cultures on suitable media and in the second, by cross-neutralization tests with anti-sera supplied through the courtesy of Dr. F. L. Horsfall.

In the case of both strains, inoculation of 1 ml of 10^{-7} and occasionally 10^{-8} dilution of consolidated lung tissue into the yolk-sacs of 5- or 6-day eggs incubated at 36° C sufficed to bring about death of the developing chicken embryos after several days' infection. The yolk-sacs of these eggs examined immediately after death were bacteriologically sterile but showed innumerable elementary bodies in Giemsa-stained smears. These findings not only confirm the microscopic observations as to the presence of large amounts of virus in the lungs of mice intranasally infected with lymphogranuloma venereum agent but also serve to reëmphasize the delicacy of the yolk-sac technic as compared with all other methods at present available for the detection of the virus. The obvious usefulness of the pulmonary infection† of mice in immunological experimentation on lymphogranuloma venereum is being further explored.

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Complement Fixation Test in Lymphogranuloma Venereum.

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Although it is generally acknowledged that the cutaneous test with the Frei antigen is of great value in establishing the diagnosis of infection with the etiological agent of lymphogranuloma venereum, many workers have sought to devise other procedures which might be employed as corroborative evidence. The serological technic most widely explored in this connection has been complement fixation but, using a variety of antigens, most investigators have been unsuccessful in their attempts to demonstrate a specific reaction (for literature see[1]). Nearly all the reports of positive findings are justly open to criticism on such grounds as inadequately detailed description of the method, lack of controls, or incomplete data on the results.

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[4] Horsfall, F. L., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

† Workers should bear in mind the possible hazards involved in the use of the intranasal technic where high concentrations of virus are concerned.

[1] Melczer, N., and Sipos, K., *Arch. f. Dermat. u. Syph.*, 1937, **176**, 176.

Since the quantitative relationships of the reagents are of paramount importance in serological tests it seemed likely that many of the failures might be due to the use of antigens of insufficient potency and that it might be worthwhile to reinvestigate the potentialities of the complement fixation test in this disease, with preparations containing higher concentrations of antigen than had hitherto been available. The following antigens have been employed: (a) the consolidated lungs of several mice, infected intranasally[2] with the agent of lymphogranuloma venereum, were pooled and ground with Pyrex fragments plus broth to a 10% suspension. This was freed of gross particles by centrifugation at 2000 RPM. The supernatant, termed "Lygranum" (M.L.) antigen, was stored at −32°C until needed, when it was thawed and further diluted with 0.85% saline to a final lung concentration of 1:100 or 1:150 for use in the test. Normal mouse lung suspension similarly prepared served as control. (b) Five- or 6-day eggs, inoculated via the yolk-sac[3] with lymphogranuloma venereum agent, were incubated at 36° C until the death of the chicken embryo. Immediately thereafter the bacteriologically sterile yolk-sacs, heavily infected with virus, were removed and ground with Pyrex fragments plus broth to 10% suspension. This was centrifuged 1 hour at 2000 RPM; the supernatant was recentrifuged in the cold for 2 hours at 12,000 RPM and the sediment obtained thereby was resuspended in saline to 10 times the volume of the original 10% yolk-sac suspension for use in the test. This was called "Lygranum" (Y.S.) antigen. The resuspended sediment from normal yolk-sacs, treated in the same way, was used as control.

The sera were inactivated at 56° C before use and dilutions were made in saline. The source of complement was pooled guinea-pig serum kept frozen at −32° C. Previous to each test the thawed complement was titrated and diluted in saline so that 2 hemolytic units were contained in 0.2 cc. In the test 0.2 cc of each reagent was added to the tubes in the following order: serum dilution, complement, and antigen. The well-shaken mixtures were placed for 1¼ hours at 37° C. Then to each tube was added 0.2 cc of 3% suspension of washed sheep cells sensitized with 2 minimal hemolytic doses of anti-sheep cell rabbit amboceptor. Readings for hemolysis were made after a further period of 30 minutes at 37° C. Controls for free complement and for anticomplementary action in each antigen and serum were always included. The titre of a given serum

[2] Shaffer, M. F., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 408.

[3] Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

was taken as the highest dilution showing complete or nearly complete fixation with specific antigen and no fixation with the antigen control prepared from the corresponding normal tissues. The results with "Lygranum" (Y.S.) ran entirely parallel to those obtained with "Lygranum" (M.L.), although under the conditions of concentration employed in these experiments, the former antigen seemed more active. On the other hand, non-specific fixation in low serum dilutions with the antigen control from normal tissue was less frequently encountered in the case of mouse-lung. In performing the test the lowest initial serum dilution was 1:2 and since, in the system for fixation, this underwent a further threefold dilution, the lowest possible titre in these experiments was 1:6.

Using the method outlined, sera from 20 individuals with clinical history of lymphogranuloma venereum and positive reactions to Frei antigen were tested. Nineteen sera fixed complement specifically in the presence of "Lygranum" antigen, at titres ranging from 1:15 to 1:600. In one case the titer was only 1:6. The possible correlation between serum titre, degree of reactivity to standardized Frei antigen and the clinical status of lymphogranuloma infection in a group of cases is being investigated in collaboration with Dr. A. W. Grace of the New York Hospital, through whose courtesy most of the lymphogranuloma sera were obtained. As a control, sera taken from 22 presumably normal individuals were tested; 20 failed to fix complement even in the lowest dilution (titre less than 1:6). Positive reactions in this group were obtained only with serum from one laboratory worker who had been exposed to contact with the virus over the period of a year and with the serum of a female child residing in an orphanage.

As a further control, the test was carried out on a group of 29 sera with strongly positive Wassermann reaction obtained through the courtesy of Mr. J. V. Mulcahy of the State Dept. of Health, Trenton, N. J. and Dr. B. Webster of the New York Hospital. Sixteen of these sera gave specific fixation with "Lygranum" antigens showing titres between 1:15 and 1:150. In view of the reports concerning positive reactions to the Frei antigen, elicited in prostitutes who were tested as a matter of routine although not suffering overtly from lymphogranuloma, as well as the positive fixation by the serum of one worker in our laboratory and 55% of syphilitic sera which we have tested, the likelihood of undiagnosed or subclinical infection appears great and is being further investigated in collaboration with Dr. A. W. Grace.

If the reaction is specific, as we believe, it will prove to be a most

useful method for the detection of lymphogranuloma venereum infection particularly in individuals who have not been tested with Frei antigen, as well as for immunological studies in infections of humans and animals with this etiological agent.

Summary. In individuals with lymphogranuloma venereum, the serum has been found to fix complement regularly in the presence of antigens containing the virus in high concentration. Such fixation was observed only once with sera taken from supposedly uninfected individuals but was obtained frequently in syphilitic sera showing markedly positive Wassermann reactions.

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Persistence of St. Louis Encephalitis Virus in the Brains of Chicks.*

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St. Louis encephalitis virus has been cultivated *in vitro* on minced chick embryonic tissue[1] as well as in the yolk[2] and on the chorio-allantoic membrane[1, 3-5] of chick embryos. On chorio-allantoic membranes, serial passage through 68[3] and more than 100[6] transfers has been possible. Brains of the corresponding embryos have been found to contain slightly more virus than the membranes, virus being also present in the livers and spleens.[3, 5] Virus has been demonstrated in the brains of chicks allowed to hatch[1] though only slight or no microscopic changes were demonstrable.[1, 3] It has been observed that embryos may survive until the time of hatching,[3] or die within a few days after inoculation.[1, 6]

Harrison and Moore[1] have reported that young chicks (4 to 6

* Studies supported by Mary Hooper Somers Fund for Filtrable Virus Research.

1 Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

2 Stimpert, F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 483.

3 Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 191.

4 Smith, M. G., and Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 323.

5 Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 799.

6 Schultz, E. W., *et al.*, unpublished work.