A SOLUBLE ANTIGEN OF LYMPHOCYTIC CHORIOMENINGITIS

II. Characteristics of the Antigen and Its Use in Precipitin Reactions

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A specific soluble substance which occurs in tissues infected with the virus of lymphocytic choriomeningitis (1) was demonstrated in earlier work by means of the complement-fixation reaction. In the present communication certain characteristics of the soluble substance are recorded and methods are described for preparing solutions of antigens with which a precipitin reaction can be elicited in the presence of hyperimmune serum. Additional observations on the specificity of the soluble antigen obtained by the aid of the precipitation reaction are also presented.

Materials and Methods

Preparation of Antigen.—Solutions of antigen were prepared from spleens of guinea pigs moribund with lymphocytic choriomeningitis, *i.e.*, from animals 6 days after a combined intracerebral and intraperitoneal inoculation with a 10 per cent suspension of brain from a guinea pig infected with the W.E. strain of virus. Spleens were removed aseptically, ground, and made into a 10 per cent suspension with physiological saline solution containing 2 per cent inactivated normal guinea pig serum. After preliminary horizontal centrifugation, the opalescent suspension was run in the concentration centrifuge of Bauer and Pickels (2) at a speed of 30,000 R.P.M. for 20 minutes. The clear supernatant fluid was then filtered through a Seitz pad. Extracts of infected spleen prepared in this manner were free of virus and contained the soluble antigen of lymphocytic choriomeningitis in amounts adequate for its ready demonstration by means of the complement-fixation reaction.

Antisera.—Hyperimmune serum obtained from guinea pigs was used throughout most of the experiments. This serum was prepared by first infecting guinea pigs with the W.W.S. strain of virus which is low in virulence, and, after their recovery, inoculating them with 2 to 3 cc. of a 10 per cent suspension of brain tissue from guinea pigs infected with the highly virulent W.E. strain. Animals which survived reinoculation were again treated with a similar amount of infective tissue material; they were bled 10 days later and their sera were pooled. In a few instances convalescent sera from guinea pigs recovered from infection with the W.W.S. strain were employed.

Complement-Fixation Reaction.—A detailed description of the complement-fixation

technique which we have employed has been given in an earlier paper (1). The essential points in the technique are as follows: Two units of guinea pig complement obtained from freshly resuspended guinea pig serum which had been dried *in vacuo* from the frozen state, were added to dilutions of the material to be tested, *i.e.*, antigen or antiserum. The mixtures were then stored at 0°C. overnight. Fixation could be demonstrated after incubation for 2 hours at 22°C. or 30 minutes at 37°C., but the titers were lower under these conditions than they were when other portions of the same materials were allowed to fix overnight in the cold. The next morning 0.5 cc. of a 5 per cent suspension of washed sheep erythrocytes and 0.2 cc. of diluted anti-sheep cell serum prepared in rabbits (2 units of hemolysin) were added to each material to be tested. The degree of hemolysis was estimated after incubation at 37°C. in a water bath for 30 minutes. Dilutions of antigen and serum were calculated on the basis of material in the 0.2 cc. used in the test; the titer was taken as the highest dilution which bound complement completely.

Precipitin Reaction.—0.2 cc. amounts of a solution of antigen were added to equal volumes of antiserum of varying dilutions. The mixtures were incubated at room temperature for 18 hours in stoppered pyrex tubes having an inside diameter of 4 mm. In order to prevent the growth of bacteria during incubation of the tests, merthiolate,[1] diluted 1:100 in saline solution, was added to solutions of antigen in such amounts that the final dilution was 1:10,000. Antisera employed in the tests had been inactivated at 56°C. for 30 minutes.

EXPERIMENTAL

Characteristics of the Soluble Antigen of Lymphocytic Choriomeningitis

The serologically active material in extracts of infected spleen was found to be relatively stable and to have certain properties which indicated that it was of a protein nature. The results of experiments showing the effect of various physical and chemical agents on the antigen are summarized in the following paragraphs.

Effect of Storage on Antigen.—It was obviously of importance to learn how well the soluble antigen could withstand storage. An antigenic solution prepared according to routine from infected spleens of guinea pigs was stored at 3°C. and tested by means of the complement-fixation reaction at various intervals. The original titer of the extract, 1:32, did not change significantly during the first 4 months; furthermore, the titer was 1:16 at the end of 12 months. This serologically active solution did not become anticomplementary after the long period of storage. Soluble antigen could be extracted as well from infected spleens which had been frozen and stored at −10°C. for several weeks as from spleens which were used immediately after their removal from acutely ill guinea pigs. In general, these observations agree with those of Howitt (3) and Lépine, Mollaret, and Sautter (4) on the complement-fixing substance in tissues infected with the virus of lymphocytic choriomeningitis.

Effects of Freezing and Drying on Antigen.—Extracts of infected spleen prepared by

[1] Product of Eli Lilly and Company.

the usual method were dried *in vacuo* from the frozen state and resuspended in an amount of distilled water equal to the original volume. Although a considerable amount of material was insoluble on resuspension from the dried state, no detectable loss of complement-fixing activity was displayed by the redissolved portion. It was possible to concentrate the antigen by this means, *e.g.*, material taken up in one-fifth of the original volume was approximately 5 times as active as that resuspended to its original volume.

Effect of Heat on Antigen.—The soluble antigen present in splenic extracts was found to be relatively heat-stable, *i.e.*, it was more resistant than the heat-labile soluble antigen of vaccinia but less resistant than the heat-stable soluble antigen found in that disease (5). Subjection of a solution of the antigen to a temperature of 37°C. or 45°C. for 30 minutes caused no demonstrable decrease in its serological activity; moreover, only a slight reduction in the amount of active antigen was detected after heating at 50°C. or at 56°C. for 30 minutes. However, when the solution of antigen was held at a temperature of 60°C. for 30 minutes, its complement-fixing activity was almost completely destroyed, while at 70°C. and 100°C. it was completely lost.

Solutions became somewhat opalescent after heating at 50°C. and 56°C., although no appreciable amount of precipitate was obtained by centrifugation. On the other hand, with the higher temperatures mentioned, particularly 70°C. and 100°C., there occurred a moderate amount of flocculent precipitate. Such a precipitate was removed by centrifugation, washed and resuspended in saline solution, and was tested for complement-fixing activity. It was so anticomplementary that any specific fixing power which might have been present was completely obscured.

Effect of Variation of Hydrogen Ion Concentration on the Antigen.—The effect of hydrogen ion concentration on the serological activity of splenic extracts was studied over a pH range of from 3 to 9.

Adjustments in the pH of extracts, determined by colorimetric methods, were made by the addition of N/10 solution of hydrochloric acid or of sodium hydroxide. Aliquots of a solution of antigen which had been adjusted to the desired pH were stored for 2 hours at 3°C., then neutralized, and finally brought to equal volumes by the addition of physiological saline solution buffered to pH 7.0. No loss of complement-fixing activity occurred in the pH range between 5 and 9, nor was there any appreciable amount of precipitation. However, at pH 4.5 a voluminous precipitate appeared which was removed by centrifugation. The clear supernatant fluid contained practically all of the antigen. About two-thirds of the material which was insoluble at pH 4.5 redissolved in saline solution at pH 7.0, but this contained little or no complement-fixing antigen. Adjustment of a portion of splenic extract to pH 3 caused only a negligible amount of precipitate, which was separated and discarded; the soluble portion of the specimen failed to fix complement.

Precipitation of Antigen by Alcohol.—The serologically active material in splenic extracts freed of virus was precipitated quantitatively by the addition of 9 volumes of cold absolute ethyl alcohol. After the removal of alcohol, resuspension of the precipitate in either saline solution or distilled water was incomplete; nevertheless, all of the original antigen was recovered in the soluble portion. The same results were obtained whether alcohol precipitation was carried out at pH 7.0 or 4.5. In this respect the antigen of lymphocytic choriomeningitis behaves differently from the heat-stable soluble substance of vaccinia which is soluble in acid alcohol (6). In several instances the alcoholic solution, cleared of insoluble material, was distilled at 40°C. under partial vacuum; on no

occasion was soluble antigen demonstrated in the redissolved portion of the residual fraction.

Precipitation of Antigen by Ammonium Sulfate.—The soluble antigen in splenic extracts was completely precipitated when ammonium sulfate was added to the point of saturation. Nevertheless, attempts to isolate the soluble antigen *in toto* in either the albumin or globulin fraction were not successful. The soluble substance was about equally distributed between the two fractions in several experiments, but on a few occasions it was recovered to a slightly greater extent in the globulin fraction. The process of fractionation with ammonium sulfate was frequently accompanied by a considerable loss of complement-binding antigen, *e.g.*, in one typical experiment in which the first globulin and albumin fractions were refractionated, it was found that the titer of the refractionated globulin was only one-eighth that of the original extract; furthermore, the refractionated albumin had the same titer as the globulin.

The soluble antigen of lymphocytic choriomeningitis was little affected by storage for long periods of time at ice box temperature, by heating to 56°C., or by mild changes in pH. It exhibited certain characteristics of a protein, *i.e.*, it was precipitated from solution by the addition of 9 volumes of absolute alcohol or by the addition of ammonium sulfate to the point of saturation. When ammonium sulfate was employed for the separation, the active material occurred in both the globulin and albumin fractions.

Precipitation of Soluble Antigen by Hyperimmune Serum

Precipitation phenomena were displayed when extracts of infected spleen containing soluble antigen but no virus were added to low dilutions of hyperimmune guinea pig serum. Little significance could be attached to reactions with these crude splenic preparations, because precipitation also occurred when normal serum, in dilutions greater than 1:4, was added to the extracts. The non-specific flocculating material could be removed from the usual splenic extract by several methods of fractionation and with such partially purified preparations a specific precipitation of the soluble antigen in the presence of antiserum was demonstrated.

Experiment 1.—50 cc. of a 10 per cent extract of infected spleen, prepared by the usual method of ultracentrifugation and Seitz filtration, were dried from the frozen state. The desiccated material was resuspended in 10 cc. of distilled water and the soluble portion was dialysed in a cellophane bag against running water for 18 hours. A voluminous tan flocculent precipitate which formed was separated from the clear brown solution by centrifugation; the precipitate was then taken up in saline solution and the portion which failed to dissolve was discarded. The soluble fraction of the dialysate was brought to physiological concentration by the addition of sodium chloride. The complement-fixing titer of the original extract was between 1:4 and 1:8; that of the soluble fraction of the dialysate was 1:32, while the redissolved portion of the material insoluble in water after dialysis contained only a negligible amount of complement-fixing antigen. 6.5 cc.

of the soluble dialysate were adjusted to pH 4.5 by the addition of N/10 solution of hydrochloric acid and the heavy precipitate which formed was removed by centrifugation after storage in the cold for 1½ hours. This precipitate dissolved completely when suspended in 3 cc. of saline solution and adjusted to neutral reaction. The supernatant fluid containing the fraction soluble at pH 4.5 was also adjusted to pH 7.0 and found to fix complement in a dilution of 1:32. Contrary to the usual experience, the pH 4.5 insoluble fraction in this experiment displayed a slight complement-binding power.

A specific precipitin reaction was obtained when dilutions of hyperimmune serum were added to this preparation from which material insoluble in water and insoluble at pH 4.5 had been removed. Results of tests with this solution of antigen (No. 1) are presented in Table I.

TABLE I

Precipitation of Soluble Antigen of Lymphocytic Choriomeningitis by Antisera

Antigen	Serum	Dilution of serum					
		1:2	1:4	1:8	1:16	1:32	1:64
No. 1 undiluted	Hyperimmune guinea pig 9585	++++	+++	+++	++	+	−
(C-F titer 1:32)	Normal guinea pig	−	−	−	−	−	−
No. 2 undiluted	Hyperimmune guinea pig 9585	++++	++++	+++	+	−	−
(C-F titer 1:64)	Immune guinea pig 22	+++	+	±	−	−	−
	Normal guinea pig	−	−	−	−	−	−
No. 3 undiluted	Hyperimmune guinea pig 7798	++++	++++	++++	+++	+	±
(C-F titer 1:16)	Immune guinea pig 30	+	±	?	−	−	−
	Normal guinea pig	−	−	−	−	−	−
No. 3 diluted 1:2	Immune guinea pig 30	+++	+	±	−	−	−

Mixtures of 0.2 cc. amounts of antigen solution and diluted serum were incubated at room temperature for 18 hours.

C-F titer of antisera: No. 9585, 1:256; No. 7798, 1:128; No. 22, 1:32; No. 30, 1:32.

Experiment 2.—16 spleens removed from moribund guinea pigs were frozen and stored at −10°C. for 2 weeks. The spleens were then thawed, ground, and suspended in 60 cc. of saline solution. In order to break up cells which were still intact the suspension was repeatedly frozen and thawed. Particles were removed by horizontal centrifugation and by two runs in the ultracentrifuge at 30,000 R.P.M. for 20 minutes; the latter procedure removed all or nearly all of the virus. The complement-fixing titer of the clear extract was 1:32. 40 cc. of this preparation were chilled and treated with an equal volume of a saturated solution of ammonium sulfate. 3 hours later the sediment was removed by centrifugation in the cold; storage at 0°C. overnight did not result in further precipitation. The precipitated globulin fraction dissolved readily in 15 cc. of distilled water; this solution which was increased in volume after dialysis overnight against running water was reduced to 10 cc. by placing the dialysing bag and its contents in a stream of air for several hours. The euglobulin fraction, insoluble in water after dialysis, was

dissolved in 10 cc. of saline solution and the solution of pseudoglobulin was brought to physiological salt concentration by the addition of sodium chloride. Albumin was precipitated from the globulin-free solution by the addition of crystalline ammonium sulfate until no more dissolved. 20 cc. of claret colored fluid containing the albumin fraction after dialysis were treated with sufficient sodium chloride to bring the concentration to 0.85 per cent. The complement-fixing titers of the various preparations were: pseudoglobulin, 1:64; euglobulin, 1:4; albumin, 1:16. The solution of the pseudoglobulin fraction gave a precipitin reaction with hyperimmune guinea pig serum; moreover, it did not precipitate non-specifically when added to dilutions of normal guinea pig serum. On the other hand, the solution containing the albumin fraction precipitated non-specifically in the presence of diluted normal serum in a manner similar to that observed with crude splenic extracts prepared according to routine. The results of precipitin titrations with the pseudoglobulin fraction (antigen 2) are presented in Table I.

Experiment 3.—20 cc. of an extract of infected spleens, prepared according to routine, were treated in the cold with 9 volumes of absolute alcohol. A large portion of the alcohol-insoluble material which failed to dissolve when suspended in 20 cc. of saline solution was discarded; nevertheless, the complement-fixing titer of the solution before and after precipitation by alcohol was 1:16. Flocculation occurred when this treated preparation was added to dilutions of hyperimmune serum up to 1:32, but no demonstrable reaction was obtained with normal serum. The results of titrations with antigen 3, summarized in Table I, show in addition that a stronger reaction was obtained with simple immune serum having a low complement-fixing titer, *viz.*, 1:32, when the solution of antigen was diluted 1:2.

The relatively low concentration of soluble antigen in the preparations from extracts of infected spleen made it desirable to employ in the precipitin reaction constant amounts of undiluted, or slightly diluted, solutions of antigen against decreasing concentrations of antiserum. Incubation of test mixtures at room temperature overnight gave results similar to those obtained following incubation for the same period of time at 37°C. and somewhat superior to those obtained at 56°C. or at 3°C.

In a previous report (1) the serological specificity of the soluble antigen of lymphocytic choriomeningitis was demonstrated by cross tests in which the complement-fixation technique was employed with materials from vaccinia and infectious myxomatosis. Therefore, it was to be expected that no cross reaction would be detected by precipitation technique when the soluble antigen of vaccinia and of myxomatosis and their specific antibodies were mixed with antisera and soluble antigen, respectively, of lymphocytic choriomeningitis. Tests carried out with such materials gave negative results.

Absorption Experiments with Soluble Antigen and Immune Serum

By means of the precipitin reaction it was possible to absorb soluble antigen from extracts of infected spleen with immune serum or hyper-

immune serum. Conversely, the absorption of anti-soluble substance antibodies from immune sera was accomplished by the addition of soluble antigen. Such absorbed sera still neutralized the virus of lymphocytic choriomeningitis, although they no longer reacted demonstrably with soluble antigen when tested by either the precipitin or complement-fixation technique. The results which are presented in the following protocol were typical of those obtained in 3 experiments.

Experiment 4.—The globulin fraction from 25 cc. of an extract prepared, according to routine, from 8 infected guinea pig spleens was dissolved in 15 cc. of saline solution. The complement-fixing titer of this solution was 1:32. Pooled serum 22, obtained from guinea pigs 7 months after infection with the W.W.S. strain of virus, had a complement-fixing titer of 1:32. It contained a sufficient amount of neutralizing antibodies to protect against the test dose of virus, *i.e.*, approximately 10,000 M.L.D., when inoculated subcutaneously into guinea pigs according to the technique used in this laboratory (7). After optimal proportions of antigen and antibody were determined by preliminary precipitation titration, absorption of antigen was carried out as follows: 2 cc. of the solution of globulin, 0.5 cc. of serum 22, and 1.5 cc. of saline solution were mixed and stored overnight at room temperature. A considerable amount of white flocculent precipitate was removed by horizontal centrifugation. Storage of the 3.5 cc. of clear supernatant fluid at 0°C. for 24 hours did not result in further precipitation. A 0.25 cc. amount of serum 22 was added to the mixture which was again left at room temperature overnight. Only a small amount of precipitate occurred after the second absorption. The clear absorbed solution of globulin no longer reacted in a precipitin test when added to dilutions of serum 22; furthermore, it did not fix complement in the presence of immune serum (see Table II). A control solution of antigen incubated under similar conditions showed no appreciable loss in titer.

Absorption of anti-soluble substance antibodies was carried out with antigen and immune serum from the same lot used in the preceding absorption experiment; however, the relative proportion of the two materials was different. 1 cc. of pooled serum 22, 1 cc. of globulin solution, and 4 cc. of saline solution were mixed and left at room temperature. A heavy flocculent precipitate was removed by horizontal centrifugation 24 hours later and after storage at 0°C. for 1 day no further flocculation occurred. A second cc. of globulin solution was added to the 5.5 cc. of clear supernatant fluid recovered from the first absorption and the mixture was again incubated as before; this second absorption yielded a small amount of flocculent material which was removed. The final absorbed serum, which represented a dilution of approximately 1:8 of the original serum, was not anticomplementary. It neither precipitated nor fixed complement in the presence of soluble antigen (see Table III). Nevertheless, the absorbed serum was still capable of completely neutralizing the usual test dose of virus, *i.e.*, 10,000 M.L.D.; this activity was comparable to unabsorbed pooled serum 22 in a dilution of 1:8. Since merthiolate had been added to the original extract of infected spleen in a ratio of 1:10,000 before fractionation was carried out, it was important to test the partially purified solution of antigen to determine whether residual merthiolate might account for the inactivation of virus by absorbed serum. The solution of globulin which served as a source of antigen in this experiment was substituted for serum in the regular neutralization test and was shown to be incapable of inactivating a standard dose of virus.

The relatively low concentration of soluble antigen in the preparation suitable for precipitin reactions made it impractical to attempt to absorb completely anti-soluble substance antibodies from hyperimmune serum. Nevertheless, this was undertaken with serum 7798, the titer of which was 1:128; after several absorptions the complement-fixing titer was lowered

TABLE II

Absorption of Soluble Antigen by Immune Serum

Material tested	Complement fixation					Precipitation				
	Dilution of antigen					Dilution of serum				
	1:4	1:8	1:16	1:32	1:64	1:4	1:8	1:16	1:32	1:64
Unabsorbed antigen 1:2 dilution	++++	++++	++++	+++	−	++++	++++	++++	++	±
Absorbed antigen 1:2 dilution of original	−	−	−	−	−	−	−	−	−	−

Absorption and precipitin tests were done with serum from guinea pig 22, immune to the W.W.S. strain of virus.

Complement-fixation tests were done with hyperimmune guinea pig serum 7798, diluted so that each tube received 4 units of antibody.

See protocol of Experiment 4 for details.

TABLE III

Absorption of Anti-Soluble Substance Antibodies from Immune Serum

Material tested	Complement fixation						Precipitation						Neutralization
	Dilution of serum						Dilution of serum						
	1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4	1:8	1:16	1:32	1:64	
Unabsorbed serum	++++	++++	++++	++++	+++$^{+}$	−	++++	++++	++++	++++$^{-}$	++	±	Positive
Absorbed serum			−	−	−	−			−	−	−	−	Positive

Complement-fixation tests were done with 2 units of antigen.

Precipitin tests were done with antigen diluted 1:2.

See protocol of Experiment 4 for details.

to 1:16 without any detectable reduction in neutralizing ability. In general, however, the evidence for the existence of anti-soluble substance antibodies which were different from neutralizing antibodies was more clearly indicated when simple convalescent sera with a comparatively low complement-fixing titer were employed.

DISCUSSION

A soluble antigen of lymphocytic choriomeningitis which is readily demonstrated in extracts of infected guinea pig spleen by means of the complement-fixation technique has been shown to be relatively stable. In solution it is little affected by storage for long periods at 3°C., by exposure to a temperature of 56°C. for ½ hour, or by changes in pH over the range from 4.5 to 9.0. The protein nature of the soluble substance is indicated by its precipitation in the presence of 90 per cent solution of alcohol or a saturated solution of ammonium sulfate. Serologically active material was recovered from both the globulin and albumin fractions when separation was carried out with ammonium sulfate. In this respect the soluble antigen of lymphocytic choriomeningitis appears to lie between the soluble substances of yellow fever on one hand and vaccinia and infectious myxomatosis on the other; thus, in yellow fever (8) the specific substance is isolated in the albumin fraction, whereas in vaccinia (6) and myxomatosis (9) the antigen precipitates with the globulin. The relative stability of the soluble antigen of lymphocytic choriomeningitis on heating at 56°C. is a common characteristic of soluble substances associated with virus infections. In myxomatosis (9) there is only a heat-labile antigen; in vaccinia (10) both a heat-labile antigen inactivated at 56°C. and one that withstands boiling occur while in other diseases, *e.g.*, bacteriophagy (11), yellow fever (8), psittacosis (12), and influenza (13), the soluble substance withstands heating at 56°C.

A spontaneous flocculation occurred when fresh clear extracts of guinea pig spleen, from either normal or infected animals, were stored at ice box temperature for several days. This probably accounted for the consistent finding that more voluminous non-specific precipitates resulted when diluted normal or immune serum was added to fresh extracts than to older ones. Furthermore, greater flocculation was observed with unheated serum that had been stored for several months than with fresh serum heated at 56°C. for 30 minutes. Our chief interest in this non-specific flocculating material has been in its elimination from extracts containing soluble antigen; this was accomplished by several methods. There is no reason to believe that a similar type of substance contributed to certain non-specific precipitations observed in the flocculation reaction obtained by Magill and Francis (14) with human sera and crude suspensions of lungs from mice infected with influenza. On the other hand, this material may have played a part in the falsely positive reactions which Dr. Lowell T. Coggeshall, of the International Health Division of The Rockefeller Foundation, encountered while carrying out complement-fixation tests with sera from human beings and

monkeys for the diagnosis of lymphocytic choriomeningitis, and which he permits us to discuss. He prepared 10 per cent suspensions of spleens from guinea pigs moribund with lymphocytic choriomeningitis, cleared them by ultracentrifugation and used them on the day of preparation. Seven of 30 sera from apparently normal human beings without a history suggestive of lymphocytic choriomeningitis, and 3 of 5 sera from normal monkeys in dilutions of 1:1 to 1:16 fixed complement in the presence of such a preparation of antigen. Moreover, the identical sera reacted similarly with fresh extracts prepared by the same method from spleens of normal guinea pigs. Completely negative results were obtained when Dr. Coggeshall tested these sera with our regular preparations of soluble antigen and with our extracts of normal guinea pig spleen. Furthermore, his sera did not neutralize the virus of lymphocytic choriomeningitis. The chief difference in the two sets of test antigen was in their age; our solutions were a number of weeks old when used, hence the spontaneously flocculating material had been precipitated by storage in the ice box.

Falsely positive reactions have not been obtained in our own experience with the complement-fixation technique employed for diagnostic purposes (15) on 150 sera from human beings and 45 sera from monkeys. It happens, however, that we have never used solutions of freshly prepared antigen in these tests. Preparations having a good titer, *e.g.*, 1:16 or 1:32, have been saved until needed for test antigen, usually a matter of several weeks or months, and then used at intervals until the supply was exhausted, a period of at least another month. These findings have been discussed in some detail because it seems desirable to call the attention of others to a possible source of error in the diagnostic test and to suggest that extracts of infected guinea pig spleens be stored for a short period before they are used for this type of work. Lépine has stated (16) that he too has invariably employed for diagnostic work antigenic preparations which had been stored for some time. Falsely positive tests have not been encountered in his complement-fixing studies on lymphocytic choriomeningitis.

The removal of complement-fixing and precipitin antibodies from antisera by absorption with preparations containing soluble antigen suggests strongly, although not conclusively, that these two types of reaction are different manifestations of union between the same types of antigen and antibody. On the other hand, the absorption experiments seem to indicate that the neutralizing substance in immune serum is not identical with the anti-soluble substance antibody. Further evidence for regarding the anti-soluble substance antibodies and neutralizing antibodies as individual entities will be presented in another report. It may be mentioned here, however, that the former appear in the blood of guinea pigs and human

beings several weeks before the latter and diminish greatly within a matter of months, whereas the neutralizing substances are generally demonstrable for years after infection. In this respect also lymphocytic choriomeningitis follows the general pattern observed in other virus infections in which a soluble antigen is found. In each disease which has been adequately investigated, *viz.*, bacteriophagy (11), yellow fever (8), vaccinia (17), influenza (18), and infectious myxomatosis (9), the soluble antigen and its specific antibody appear to play, at the most, a minor rôle in immunity to infection.

SUMMARY

The soluble antigen of lymphocytic choriomeningitis which is readily separable from the virus is a relatively stable substance and appears to be of a protein nature. A specific precipitin reaction can be demonstrated when immune serum is added to solutions of antigen which have been freed of certain serologically inactive substances. The complement-fixation and precipitation reactions which occur in the presence of immune serum and non-infectious extracts of splenic tissue obtained from guinea pigs moribund with lymphocytic choriomeningitis seem to be manifestations of union of the same soluble antigen and its antibody. On the other hand, the anti-soluble substance antibodies and neutralizing substances appear to be different entities.

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