ATTEMPTS TO DEMONSTRATE TYPE TRANSFORMATION WITH KLEBSIELLA PNEUMONIAE

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Since Griffith's demonstration of capsular type transformation in pneumococci a vast amount of research has been done on pneumococcal and other type transformations with a variety of genetic markers. (For general reviews see Ephrussi-Taylor, 1951; Austrian, 1952.)

Type transformations in pneumococci have been obtained by in vivo and in vitro methods. For in vitro transformations competent cells, a suitable environment, a transforming principle, and experimental methods adequate to demonstrate transformations which may occur at a low frequency have been found to be necessary. Only certain strains yield competent cells, and competency can be determined only by testing the cells in a transforming system. For a suitable environment anti R serum, serous fluid, bovine serum albumin, or Fraction V of bovine serum albumin has been found to be necessary. The transforming principle has been found to be a biologically active desoxyribonucleic acid (DNA). Various methods of extracting and purifying this principle have been devised.

Analogous transformation reactions have been described for Escherichia coli, Hemophilus influenzae, and Neisseria meningitidis (Alexander and Redman, 1953). Broth that will support growth has been found to be adequate for the environment. In some cases the environment does not have to support growth.

Reports of transformations which are based on less complete studies include: sulfonamide resistance in E. coli, streptomycin resistance in Salmonella typhosa, pigment production in Staphylococcus aureus, colony morphology in Shigella, and Vi antigen in Salmonella.

How extensive is type transformation? It has been demonstrated for only a limited number of species. Apparently failures to obtain type transformation have been common although they have not always been reported. One such failure was that of Atchley (1951) who attempted to transform phage resistance or synthetic ability in four strains of E. coli by employing several well-known techniques.

This paper reports on unsuccessful attempts to produce capsular type transformation in Klebsiella pneumoniae. This organism was thought to be especially suitable for study since its capsular polysaccharides provide a means of serological identification (Julianelle, 1926; Kauffmann, 1949; Edwards and Fife, 1952).

EXPERIMENTAL METHODS AND RESULTS

The studies carried out are shown in the outline which follows. (For details of procedure see Balows, 1952.) In general the techniques used were those which had been used successfully by other workers with other organisms, or slight modifications of such procedures. When these techniques failed, since it was thought that the heavy capsule of K. pneumoniae might have interfered with the extraction of the postulated transforming principle, extractions were tried by several procedures using cells that had been decapsulated by C lysin (Humphries, 1948). Decapsulated living cells were also tried as a source of competent cells.

I. In vivo studies:

A. Strains used: 108 (Type A, Julianelle), 83 (Type B, Julianelle), 47 (Type B), 2 (Type B).

B. Injections: Cell suspensions from mucoid strains killed by formalin, toluene, or heat + living S cultures of other strains.

C. Experimental animals: white mice, injected intraperitoneally or subcutaneously.

D. Results: no demonstrable transformation.
II. In vitro studies:
   A. Strains used as source of postulated transforming principle: Same as for in vivo studies.
   B. Preparations tested for transforming principle:
      1. Tryptone-glucose broth culture filtrate.
      2. Chloroform autolysate of tryptone-glucose broth culture (Vendrely, 1947).
      3. Absolute ethanol precipitate of chloroform autolysate dissolved in slightly alkaline saline (positive Stumpf, 1947, and Dische, 1944; reactions for DNA; reactions negative after treatment with DNAase).
      4. Absolute ethanol precipitates of deproteinized (Sevag, Lackman, and Smolens, 1938) sodium deoxycholate and sodium hypochlorite lysates, and alumina disrupted cell suspensions (positive Stumpf and Dische reactions, negative or slight reactions for carbohydrates and proteins).
      5. Chloroform autolysates and precipitates of sodium deoxycholate lysates of cell suspensions from C lysin-treated cultures (Humphries, 1948) (DNA positive).
      6. Bacteriophage lysates of mucoid strain 108 cells; bacteriophage removed by ultrafiltration or inactivated by heat.
   C. Test strains (source of postulated competent cells): Two smooth and one rough substrain each of strains 108, 83, 47, and 2; one smooth strain each of Kauffmann (1949) types 6, 7, 9, 10, 11, 13, and 14, and Worfel-Ferguson (1951) type 15, C lysin-decapsulated cells of mucoid strain 108.
   D. Environments tested:
      1. Tryptone glucose broth.
      2. Tryptone glucose broth + polyvalent S antiserum.
      3. Tryptone glucose broth + bovine albumin.
      4. Tryptone glucose broth + Fraction V of bovine albumin.
   E. Results: no demonstrable transformation. Attempts to obtain DNA positive precipitates from Tergitol 7, Tergitol 08, sodium lauryl sulfate PT, and glycine lysates failed.

DISCUSSION
Several interpretations of the results of this study suggest themselves: (1) K. pneumoniae is genetically stable in that it is not subject to type transformation as are other bacterial species, (2) the DNA extracts prepared were inactive, (3) the environmental conditions furnished were unsuitable for type transformation, (4) none of the strains used furnished competent cells. The second and third of these interpretations appear to be highly unlikely since the methods of DNA extraction and the environmental conditions included those which have been used successfully for type transformation of other bacterial species. To accept the first interpretation as correct it is necessary to postulate that type transformation is not a universal phenomenon, as has frequently been observed in recent years. In spite of the number of different strains that we have employed, the last interpretation appears to be the most probable since, with other species of bacteria that have been studied, competent strains appear to be in the minority.

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SUMMARY
An extensive series of in vivo and in vitro experiments was conducted to determine if capsular type transformation could be produced with a representative group of strains of Klebsiella pneumoniae. As most of the procedures used were those which have been successful with other organisms, failure to obtain type transformation is thought to have been due either to the unsuitability of K. pneumoniae for the demonstration of type transformation or to the absence of competent strains among those studied.

REFERENCES