearance of clones of such variants when they are grown on solid media. To describe such differences, the designations mucoid (M), smooth (S), and rough (R) have frequently been employed. It is regrettable that the same designations have not always been applied in the naming of analogous variants in different bacterial species. In describing pneumococcus, the term smooth (S) has been widely used to indicate encapsulated forms and the term rough (R) to designate the unencapsulated variant described by Griffith (18). By analogy, these forms correspond respectively to the mucoid and smooth variants in certain other bacterial

species, but there has been hesitancy on the part of those interested in the study of pneumococcus to change the nomenclature applied to variants of this organism lest confusion be created thereby. In 1934, Dawson (12) described a second unencapsulated variant of pneumococcus which resembled the rough variants of other bacterial species by virtue of its morphology and the appearance of its growth both in liquid and on solid media. Taylor, in studies employing a similar variant of pneumococcus, has referred to it as "extremely rough" (ER). While duplications of nomenclature are not altogether desirable, the use of the term ER to indicate the rough pneumococcal variant described by Dawson provides an unambiguous designation for current use.

Of the cellular characteristics of pneumococcus responsible for differences in colonial appearance, the factor distinguishing R from S is best understood. The glistening mucoid appearance of S colonies derives from the production by cells of this form of appreciable quantities of capsular polysaccharide. It is noteworthy, however, that the phenomenon of "smoothness" involves certain quantitative considerations. A strain of pneumococcal cells may produce type-specific capsular polysaccharide in such small amounts that, when grown on solid media, its colonies are indistinguishable from those of R variants which fail to produce any capsular polysaccharide. Such a strain, from a morphologic point of view, may appear to be rough, whereas from an immunochemical and genetic point of view, it is better classified among smooth forms. Such considerations make any rigid classification of morphologic variation undesirable until the underlying bases thereof are understood.

Little is known concerning the basic differences between the R and ER variants of pneumococcus. Each variant can be obtained from the other by appropriate selection of spontaneously occurring mutants, and a variety of forms intermediate between the two extremes of colonial form can be recognized. Viewed morphologically at the cellular level, the most readily observable difference resides in the separation of cells after division, those of the R variant separating into pairs or short chains while those of the ER variant tend to remain attached to each other forming chains of great length which may be composed of several hundreds of cells. The roughness of growth on solid media (7) and the autoagglutinable growth in liquid media of the ER variant can be attributed to this property. The mechanism whereby chain length is determined in the growth of pneumococcus is at present unknown. The spontaneous variability of growth in the direction of either increasing or diminishing chain length presents, however, certain analogies to quantitative variation in capsular polysaccharide synthesis.

Strains of the ER variant are capable of transformation to the R variant and ultimately to the encapsulated S variant (4, 38). Working with an ER strain derived from pneumococcus type II, Taylor showed that the ER variant could be transformed to the R variant with the transforming principle of either R or S strains under conditions which permitted but rarely the detection of spontaneously occurring R mutants. The reaction required the presence of serum factor in addition to nutrient broth and a sensitizing period of approximately five hours during which the addition of desoxyribonuclease would inhibit the

activity of the transforming principle. It was noted also that the presence of rough cell agglutinins in the transforming system inhibited the transformation of Taylor's ER strain to the R variant. If, however, such antibodies were introduced into a system containing ER cells and transforming principle from an S strain after an initial growth period of five and three-quarter hours during which transformation from ER to R had occurred, a second transformation of R to S would take place under the influence of the same transforming principle. In this fashion, the presence of at least two distinct transforming activities in highly purified transforming principle derived from a single strain of pneumococcus has been demonstrated. The inhibiting effect of rough cell agglutinins upon the transformation of ER cells to the R variant varies among different ER strains, for certain strains may be transformed to R or to S forms in the presence of R agglutinating antibody (4). The reasons for this difference in the behavior of different strains are not apparent.

Of great interest is the observation of Taylor (39) that an R strain of pneumococcus could be transformed to the ER variant under the influence of the desoxyribonucleic acid fraction derived from the ER strain studied by her. The reaction was carried out in the presence of anti-R agglutinins but under conditions which do not lead to the detection of spontaneously occurring ER variants. To explain the reciprocal nature of the R—ER transformation, Taylor has suggested that both variants may possess the same number of genetic determinants and that the transformation may result from exchange of a determinant within the cell for one present in the transforming principle in the presence of which the cell is grown. The experimental observation just described has been confirmed by the writer, and the transformation of R variants to the ER form has been effected with R cells derived from one capsular type and transforming extracts of ER cells obtained from an heterologous capsular type (5).

The experiments previously reviewed show clearly that variation of colonial morphology in pneumococcus, at least, is subject to a type of genetic control.

4. *Drug Resistance.* The resistance developed by bacteria to the inhibiting or lethal effects of certain antibacterial compounds has been recently a subject commanding considerable attention among biologists, and the importance of this problem to clinical medicine has made knowledge of the mechanisms whereby drug resistance develops a topic of especial interest. Studies of bacterial populations during the process of acquiring resistance to any one of several antibacterial agents have suggested that such insensitivity may be genetically determined and that the emergence of a population resistant to a given drug may result from the selective effect of that agent upon mutants arising spontaneously from the original population (9). Additional scrutiny of the phenomenon has suggested also that, in certain instances, increasing resistance may be the sequel to selection of organisms having undergone a multiplicity of mutational changes.

The first reported study of the problem of drug resistance with transformation reactions was that of Langvad-Nielsen (24). Inoculating an unencapsulated living, sulfapyridine-sensitive R pneumococcus together with a heat-killed vaccine of an encapsulated, sulfapyridine-resistant pneumococcus subcutaneously

into mice, he looked for sulfapyridine-resistant organisms in animals succumbing to infection. That alteration of resistance was not observed is not altogether surprising in view of the fact that the pneumococci would have had to have undergone a double transformation reaction acquiring the factors controlling both drug resistance and encapsulation.

More recently, the transfer of penicillin resistance from resistant to sensitive pneumococci by means of transformation reactions has been described by Hotchkiss (21). By growing sensitive pneumococci in the presence of penicillin, he was able to select spontaneously occurring penicillin resistant mutants from such populations. When penicillin sensitive, unencapsulated pneumococcal cells were grown in a transforming system containing the desoxyribonucleic acids of a selected resistant strain, resistant cells were recovered in ten thousand times the numbers found to arise through spontaneous mutation. It is significant that resistance to penicillin acquired by pneumococci in this fashion developed in the absence of contact with the drug and was maintained by the transformed cells indefinitely. That the reaction was controlled by desoxyribonucleic acids was demonstrated by the ability of desoxyribonuclease, when introduced into systems containing highly purified transforming principle, to block the acquisition of penicillin resistance. It was shown, moreover, that, when sensitive cells were transformed in the presence of desoxyribonucleic acids of a strain thirty or more times as resistant to penicillin as the sensitive strain, resistance was acquired in stepwise fashion, transformed cells selected after single exposure to transforming principle never equalling in resistance that of the strain from which the transforming principle was derived. Successive exposures of cells having acquired demonstrable but slight resistance to penicillin to the same transforming principle responsible for the initial change resulted in the demonstration of the acquisition of two additional increments of drug resistance. These experimental data present certain analogies to the quantitative variations in capsular polysaccharide production discussed previously, and it is of interest to speculate whether the mutational steps concerned with drug resistance may involve successive modifications of a single genetic factor and/or alterations in several unrelated genetic units. However that may be, the observations are in accord with those describing certain patterns of development of drug resistance through selective cultural techniques.

Study of unencapsulated and encapsulated, penicillin resistant pneumococci showed either to be a satisfactory source of desoxyribonucleic acids for the transfer of penicillin resistance to sensitive cells. When an encapsulated resistant strain was the source of the transforming principle, the factors controlling capsule formation and drug resistance were shown to be acquired independently and with different frequencies, penicillin resistance appearing ten to fifty times more often than encapsulation.

In describing the transfer of penicillin resistance, Hotchkiss has reported also an important modification in the technique for carrying out transformation reactions in which a simple pneumococcal lysate was employed in place of more highly purified transforming principle. When a strain of pneumococci sensitive

to penicillin was grown in the presence of that drug, the cells soon died and lysed. If competent penicillin resistant cells lacking characters possessed by the sensitive strain were grown in the presence of a lysate of the latter, some acquired one or another of the characters controlled by the transforming principles yielded by the lysed, sensitive cells. The lysate could be added to the resistant cells or could be formed in their presence by the addition of penicillin to a culture containing both sensitive and resistant strains. The dependence of the reaction upon desoxyribonucleic acid was demonstrated by the effect of desoxyribonuclease which destroyed completely the activity of such lysates. Because transformation with lysates requires only small numbers of cells (*ca.* 10^5) and obviates the necessity for isolating transforming principle in a more or less highly purified form, it is an extremely efficient technique for investigating those characters of the pneumococcal cells the inheritance of which is controlled by desoxyribonucleic acids and one which should find wide application. By growing an unencapsulated, streptomycin sensitive, penicillin resistant pneumococcus in the presence of lysates of an encapsulated, streptomycin resistant, penicillin sensitive pneumococcus, Hotchkiss succeeded in demonstrating the acquisition through transformation of streptomycin resistance and of ability to produce capsular polysaccharide by the penicillin resistant strain.

Of equal interest is the suggestion of this investigator that conditions for transformation with lysates may arise in nature. Through such reactions, acquisition of characters of strains unable to survive in certain environments by resistant cells concomitantly present might prevent the biological extinction of traits possessed by the sensitive cells. Such a view is in accord with the observed antigenic combinations in pneumococcus described previously (3).

5. *Other Bacterial Characters.* In pneumococcus, failure to induce the acquisition of ability to ferment mannitol and inulin concomitant with transformation of capsular type *in vivo* has been reported by Langvad-Nielsen (24). Loss of sucrase activity by *E. coli* (8) and of indole production by *S. paradysenteriae* (42) during transformation reactions has been reported also, but the significance of these changes in activity is obscure. Whether they represent selective transformation of spontaneously occurring, deficient mutants or the acquisition of factors masking previously demonstrable activities is unknown.

Recently two reports by Dianzani have appeared describing "mutation in the enzymatic equipment of *Escherichia coli* and *Proteus* OX19 directed by desoxyribonucleic acid isolated from bacteria of the same and of different species" (15, 16). Ability to oxidize sucrose was manifested by one strain of *E. coli* lacking previously this ability after multiple passages in the presence of the desoxyribonucleic acid fraction of heterologous strains of the same species and of *Proteus* OX 2 and of two species of *Salmonella*. Ability to attack sucrose was manifested also by the same strain after 22 passages in the presence of this sugar. Analogous alterations in oxidative capacity were shown by a strain of *Proteus* OX19 transferred repeatedly in the presence of desoxyribonucleic acids from *Proteus* OX 2, from two strains of *E. coli*, and from two strains of *Salmonella*. The results are somewhat difficult to compare with transformation reactions described by others

for the manifestation of the new character developed only after repeated passage of the mutating strain in the presence of the nucleic acids. In commenting upon these experiments, Lederberg (26) has called attention to the fact that tests for enzymatic activity were carried out with cells harvested from plain agar so that the possible role of enzymatic adaptation to the different carbohydrate substrates could not be evaluated. In addition, Dianzani's report of spontaneous adaptation of control cultures to certain of the carbohydrates studied has raised questions concerning the stability of such cultures grown in the absence of desoxyribonucleic acid. Comparison by Lederberg of one of Dianzani's induced sucrose-oxidizing cultures with its parent culture and with the sucrose-positive transforming culture in fermentation tests failed to reveal any distinction between the first two cultures, both of which were quite different from the third. Further data are needed, therefore, before the phenomena reported can be related properly to other observations in the field of bacterial transformations.

The description of the acquisition of pigment production by a nonchromogenic strain of *Staphylococcus aureus* following incubation in the presence of an extract of a chromogenic strain (10a) presents similar problems of evaluation. The method of preparation of the extract, the inconstancy of results, and the transitory nature of the induced chromogenesis together with the paucity of evidence for the presence of desoxyribonucleic acid in the bacterial extract make necessary reservations in accepting the observations as an example of bacterial transformation.

From the data discussed, it is apparent that considerable progress has been made in the field of bacterial transformations since the subject was reviewed by McCarty (31). A growing body of increasingly precise data gives continuing support to the view that desoxyribonucleic acids are indeed the biochemical determinants of inheritable characters in pneumococcus and that they function probably as such in at least two other, if not in all, bacterial species. Much indirect evidence supports the view that desoxyribonucleic acids are concerned with genetic mechanisms in all living forms, and any experiments relating their presence directly to inheritable characters of any cellular form are of general biological interest. For this reason, bacterial transformation reactions take on additional significance.

Continued study of pneumococcus has revealed that an increasing number of its inheritable characters are susceptible to control through transformation reactions. Ability to modify predictably certain cellular characters in quantitative or qualitative fashion has permitted a better understanding of the complexity of the factors concerned with their inheritance. It has enabled also a better appreciation of the role of such characters in pneumococcal infections and has given insight into the relationships of different classes of antigens within a single bacterial species. These latter observations when related to the antigenic structure of naturally occurring pneumococcal strains are compatible with the view that transformation reactions may take place in nature. If such reactions do take place between strains of pneumococci or of other bacterial species, it is conceivable from the studies of Taylor concerned with relatively avirulent capsular

mutants of pneumococcus that a reaction involving two strains of negligible virulence might result in the emergence of one of high virulence. Whether or not bacterial transformations do occur in nature under other than artificial circumstances remains an unanswered question.

The demonstration of transformation reactions in bacterial species other than pneumococcus gives this reaction wider significance than it possessed heretofore. Its description in *E. coli*, in which genetic and morphologic studies have revealed the probable presence of a chromosomal structure, gives promise that the genetic activity of soluble desoxyribonucleic acids may be linked ultimately to a discrete morphologic unit directly concerned with the control of inheritance. The ability of this species to grow and to undergo transformation in relatively simple media and the wide variety of biochemical mutants obtainable from it make it a potentially useful subject for the study of the hereditary control of a number of cellular components.

It may be anticipated that future investigations in the expanding field of bacterial transformations will yield additional data of both general and special biological interest.

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