It appears as if Griffith may have been exploring the qualities of various immune environments, including concurrent vaccination, when he discovered the cell --x cell effects. If he had tried mixed culture in vitro (in line with paragglutination precendents) he does not say so. And without a special selective environment it would of course not have worked.

System 1930 [White] ~1972

diffused uniformly throughout the serum. R colony cultures obtained at any stage of the passage through serum are incapable of multiplying in the mouse, and do not produce a fatal septicæmia (unless reversion to the S form takes place), though large doses intraperitoneally may cause death from toxic action. There is some evidence, as shown by differences in capacity to revert to the virulent form, that strains from individual R colonies are not equally attenuated.

2. Growth in bile causes attenuation and changes in the character of the colonies similar to those obtained in immune serum.

3. Other methods of producing attenuation are by growth in optochin, in meat infusion, in acid broth and at temperatures over 39° C.

4. Attenuated R strains have also been obtained from the blood of highly immunized horses inoculated with living virulent pneumococci.

Pneumococcus cultures which have become partially attenuated in the course of cultivation on artificial media and have dissociated into a mixture of R and S forms regain their virulence after passage through animals. The effect is in part the result of the elimination of the attenuated forms, and the survival of those best suited for multiplication in the animal body.

Selection of particular cells, however, is not the only mechanism involved in increase of virulence. Avirulent strains derived from single pneumococci, and wholly composed of R forms, may revert to the virulent S form after inoculation into animals (see p. 209). It must also be mentioned that Dawson and Avery (1927) and Dawson (1928) found that virulence and type-specificity could be restored to R pneumococci by growth in an antiserum prepared by immunizing rabbits with avirulent R pneumococci. Moreover, Felton and Dougherty (1924) have reported that virulence may be increased *in vitro* by frequent transfers in milk at 4-hourly intervals; they also found that peptone in 2 per cent. solution maintained and even increased the virulence of a strain of pneumococcus. The possibility of determining virulence by the supply to the growing organism of definite chemical substances is a subject worthy of further study.

as far as he perceived!

Pneumococci obtained from the lesions of pneumonia patients are generally of high virulence for mice, causing fatal septicæmia within 48 hours in a dose of 10^{-7} c.cm. of broth culture. On the other hand, these same sources have yielded cultures which failed to kill in much larger doses, and Neufeld and Händel found strains of pneumococci which killed mice when injected in very small doses, but only after a period of 5 to 7 days. As a rule pneumococci freshly isolated from human beings only attain their maximum virulence for a particular species after several passages through animals of that species. Moreover, high virulence for one species does not necessarily signify equally high virulence for another species. Tillet (1927) examined 11 strains of Type III, obtained from cases of pneumonia, 10 of which were of low virulence and 1 of high virulence for rabbits, while all killed mice in a dose of 10^{-7} c.cm.

artificial cultivation under suitable conditions. After many years sub-cultivation the chief type strains show no divergence from their original characters, provided that their virulence is maintained by occasional passage through animals. Whether pneumococci may change from one type to another under natural conditions, e.g. in human infections, has not been shown, though it is difficult to conceive that the chief types, as well as the innumerable serological varieties of Group IV, are absolutely fixed and unalterable. It is well known that after recovery from pneumonia the infecting type, I or II, tends to disappear from the respiratory tract and is often replaced by a Group IV strain. Frequently several different varieties of Group IV may be found in the sputum of a convalescent pneumonia patient. According to the more generally accepted view, the chief types die out and the Group IV strains, originally present in the nasopharynx before the development of pneumonia, can alone be demonstrated. As an alternative to the above hypothesis one may consider the possibility, as the author has done (Griffith, 1928), that under the influence of the immune substances developed during recovery the pneumococcus responsible for the pneumonia may assume different serological characters, that is to say, the Group IV strains may be derived from one or other of the chief types.

Modification Experiments.

Morgenroth, Schnitzer and Berger (1925) have reported that they were able by special methods to transform pneumococci into streptococci; Reimann (1927), following the methods devised by Morgenroth and his collaborators, repeated their experiments and also obtained variants. These he identified with the R form of pneumococci obtained by various other methods. The R pneumococcus produced on blood-agar colonies resembling those of *S. viridans*, but, unlike the latter, the R colonies were invariably bile-soluble though slightly more resistant to the action of bile than the type-specific pneumococcus.

The writer (Griffith, 1928) has described a method by which one type of pneumococcus can apparently be converted into another. The principle of the method is to supply to the living R form of one type of pneumococcus a pabulum consisting of a dense suspension, killed by heat, of a virulent pneumococcus of another type from which to build up its type-specific antigen. The change of type has been obtained only when the mixture of living and heated cultures are injected into mice, and not in the test-tube. A preliminary observation which suggested the procedure was the discovery that the attenuated R form of the pneumococcus when inoculated subcutaneously into a mouse together with a mass of killed virulent culture of the same type readily reverted to the original type-specific form. The R form inoculated alone rarely reverted unless very large doses were given, e.g. the deposit of 50 to 100 c.cm. of broth culture. The temperature to which the virulent culture was heated exercised an important influence on the results and conversion from one type into

i.e. a vaccine

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