STUDIES OF MICROORGANISMS IN SIMULATED ROOM ENVIRONMENTS

V. The Effect of Survival on the Pathogenic Properties of Streptococci: Properties Other than Mouse Virulence[1]

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In the previous paper (1941) it has been shown that exposure to the environment of an experimental chamber for periods of time up to ten days had slight or no influence on the titratable mouse virulence of a highly mouse-virulent strain of beta hemolytic streptococcus. However, the attempted correlation of any single laboratory test for the virulence of streptococci with actual human virulence might be regarded as a risky matter. The experiments reported here were undertaken, therefore, to determine whether other pathogenic properties of streptococci were affected by a similar environment. The properties studied were (1) ability to grow in defibrinated human blood; (2) ability to lyse human blood clot; (3) ability to produce a skin toxin for rabbits; (4) susceptibility to agglutination by homologous immune serum.

ABILITY TO GROW IN DEFIBRINATED HUMAN BLOOD

Two strains of Group A streptococci were used: C3 of the New York City Department of Health, and a strain recently isolated from a human throat. The cultures were sprayed into and recovered from the artificially illuminated chamber as described previously (1941). Cultures recovered at regular intervals for

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seven days were tested for their ability to grow in defibrinated human blood. The test was performed as follows: 0.02 ml. of a 1×10^{-8} dilution of the second 18-hour blood-broth transfer was mixed with 0.2 ml. of freshly drawn defibrinated human blood, 0.05 ml. of the mixture was then plated in sheep's blood agar as a control. The remainder was sealed in a tube 75 × 10 mm. with a freshly paraffined cork and rotated at 37°C. for 3 hours, when 0.05 ml. of the mixture was plated out to determine the outcome.

Ninety-one cultures of strain C3 recovered from the experimental room were studied. They were distributed as regards duration of exposure as follows: one day, 16; two days, 16; three days, 16; four days, 14; five days, 13; six days, 12; seven days, 4. In addition, seven single colony isolations of the stock culture which had not been exposed were tested as controls.

It was found that in each instance the recovered cultures as well as the controls increased from an average number of about 600 organisms per unit volume to more than 50,000.

Twenty-nine cultures of the freshly isolated throat strain were recovered from the experimental room at daily intervals for five days. They were exposed as follows: one day, 5; two days, 6; three days, 6; four days, 6; and five days, 6. In addition, six single colony isolations of the unexposed culture served as controls.

In every instance it was found that the recovered cultures as well as the controls increased from an average count of slightly less than 300 per unit volume to variable numbers ranging from two to more than 100 times the initial concentration. The average increase was about 40 times. No apparent difference between control and recovered cultures was noticed.

ABILITY TO LYSE HUMAN BLOOD

The strain C3 was used and the recovered cultures were those described above. The technique was that of Tillet and Garner (1933). It was modified to the extent that the 18-hour whole-broth culture was diluted one to fifty rather than undiluted. This was done so that lysis of the control cultures would occur in two hours rather than 20 minutes, which may have served as a form of titration.

It was found that of 99 cultures recovered over a period of seven days, all lysed human blood clot in about two hours, as did eight control cultures. The earliest lysis occurred in one hour and fifty-seven minutes and the latest in two hours and ten minutes.

ABILITY TO PRODUCE A SKIN TOXIN

The same recovered cultures of strain C3 were used. After one or two transfers in blood broth the recovered cultures were inoculated into Malcolm broth and incubated for four days at 37°C. They were then filtered through Seitz pads and stored in the refrigerator for one or two months. All the toxins were tested by intracutaneous inoculation into each of two rabbits of 0.1 ml. quantities of a 1 to 200 dilution in 0.85 per cent saline. The reactions were read in 20 hours. A total of 70 recovered cultures were tested. Seven single colony isolations of the unexposed stock cultures served as controls.

It was found that all toxins prepared from the recovered cultures as well as those from the control cultures produced a definite skin reaction in each rabbit. The size of the erythematous zones varied, the diameters ranging from 15 to 20 mm. There were no apparent significant differences between the reactions to the recovered and those to the control cultures.

SUSCEPTIBILITY TO AGGLUTINATION BY HOMOLOGOUS IMMUNE SERUM

A group C strain, F 132, was sprayed into the darkened chamber and cultures were recovered at several hourly intervals for a period of 24 hours as follows: One hour, 5; five hours, 5; seven hours, 5; twenty-four hours, 3. Ten colonies recovered immediately after spraying served as controls. The slide agglutination technique was used with a homologous immune serum having a titer of 1 to 1280. It was found that each recovered and control culture agglutinated to the 1 to 1280 dilution of the serum.

SUMMARY AND DISCUSSION

The effect of prolonged room environment (maximum seven days) on several pathogenic properties of beta-hemolytic strep-

tococci was studied. The properties examined were (1) ability to grow in defibrinated human blood—two group A strains; (2) ability to lyse human blood clot—one group A strain; (3) ability to produce a skin toxin (rabbit)—one group A strain; (4) susceptibility to agglutination by homologous immune serum—one group C strain.

Despite the fact that efforts were made to guage minimal losses of pathogenic power no evidence was adduced that the properties of any of the strains were adversely affected by exposure to room environment.

These findings, which tend to corroborate those of the previous paper on the property of mouse virulence, thus serve to support the conclusion there set forth, namely, that by the means at our disposal it is difficult if not impossible to garner evidence of any significant attenuation of the pathogenic properties of streptococci as a result of exposure to room environment.

REFERENCES

BUCHBINDER, L., SOLOWEY, M., AND SOLOTOROVSKY, M. (With a statistical appendix by E. B. Phelps) 1941 Studies on microorganisms in simulated room environments. IV. The effect of survival on the pathogenic properties of streptococci: mouse virulence. J. Bact., **42**, 615–630.

TILLETT, W. S., AND GARNER, R. L. 1933 Fibrinolytic activity of hemolytic streptococci. J. Exptl. Med., **58**, 485–502.