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The rate of growth of virulent and avirulent streptococci in serum media.

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In a series of experiments which we reported in 1921[1] we found that after strains of beta type streptococci had been rendered virulent for mice by animal passage cultures of the virulent streptococci grew more slowly during the first few hours after they were inoculated into broth to which horse serum had been added than did cultures of the avirulent forms of the same strains. In comparing the growth of the virulent and the stock cultures of these streptococci bacterial counts were made at hourly intervals and the amount of hemolysin produced in the cultures was titrated with standardized suspensions of erythrocytes obtained from defibrinated horse blood. The amount of hemolysin produced at these intervals in these cultures corresponded with the rate of growth of the bacteria. The virulent streptococci grew slowly after they were inoculated into the broth, then after a latent growth period of several hours grew very rapidly until the sixteenth hour, after which the bacterial count gradually fell. The maximum amount of hemolysin was produced during the period of very rapid growth. The avirulent cultures showed a much shorter latent growth period. They began to grow rapidly after they had been inoculated into the broth about three hours and reached the maximum bacterial count from one to two hours before the cultures of the virulent bacteria had attained their maximum. The maximum amount of hemolysin was produced from one to two hours earlier in these cultures than in the cultures of the virulent streptococci although the maximum titers of hemolysin were equal. The avirulent forms of the streptococci always grew more rapidly under the conditions laid out for these experiments and the curve of the hemolysin titers was a constant index of the length of the latent growth period.

[1] Stevens, F. A., *J. Exp. Med.*, 1921, xxxiii, 223.

In these experiments there were several factors which may have been responsible for these differences in growth. It was possible that the species of animal from which the serum in which the bacteria were grown was responsible. To eliminate this possibility, virulent and avirulent cultures of the same strain of streptococci have been compared in the serum of the animal for which they were rendered virulent.

Five strains of streptococci were inoculated consecutively into rats until .0001 cc. of 18 hour broth cultures killed these animals on repeated tests. Following out the technique of the previous experiments we have compared the growth and the production of hemolysin with these strains in broth enriched with rat serum. The hemolysin produced was titrated with standardized suspensions of cells obtained by defibrinating rat blood. The same results were obtained in these experiments as were found in those previously reported. The avirulent strains grew more rapidly immediately after inoculation and produced the maximum amount of homolysin earlier than the strains which had been rendered virulent for mice.

Since similar results were obtained in both series of experiments it appears that the differences in the rates of growth depend on the differences in the virulence of the strains. The experiments suggest that an analogy exists between the behavior of these strains of streptococci and saprophytic and parasitic bacteria. Saprophytes which grow readily *in vitro* resemble the less virulent cultures of streptococci, while parasitic, pathogenic bacteria which are grown with greater difficulty in media resemble the more slowly growing virulent cultures of streptococci.

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Studies in narcosis.
IV. A simple and rapid method for the determination of ether in blood.

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The principle of the method here described depends upon the separation of ether from blood by boiling a tannic acid blood