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A method for demonstrating growth-inhibitory and bactericidal action on the pneumococcus of a normal serum-leucocyte mixture.

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Methods heretofore employed in testing for growth inhibiting and bactericidal action of the blood on pneumococcus have consisted in suspending small numbers of pneumococci in whole blood, serum or serum-leucocyte mixtures, contained in the capillary pipette, test tube or hanging drop. Results of tests on serum and serum-leucocyte mixtures have shown a general agreement that neither serum nor serum and leucocytes together inhibit the growth of the pneumococcus. Studies on whole blood, however, have resulted in most divergent findings. Certain workers (Wright, Heist and Solis Cohen) report the finding not only of growth inhibition, but also of pneumococcidal activity in the blood of animals resistant to pneumococcus infection. Other investigators, (Barber, Bull and Bartual) using the same methods on the blood of the same and other resistant animal species failed to find anything more than growth retardation. A review of the more important literature on this subject leads one to the conclusion that with the methods heretofore employed it is not possible to demonstrate with any degree of constancy either growth inhibitory or bactericidal activity of the normal blood for the pneumococcus.

It seemed probable to the writers that further information on this subject could be obtained were it possible to work out a method that should incorporate certain conditions under which inhibition of growth with subsequent destruction of the pneumococcus might be expected to occur in the body. In the tissues of the animal body fluid currents operate to bring the leucocytes and implanted micro-organisms into intimate contact. In the capillary pipette or test tube this constant mixing process is absent. Growth inhibition and bacteriolysis probably occur only if

all the organisms come into contact with and are phagocyted by actively functioning leucocytes.

A method has been devised for carrying out growth inhibition tests with the pneumococcus by which a constant and thorough mixing of leucocytes and micro-organisms is obtained during incubation. For this purpose mixtures of serum and washed leucocytes placed in small glass tubes are seeded with varying numbers of pneumococci. All the constituents are added in known quantities. The tubes are then sealed with paraffined corks and attached to an agitating apparatus placed in the incubator.

The apparatus consists of two solid wheels made of wood and mounted on an axle which rests on a central pivot-bearing placed midway between the two wheels. The axle is made to oscillate in a vertical plane by means of an eccentric. A small motor supplies the power, the speed being reduced to any desired rate by means of a series of pulleys. A leather belt with a piece of tape run through a series of slits cut in the leather serves as a holder for the tubes. This is attached to the wheel. When the apparatus is in operation, the motion given to the tubes, rotation plus oscillation, serves to mix their contents thoroughly and at the same time keeps all parts of the inside surface of the tubes moistened. The importance of a continuous washing of the tubes' contents over all parts of the inside surface is readily seen since the occurrence of any drying would probably mean the escape of some of the organisms from phagocytosis. Rotation and oscillation are maintained at a slow rate so as to reduce to a minimum mechanical injury to the leucocytes.

The pneumococci used for the test were suspended in Locke's solution P_H 7.8-8.0, to which 0.125 per cent. of gelatin had been added for the purpose of better preserving the organisms. The suspension was standardized by means of Gates' turbidimeter method with preliminary bacterial counts. A standard suspension of approximately 1,000 million pneumococci was used. Dilutions were made from the suspension in gelatin Locke's solution. Only organisms in the active growth phase were employed in the tests. The strain used was of low virulence for the cat, but highly virulent for rabbits, guinea pigs and mice. The leucocytes were obtained from pleural exudate produced by the injection of aleuronat. The exudate was mixed in the pleural cavity with equal parts of 1 per cent. sodium citrate in normal salt solution. After

centrifugation the cells were suspended in gelatin salt solution and a count of the white blood cells made. A second washing with gelatin Locke's solution was done and the cells finally suspended in this solution in known concentration. Solutions for washing and suspending leucocytes were adjusted to P_H 7.2-7.6. In addition to the leucocytes washed red blood cells were added as indicators of pneumococcus growth.

Results

It was found that pneumococci seeded into 0.3 c.c. cat's serum plus 0.1 c.c. leucocyte suspension containing 50,000 white blood cells per c.mm. (the equivalent number of white blood cells contained in 0.5 c.c. of blood with a count of 10,000 per c.mm.) failed to grow in numbers less than 0.001 c.c., or at most 0.0001 c.c., whereas the control tubes containing serum alone showed growth with 0.0000001 c.c. of organisms. The tubes were allowed to incubate for varying lengths of time, from 24-72 hours before microscopic examination was made. The contents of those tubes showing no growth were transferred into 1 per cent. dextrose blood broth P_H 8.0 and rabbit blood agar plates P_H 7.8 in order to determine the presence or absence of living pneumococci. Organisms could not be recovered from the tubes which failed to show growth. The media used for these tests had been determined beforehand to be highly favorable for the growth of very small numbers of pneumococci. Further experiments were made in which mice were employed as a culture media. The results of these tests showed that after 24 hours sojourn in the cat serum-leucocyte mixture as much as 10,000 times the killing dose of pneumococci failed to kill the test mice. Tests carried out on the dog serum and leucocytes gave similar results. On the other hand, the serum-leucocyte mixtures of susceptible animals, the rabbit and guinea pig, showed no growth inhibiting action against pneumococci. Even such a small number of organisms as 0.0000001 c.c. of the standard suspension grew readily in the blood elements of these animals.

The results of the above experiments seem to warrant the conclusion that with the technique employed, a mixture of serum and leucocytes from resistant animals can be shown to exert not only a growth inhibiting but also a bactericidal action on the pneumococcus.