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Complement-Fixation in Infections with the Virus of Lymphocytic Choriomeningitis.

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The demonstration by Howitt[1] of complement-fixation in the presence of mixtures of suspensions of guinea-pig brain infected with the virus of lymphocytic choriomeningitis and hyperimmune sera from guinea pigs suggested a simple serological method of diagnosing the malady in man and lower animals. We conduct the test as follows: 0.2 cc each of antigen, inactivated immune serum, and diluted guinea-pig serum (2 units of complement), are mixed and incubated overnight at 3°C. Two units of hemolysin (0.2 cc) and 0.5 cc of a 5% suspension of washed sheep-erythrocytes are added. Tests are read after ½ hour at 37°C.

Complement-fixing antigen occurs in spleen, consolidated lung, liver and brain of acutely ill guinea pigs infected with the virus, but not in tissues from normal animals. The antigen is most readily obtainable from spleen and least from brain; splenic suspensions containing 1 part (dry weight) in 6000 give complete fixation. Ten percent suspensions of fresh organs are run at 30,000 rpm for 20 minutes in the concentration-centrifuge[2] and the supernatant fluid is filtered through a Seitz pad. Such preparations of spleen are not anticomplementary after storage; the contained antigen is little affected by storage at 3°C for 6 months, by heating at 56°C for ½ hour, by variations in pH range from 4.5 to 9, or by dialysis. Antigen has been obtained from guinea pigs infected with 4 strains of virus maintained in this laboratory and from mice inoculated with the strain most commonly used.

Centrifugation at 30,000 rpm for 20 minutes sediments the virus but leaves the complement-fixing antigen in the supernatant fluid. The virus can be repeatedly washed without appreciable loss of infectivity, provided the suspending medium contains 2% normal serum. Ultra-sediment, washed 2-4 times, diluted to the initial volume, and centrifuged at 10,000 rpm for 10 minutes without appreciably sedimenting the virus, gives a supernatant fluid that is

[1] Howitt, B. F., *J. Immunol.*, 1937, **33**, 235.

[2] Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 503.

not anticomplementary, and, although infective in dilutions of 10^{-6}, rarely fixes more than partially in dilutions of 1:2 and 1:4. On the other hand, Seitz filtrates of the supernatant fluid from the original ultracentrifugation, possessing infective titers of 10^{-1} or less, contain as much complement-fixing antigen as do filtrates of the original splenic suspension and give complete fixation when diluted 1:8 to 1:32. This indicates that the complement-fixing antigen in infected tissue can be separated from the virus. Thus, a soluble substance appears to be associated with this virus infection as well as with bacteriophagy, influenza, psittacosis, vaccinia, yellow fever, and myxomatosis.[3]

Howitt[1] concluded that the reaction is specific for lymphocytic choriomeningitis, since she found no fixation in cross-tests with materials from animals infected with the viruses of St. Louis encephalitis, and several types of equine encephalomyelitis. We have also found no evidence of fixation in cross-tests with materials from vaccinia, myxomatosis, and influenza.* We have found that guinea pigs develop complement-fixing antibodies before their sera neutralize the virus; the former are usually demonstrable 2 weeks after infection, while the neutralizing antibodies appear in 4-5 weeks and are present in appreciable amounts by the 6th week. Furthermore, the former reach a peak (titer 1:64-1:512) at about 5-6 weeks, and then begin to decline.

We have tested a number of sera from patients with lymphocytic choriomeningitis.[4, 5] Many of the original specimens were found to be anticomplementary after storage; nevertheless, sera from 6 patients taken 6-8 weeks after onset of the disease were suitable and gave positive fixation in the presence of lymphocytic-choriomeningitis antigen. Moreover, the sample taken at the onset of meningitis from one of these individuals failed to fix complement. Blood drawn 1½ to 3 years after the disease still neutralized the virus in all but one instance, but none of the samples definitely fixed complement. Twelve patients who failed to develop neutralizing antibodies in their sera following benign lymphocytic meningitis also failed to develop complement-fixing antibodies. In addition, human sera known to give positive Wassermann reactions or fixation with gonococcal antigen failed to react with lymphocytic-choriomeningitis antigen. Lepine and Sautter[6] have also demonstrated comple-

[3] Rivers, T. M., Ward, S. M., and Smadel, J. E., *J. Exp. Med.*, 1939, **69**, 31.

* Influenzal antiserum and antigen were provided by Dr. Frank L. Horsfall.

[4] Scott, T. F. McN., and Rivers, T. M., *J. Exp. Med.*, 1936, **63**, 397.

[5] Baird, R. D., and Rivers, T. M., *Am. J. Pub. Health*, 1938, **28**, 47.

[6] Lepine, P., and Sautter, V., *Ann. Inst. Pasteur*, 1938, **61**, 519.

ment-fixing antibodies in the serum of a laboratory worker recovered from infection with the virus of lymphocytic choriomeningitis.

Summary. A soluble specific antigen, which occurs in tissues of animals infected with the virus of lymphocytic choriomeningitis, fixes complement in the presence of immune serum; the virus freed from the soluble antigen fixes complement poorly or not at all. Patients recovered from lymphocytic choriomeningitis develop in their serum antibodies that react with the soluble antigen to fix complement.

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Sulfanilamidopyridine (2-Para-aminobenzenesulfonamidopyridine) in Experimental Infections with Type XXII Pneumococcus.*†

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Our recent observations[1] indicated that sulfanilamide had definite therapeutic properties in experimental pneumococcus infections in mice, and that the effectiveness of the drug varied with the type of pneumococcus used as the infecting agent. A marked curative action was obtained in infections with 19 of the 30 types of pneumococci (I, V, VII, IX, X, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XXI, XXIII, XXV, XXVIII, XXXI, and XXXII); in infections with the remaining 11 types (II, III, IV, VI A and B, VIII, XI, XX, XXII, XXIV, XXVII, and XXIX) the drug prolonged life but did not lead to recovery.

Recently, Whitby[2] reported that sulfanilamidopyridine (2-para-aminobenzenesulfonamidopyridine) had curative properties when administered to mice infected with Types I, VII and VIII pneumococci and prolonged life in infections with types II, III and V. In experiments on Type I, he found that sulfanilamidopyridine was a more effective therapeutic agent than sulfanilamide. This observa-

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1 Schmidt, L. H., and Hilles, C., in press.

2 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.