

without an initial cessation of glomerular circulatory activity. With successive administrations of angiotonin, the blanching effect disappeared but the glomerular engorgement was observed consistently.

Renin had no detectable effect on the renal circulation in the frog.

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Agglutination of Encephalitis Virus-Coated-Bacterial Cells by Virus Antisera.

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Details of the technic involved in satisfactorily coating bacterial cells with serum antigen and the use of such cells in an agglutination test have been given in a previous report.¹ A particular advantage of the procedure is that very minute amounts of serum antibody specific for the adsorbed antigen can be detected, even though the amount of antibody is too small to be revealed by the conventional precipitation test. The use of this very sensitive type of an antigen-antibody reaction, for the detection of 'antibodies' in the blood of man and of animals against the virus of St. Louis type of encephalitis, is the basis of the present report.

The microorganisms (*Serratia marcescens*) were prepared for use in the agglutination reaction by incubating them with virus as contained in a saline emulsion of infected mouse brain. That virus was adsorbed by the bacteria in significant quantity was indicated by the observation that such cells, after repeated washing with saline, would subsequently reproduce the disease in mice upon intranasal implantation.* Suspensions of virus-coated bacteria were employed as antigens in making agglutination tests with the sera of (a) human beings convalescent from encephalitis, (b) normal individuals, and (c) of rabbits immunized against encephalitis virus.

With the sera of convalescent patients[†] agglutination reactions

¹ Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **47**, 11.

* Subsequent testing of the brains of such infected mice established the specific identity of the virus.

† For supplies of sera and for data pertaining to mouse neutralization tests we are indebted to Dr. G. O. Broun of the Department of Medicine of St. Louis University.

occurred in dilutions ranging from 1:16 to 1:128. The specific nature of the agglutination reaction was indicated by the observation that after adsorption of these sera with virus-coated bacterial cells they failed to elicit the agglutination reaction when tested against additional preparations of virus-coated cells. On the other hand, sera adsorbed with bacterial cells coated with normal mouse brain were capable of agglutinating virus-coated cells to the original titer. In addition, similar adsorption of these sera with the brain substance of normal mice did not remove the capacity of the sera to react with cells coated with mouse brain-virus. Although agglutination was observed in all cases, the titers of sera of convalescent patients were not in complete agreement with their virus neutralizing capacities (as determined by mouse protection tests). Further observations are required to clearly indicate this relationship.

The bacterial agglutination (B.A.) method of detecting encephalitis virus antibody has been applied likewise to the blood sera of a group of 18 supposedly normal individuals, wherein it was found that the sera of 80% of those tested gave a reaction in dilutions of 1:8 or higher. The results of the B.A. method of antibody detection were correlated in most instances with the presence of neutralizing antibodies, although the neutralizing capacities were not in complete agreement with the B.A. titers. A more extensive study of the incidence of antibodies in the sera of normal individuals, as revealed by the B.A. method is under way.

The B.A. method of antibody detection was similarly applied to two lots of rabbit antisera which had been prepared by immunization of the animals with mouse brain-encephalitis virus suspensions.[†] These antisera gave B.A. reactions in dilutions of 1:512. The specific nature of the reaction was indicated by the failure of normal rabbit sera to elicit the B.A. reaction and by differential adsorption of the rabbit immune sera with (a) normal mouse brain (which did not remove the antibody involved in the B.A. reaction) and (b) encephalitis virus-mouse brain (which completely removed the antibody involved). These antisera, when adsorbed with bacterial cells coated with normal mouse brain, were capable of agglutinating virus-coated cells to full titer. On the other hand, immune rabbit sera adsorbed with bacterial cells coated with mouse brain-virus were devoid of agglutinating antibody.

Additional experiments are under way seeking to make a further application of the B.A. method to the detection of humoral antibody involved in experimental poliomyelitis of animals and in the naturally occurring disease of man.