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Thomsen's Hemagglutination Phenomenon. Isolation of a "J-Like" Bacillus.

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O. THOMSEN (1927) was the first to report upon a propagatable agent capable of rendering human red corpuscles agglutinable by their homologous (or any other human) serum. Friedenreich (1928, 1930) investigated this phenomenon and showed that the action of bacteria upon the red cells was the cause. Special attention was paid to two germs, designated the "J" and the "M" bacillus, respectively, isolated from somewhat old blood samples.

The following report records the isolation of a germ similar to the "J" bacillus.

In experiments concerned with blood groups in the horse the writer's colleague, Dr. P. J. J. Fourie, noted that the red cells of a certain horse were agglutinated by the homologous serum. This phenomenon occurred after the sedimented cells, in Ringer-Locke solution, had been standing for 48 hours at room temperature; when freshly drawn no hemagglutination had taken place. Further, when a drop of this suspension was added to the red cells of other horses, and 24 to 48 hours allowed to elapse, these cells, in turn, were agglutinated by their homologous sera, controls being negative.

The "J-like" bacillus was isolated by plating a loopful of the suspension on 5 per cent. horse-serum agar at 37° C. for 48 hours. Six different types of colonies were noted and numbered, and from each a small portion was sewn into a red cell suspension obtained under sterile conditions from a horse. The effect of adding homologous serum was noted at 1, 2 and 4 day periods, the cells being all the time at room temperature. After 1 and 2 days one lot of cells was agglutinated and after 4 days a second sample was clumped, the control and the other remaining suspensions of cells being negative. It was decided to more fully investigate the germ responsible for producing the early hemagglutination.

The " J-like " bacillus.

Morphology.—After 24 hours on 5 per cent. horse serum agar, the organism was noted to be a rather small bacillus, showing branching (whether true or false was not investigated) and a large number of V forms. Further incubation (2-3 days) produced much pleomorphism; the majority of the germs were pyriform, but coccoid, bacillary and swollen forms were encountered. It stained readily with methylene and thionin blue, was Gram positive and showed no irregular or beaded staining with Neisser.

Cultivation.—Growth was readily obtained after 24 hours (at 37° C.) on the usual laboratory media, being much sparser and slower at room temperature. On serum-agar after 48 hours, a white, smooth, shining, fairly luxurious growth, resembling a thin streak of paint was obtained, the individual colony being round, raised, white, smooth, and glistening, with an entire edge. In broth and serum broth, a faint to moderate uniform turbidity was noted. Litmus milk was rendered very slightly alkaline and gelatine was not liquefied. None of the following " sugars ", sorbite, inosite, glucose, laevulose, sacharrose, lactose, maltose, dulcite, mannite, galactose, salicin, adonite, inulin, raffinose (1 per cent. in 1 per cent. peptone water) was fermented (as judged by acid and/or gas formation) in 14 days at 37° C.

Young (overnight) broth cultures showed no motility, and 0·5 c.c. of such a culture, injected intraperitoneally into a mouse, produced no ill effects within 7 days.

Effect of Adding the Bacillus to Red Cells.

A loopful or a drop of culture, either broth or agar, was added at different times and as opportunity arose, to cell suspensions of 32 different horses. After standing at room temperature for 24 hours, the homologous serum was added to each sample; in every instance hemagglutination was produced, uninoculated controls being negative.

Effect of Adding Filtrate to Red Cells.

Three 100 c.c. flasks of ordinary broth were sewn with the bacillus and allowed to stand at room temperature. After 1, 3 and 7 days the contents of one bottle were filtered through a Berkefeld candle, tested for sterility and, if sterile, 1·0 c.c. added to 10·0 c.c. quantities of red cell suspension. Such suspensions, after 48 hours at room temperature, were tested, with homologous serum, for hemagglutination. In each instance, hemagglutination was produced, control uninoculated suspensions, standing under the same conditions being negative.

Discussion.

The " J " bacillus as described by Friedenreich is very similar to the germ noted here. The agglutinative effect of both appears to be identical. In their angular type of growth, their biochemical reactions and their lack of action upon such media as gelatin and milk, no difference can be detected. The " J " bacillus would appear to grow less vigorously on agar and to retain Gram's stain less firmly

than the organism here described. Friedenreich stresses that his bacillus does not grow at 37° C., the optimum temperature being about 20° C. The bacillus obtained from the horse cell suspension was isolated at 37° C. and grown as a routine measure, at this temperature. However, on two occasions this did not hold—(1) a subculture from a month old sealed-off agar slope did not grow at 37° C. but did so at about 23° C. and was then subcultivable at 37° C. and (2) three 100 c.c. flasks of broth inoculated in parallel with those mentioned under the "effect of adding the filtrate to red cells" showed no signs of growth (and no agglutinative effect) after 14 days in the incubator; further sojourn at room temperature produced no growth. It is thus possible that the optimum growth temperature may be determined by a phase of the germ or by some factor unknown.

Conclusions.

The isolation from a horse red cell suspension of a micro-organism resembling Friedenreich's "J" bacillus, is described. This germ, added to red cells, produced Thomsen's hemagglutination phenomenon.

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