more than did the addition of alum (Experiment 5). Of significance also is the fact that zinc sulphate when used alone has afforded over 95% protection in a total of 53 animals (Experiments 7 to 13). Thus far, only 2 animals treated with zinc sulphate alone have succumbed to poliomyelitis. Six animals have survived repeated virus instillations for 3 months.

It will be noted that the protection afforded by picric acid is distinctly greater in Experiment I than in Experiments 2, 3, and 4. This is accounted for by the fact that in the latter experiments, virus was instilled intranasally almost daily during the entire month; an exposure which brought down 100% of the controls. Even under these drastic conditions, none of the 9 monkeys treated with 0.5% $ZnSO_4$ (Experiment 7) developed the disease.

Studies are in progress to determine the lowest dilutions of zinc sulphate which will protect monkeys for at least one month and to determine the factors which influence the effectiveness and duration of the protection.

In presenting these observations, we do not wish to imply that any of the agents described thus far will necessarily prove effective in the prevention of poliomyelitis in man. The results, however, lay a foundation for similar studies in man during an epidemic period.

9038 P

Complement Fixation Test Differentiating 3 Strains of Equine Encephalomyelitic Virus and the Virus of Lymphocytic Choriomeningitis.

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Although most of the attempts to apply the complement fixation test in the field of filterable viruses have either been negative or inconclusive, within recent years a few favorable reports have lent more encouragement. The work of Craigie[1] and his coworkers has been of especial value in suggesting a new method of attack. Based on their reports, work was undertaken to extend the test for the differentiation of strains of equine encephalomyelitic virus, representing the so-called eastern and western American types and that

[1] Craigie, J., and Tulloch, W. J., *Sp. Rep. Ser. Med. Res. Coun. London*, No. 156, 1931; Craigie, J., and Wishart, F. O., *Can. Pub. Health J.*, 1936, **27**, 371.

of Moscow No. 2. All 3 of the strains may be differentiated serologically by the neutralization test.[2] The virus of lymphocytic choriomeningitis isolated by Dr. C. Armstrong of the National Institute of Health, was also included in the work.

With some slight modifications the antigens were prepared according to the method of Craigie and Tulloch[1] for vaccinia. The equine viruses were carried in the guinea pig and that of the "l.c.m." virus in the mouse. The brains were removed aseptically from the respective infected animals, were frozen in an ice-salt mixture and dried in a desiccator evacuated by the Cenco-Hyvac pump. The dried material was weighed and ground with ether in the desired proportions. The mixture was shaken, stored on ice for several hours. Then the ether was removed; the residue was ground with 0.85% saline, left for 6-12 hours on ice, frozen several times in an ice-salt mixture and thawed at 37°C. Finally the material was centrifugated and the supernatant fluid removed, which was then ready for use after a preliminary titration for anticomplementary, hemolytic and antigenic properties.

After a careful titration of the complement with the antigen, the test was set up with 0.1 cc. of serum from guinea pigs hyperimmunized to each strain. 0.2 cc. of antigen and 0.2 cc. of complement containing 2 full hemolytic units were then added and the mixture left for 16 hours at refrigerator temperature. Five per cent washed sheep cells sensitized with 2 units of hemolysin (final dilution 2.5% RBC) were added and the tubes left for 15 to 20 minutes in the water bath at 37°C. or until all the control tubes except that for the sheep cells showed complete hemolysis. Readings were made immediately and again some time later.

Cross immunization tests were performed with immune serums from each strain used. The various serums were also tested against antigens prepared in a similar manner from rabbit brain containing the Borna type of European equine encephalomyelitic virus, with normal guinea pig brain and with mouse brain containing the virus of lymphocytic choriomeningitis of Armstrong and Lillie.[3] Positive fixation occurred between hyperimmune serums and the homologous antigens of each type of virus but not between the same serums and heterologous antigens; it was not observed in the presence of normal guinea pig brain or Borna antigens. Specific fixation took place between immune serums and the homologous antigen of the "l.c.m." virus but there was no cross reaction whatever between this virus and

2 Howitt, B. F., *J. Immunol.*, 1935, **29**, 319.

3 Armstrong, C., and Lillie, R. D., *Pub. Health Rep.*, 1934, **49**, 1019.

those of the equine strains. No immune serum was available for the Borna virus. It was noticed that while not all immunized guinea pigs responded by a marked antibody formation the serum of certain animals consistently gave positive results over varying periods of time with antigens made on different occasions. This was particularly true for serums of the Moscow No. 2 strain.

Two series of animals were immunized with live virus of the eastern and western American and the Moscow No. 2 strains, respectively, over a period of several months. Blood was removed from the heart and increasing doses of virus were administered at weekly intervals. One series was given intravenous and the other subcutaneous injections of virus, respectively. Neutralization and complement fixation tests were performed on serums collected weekly with results indicating a parallelism between the 2 reactions, although the neutralization test usually became positive at an earlier period than the complement fixation. As a rule the latter failed to take place with serums of the American equine strains until after 2 months of immunization and then was not strongly positive until massive doses of virus had been given. The animals inoculated with the Russian strain gave the strongest reaction in the shortest time.

From the results it seems apparent that the complement fixation test may be applied to the differentiation of the strains of equine encephalomyelitic virus and to that of the lymphocytic choriomeningitis virus of Armstrong and Lillie. So far the test seems specific for the homologous strains when strongly hyperimmunized serums are used together with a potent antigen. Further work is contemplated in this field with other viruses and a more comprehensive account of the results already obtained will be reported later.

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Total Nitrogen Content of Skeletal Muscle of the Rat in Various Nutritional States.

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It is generally conceded that one important function of the growth hormone of the anterior hypophysis is the promotion of protein anabolism. Evidence for this has been obtained from the results of numerous nitrogen balance studies which have been published in