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***Listerella monocytogenes* in Relation to the Wassermann and Flocculation-Reactions in Normal Rabbits.**

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The etiology of acute infectious mononucleosis of man is unknown. Murray, Webb and Swann¹ described a small gram-positive non-sporing bacillus, called *Bacterium* or *Listerella monocytogenes*, responsible for an acute generalized infection of rabbits characterized by large mononuclear leucocytosis with multiple focal necrosis of the liver and lymphatic glands, myocardial abscesses, and meningitis, which has been considered as a possible cause for acute infectious mononucleosis of man. Others have attempted to incriminate *Spirocheta vincenti*, while Bland² claims to have produced a disease in rabbits and monkeys, resembling infectious mononucleosis, with a protozoön of the genus *Toxoplasma*, isolated from the blood of a human case. Tidy,³ McKinley⁴ and others have thought that the disease may be due to a virus.

Whatever the cause may be, it is now well-established⁵⁻¹⁰ that transitorily positive Wassermann reactions may occur in the disease in man and these may be independent of the production of antibodies (agglutinins and hemolysins) for sheep erythrocytes.

Under the circumstances we thought it advisable to determine whether or not *Listerella monocytogenes* was capable of producing in rabbits the reagin or antibody-like substance responsible for the Wassermann and various flocculative reactions occurring in syphilis. If such occurred it would lend some support to the assumption that this organism may be in etiological relationship to acute infectious mononucleosis of man and at all events prove useful for determining

¹ Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, **29**, 407.

² Bland, J. O. W., *Brit. J. Exp. Path.*, 1931, **12**, 311.

³ Tidy, H. L., *Lancet*, 1934, **2**, 236.

⁴ McKinley, C. A., *J. A. M. A.*, 1935, **105**, 761.

⁵ Löhe, H., and Rosenfeld, H., *Dermat. Z.*, 1928, **53**, 373.

⁶ Radford, M., and Rolleston, J. D., *Lancet*, 1930, **2**, 18.

⁷ Weber, F. P., and Bode, O. B., *Münch. Med. Wchn.*, 1931, **78**, 1598.

⁸ Gooding, S. E. F., *Practitioner*, 1931, **127**, 468.

⁹ Bernstein, A., *Am. J. Med. Sci.*, 1938, **106**, 79.

¹⁰ Hatz, B., *Am. J. Clin. Path.*, 1938, **8**, 39.

whether or not the bacillus carried an antigen capable of producing non-specific Wassermann and flocculative reactions.

Two cultures were employed kindly furnished by the American Type Culture Collection, namely, No. 4428, isolated by Murray¹ from the spontaneous mononucleosis of rabbits, and No. F.302 (source not stated).

Eight rabbits were selected giving negative preliminary Wassermann reactions according to the method of Kolmer;¹¹ one of these gave a weakly positive Kahn (standard) and 2 weakly positive Kline (diagnostic) reactions.

Four of the rabbits were then given 6 intravenous injections of approximately 40 million living bacilli of strain 4428 per kilo every 5 to 7 days and 4 additional animals were given similar doses of heat-killed organisms (60°C for 1 hour).

One week after the last inoculation the serological tests were repeated. All 8 rabbits gave negative Kolmer complement-fixation reactions. The 7 giving preliminary negative Kahn reactions gave negative reactions after immunization as likewise the 6 which gave negative Kline reactions before immunization.

Eight additional rabbits were selected giving preliminary negative Kolmer reactions; 4 of these gave positive Kahn (standard) and Kline (diagnostic) reactions.

Four of the rabbits were then given 6 intravenous injections of approximately 40 million living bacilli of strain F.302 per kilo every 5 to 7 days and 4 additional animals were given similar doses of heat-killed organisms (60°C for 1 hour).

One week after the last inoculation the serological tests were repeated. All 8 rabbits gave negative Kolmer complement-fixation reactions. The 4 giving preliminary negative Kahn reactions gave negative reactions after immunization as likewise the 4 which gave negative Kline reactions before immunization.

It would appear, therefore, that the immunization of normal rabbits with the two strains of *Listerella monocytogenes* (4428 and F.302) employed did not produce the reagin responsible for complement-fixation and flocculative reactions with antigens commonly employed for the serum-diagnosis of syphilis. Whether or not the negative results were due to the absence of an antigen or other substance capable of producing the antibody or reagin involved in the complement-fixation and flocculative reactions observed in syphilis,

¹¹ Kolmer, J. A., and Boerner, F., *Approved Laboratory Technic*, D. Appleton and Company, second edition, 644, 1938.

we are unable to state. At least the immunization of normal rabbits with living and heat-killed organisms of the two particular strains employed in this study did not produce detectable amounts of the reagin or antibody concerned in the serum-tests for syphilis.

Preliminary macroscopic agglutination-tests were conducted with the bacilli of both strains before immunization in final dilutions of serum ranging from 1:10 to 1:320 and an incubation of 2 hours at 56°C followed by refrigeration over night, but with completely negative results. The tests were repeated one week after the sixth inoculation and the results showed that all produced agglutinins for homologous strains in final dilutions of serum ranging from 1:80 to 1:1280. As a general rule the titers were somewhat higher with both strains with the sera of rabbits immunized with living bacilli.

Preliminary complement-fixation tests were conducted with the sera of all rabbits employing as antigens heat-killed suspensions of both strains in saline solution in dose corresponding to one-fourth their anticomplementary units, according to the method of Kolmer;¹¹ all gave completely negative reactions. One week after the sixth inoculation the tests were repeated and all gave positive reactions of varying degree with their homologous antigens, indicating that both strains were antigenic in the production of complement-fixing antibody but, as previously stated, without producing complement-fixing antibody for antigens of alcoholic extracts of beef heart commonly employed in the serum-tests for syphilis.

The sera of all rabbits were tested for agglutinin for sheep corpuscles before and after immunization according to the technic of Davidsohn with human sera,¹² but all gave negative reactions in final dilutions of serum ranging from 1:7 to 1:7168, indicating that both strains apparently did not carry the heterophilic antigen producing antisheep agglutinin.

All of the rabbits withstood immunization with but little loss of weight. At necropsy none showed focal necrosis of the liver, enlargement of lymphatic glands, or evidences of meningitis, indicating that both strains were avirulent for these animals.

Three preliminary total and differential leucocyte counts were made on all animals before immunization and thereafter 5 days after each inoculation. All showed slight to moderate leucocytosis after 6 inoculations mainly due to an increase of polymorphonuclears. The monocytes were slightly increased in some of the animals.

Summary. (1) The intravenous immunization of rabbits with living and heat-killed avirulent strains (4428 and F.302) of *Lis-*

¹² Ref. 11, page 110.

terella monocytogenes did not produce complement-fixing or flocculating reagins for antigens commonly employed in the serum-diagnosis of syphilis. (2) Both produced complement-fixing antibody and agglutinins for antigens of homologous strains of *Listerella*. (3) Neither strain produced agglutinin for sheep erythrocytes in the immunization of rabbits. (4) Both strains produced moderate leucocytosis mainly due to an increase of polymorphonuclears with a slight increase of the monocytes in some of the animals.

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General Anesthesia by Cooling.

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It is often desirable to induce in animals a general anesthesia for operative purposes without the use of drugs. This is particularly true where, as in the study of chromatophores, the effectors are especially open to drug stimulation and may remain responsive to minute amounts of the reagent for relatively long periods of time. As an effective means to a drugless anesthesia cold is very convenient. Fishes, amphibians, and reptiles may be immersed in cracked ice and water or simply in cracked ice till they are thoroughly chilled, which occurs ordinarily in from 10 to 15 minutes. They will then remain quiescent either on an operating board or on a bed of cracked ice till an operation can be performed. From this treatment the animals recover quickly at room temperatures and are soon quite free from any after-effects of the cold. They may, therefore, be tested, if this step is desirable, almost at once, for they contain no residual reagent, the complete disappearance of which must be awaited before further work can be done.

For some time fresh-water and salt-water fishes have been treated successfully in the Harvard Laboratories by this method (Parker,¹ Abramowitz,² Osborn³). Recently Abramowitz has employed it on a larger scale in that dogfishes as much as a meter in length have been immersed in a tub containing cracked ice and seawater and there

¹ Parker, G. H., *Proc. Am. Philos. Soc.*, 1937, **77**, 223.

² Abramowitz, A. A., *Science*, 1937, **85**, 609.

³ Osborn, C. M., *J. Exp. Zool.*, 1938, **79**, 309.