A SOLUBLE ANTIGEN OF LYMPHOCYTIC CHORIOMENINGITIS

I. Separation of Soluble Antigen from Virus

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Soluble antigens separable from the etiological agents have been found in a number of virus infections, *e.g.*, bacteriophagy (1), yellow fever (2), vaccinia (3), psittacosis (4), influenza (5), and myxomatosis (6). On the other hand, in certain virus diseases, *e.g.*, papillomatosis of rabbits (7) and tobacco mosaic of plants (8), all the *in vitro* serological phenomena appear to be the result of reactions between immune sera and the viruses. The occurrence of a complement-fixing substance in extracts prepared from brains of guinea pigs and mice infected with the virus of lymphocytic choriomeningitis has been reported by Howitt (9). However, she did not mention any attempts to determine whether complement fixation was due to the presence of virus or to a soluble material separable from the virus. Separation of a complement-fixing antigen from the virus has been reported by us in a preliminary publication (10). The purpose of this paper is to present in detail the evidence for the existence of a specific soluble antigen associated with the virus of lymphocytic choriomeningitis, and to record its distribution in organs of infected animals.

For experiments of this type, it is essential to have materials rich both in virus and in complement-fixing antigen. The virus of lymphocytic choriomeningitis is not only neurotropic, but is also highly viscerotropic, since it is found in relatively large amounts in organs other than the brain and occurs even in the blood of acutely ill animals (11, 12). Preliminary experiments indicated that spleens from infected guinea pigs were suitable for our needs; hence, material from this source has been extensively used.

Materials and Methods

Source of Virus and Complement-Fixing Antigen.—Guinea pigs were inoculated intracerebrally with 0.1 cc. and subcutaneously and intraperitoneally, respectively, with 1.0 cc. of a 10 per cent suspension of fresh brain from guinea pigs infected with one of four strains of the virus of lymphocytic choriomeningitis maintained in Dr. Rivers'

laboratory (W.E., F.A., R.E.S., and W.W.S.). Animals were sacrificed at the height of the disease, that is, on the 5th or 6th day when infected with the W.E. strain, and on the 9th or 10th day when infected with the W.W.S. strain; organs were removed aseptically, weighed, ground, and made up to a 10 per cent suspension with a physiological saline solution which usually contained 2 per cent normal inactivated guinea pig serum. Mice were inoculated with the W.E. strain of virus by the intracerebral and intraperitoneal routes and sacrificed on the 7th day.

Titration of Infectivity of Virus.—The infective titer of virus preparations was determined in mice by intracerebral inoculations of 0.025 cc. of tenfold dilutions of material. Three mice as a rule received each dilution and survivors were discarded 21 days after inoculation. In experiments in which the virus was concentrated or washed, the various materials to be tested were injected into animals at about the same time. Thus, for control a portion of the original tissue suspension was kept at room temperature, usually for 6 to 8 hours, until the final preparation of virus was obtained.

Preparation of Complement-Fixing Antigen.—Coarse particles were removed by centrifugation at slow speed from 10 per cent suspensions of infected organs which were prepared as described above. The turbid supernatant fluid was then run in the air-driven, concentration centrifuge of Bauer and Pickels (13). The clear, claret colored ultrasupernatant fluid, carefully separated from the floating material and the tan sediment by means of a capillary pipette, was filtered through a Seitz pad which had been previously prepared with saline solution containing 10 per cent inactivated normal guinea pig serum.

Antisera.—Antisera used most extensively were obtained from guinea pigs hyperimmunized in the following manner with the virus of lymphocytic choriomeningitis. Animals which had recovered from infection with the W.W.S. strain of virus, which has a low pathogenicity for guinea pigs, were inoculated intraperitoneally 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 this reinoculation for 10 days were again injected with a similar amount of active material; 10 days later they were bled from the heart under light anesthesia and their pooled serum constituted the hyperimmune serum used. Simple immune serum was obtained from guinea pigs which had recovered from infection with the W.W.S. strain of virus.

Complement-Fixation Reaction.—Materials employed in the hemolytic system were as follows: anti-sheep cell hemolysin, prepared in rabbits, was diluted so that 0.2 cc. contained 2 units (generally a 1:800 dilution). Guinea pig serum for complement was obtained in large amounts, desiccated *in vacuo* from the frozen state (14), and stored at 0°C. At the time of the test, dried serum was brought back to its original volume by the addition of distilled water. Two units of hemolysin and 0.5 cc. of a 5 per cent suspension of washed sheep erythrocytes were added to varying dilutions of resuspended serum in order to determine the titer of complement. Estimation of hemolysis was made after the tubes had been incubated in a water bath at 37°C. for 30 minutes. The titer of the resuspended complement was usually such that 0.03 cc. represented 2 units.

The test was performed in the following manner: 0.2 cc. of a solution of antigen, 0.2 cc. of inactivated hyperimmune serum, and 0.2 cc. of diluted complement (2 units) were mixed and stored overnight at 2°C. 0.7 cc. of a suspension of sensitized erythrocytes were added to each tube the following morning; this suspension was prepared by mixing an appropriately diluted solution of hemolysin with a 5 per cent suspension of sheep

erythrocytes, washed 3 times, in a proportion of 2:5. The tubes were kept in a water bath at 37°C. for 30 minutes, after which time the degree of hemolysis was estimated. The activity of the hemolytic system was such that complete lysis of appropriate controls usually occurred in 10 to 15 minutes. Suitable anticomplementary and hemolytic control systems were included in each test. 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.

EXPERIMENTAL

Concentration of Virus

The particles of the virus of lymphocytic choriomeningitis have been reported to possess diameters not greater than 100 to 150 mμ (15). We have found that this medium sized virus was not appreciably sedimented by a technique adequate for concentration of the virus of vaccinia (16), namely, that of centrifugation in narrow flat tubes in a Swedish angle centrifuge at 3500 R.P.M. for 1 hour. On the other hand, we have been able to concentrate the virus by high speed centrifugation (13), 30,000 R.P.M. for 20 minutes, or 20,000 R.P.M. for 30 minutes being sufficient to sediment the virus.

Early attempts to wash the ultrasediment with saline solution were unsuccessful, inasmuch as repeatedly washed material possessed little virus activity. It was found, however, that tissues heavily ladened with virus rapidly diminished in infectivity when suspended in saline solution for a few hours at room temperature. In view of the toxicity of sodium chloride solution for the virus of herpes simplex (17), diluents other than saline solution were tried, *e.g.*, dilute sodium phosphate-citric acid buffer solution at pH 7.2, dilute potassium phosphate-citric acid buffer solution at pH 7.6, and bacteriological broth. Although none of these diluents was satisfactory, the least inactivation of virus occurred when broth was used. Since the addition of normal serum to saline suspensions of the virus of yellow fever (18) renders this infectious agent more stable on storage, a similar procedure was followed in our experiments. Physiological saline solution containing 2 per cent normal inactivated guinea pig serum proved to be a satisfactory menstruum for our purpose, since with it infectious sediments could be repeatedly washed, as shown by the following work, without appreciable loss of activity.

The ultrasediment from 50 cc. of a 10 per cent suspension of infected splenic tissue which had previously been run in the horizontal machine usually amounted to 0.5 or 0.6 cc. When this sediment was resuspended in 10 to 20 cc. of diluent and then partially cleared by horizontal centrifugation at 2500 R.P.M. for 30 minutes, a considerable amount of sediment (±0.3 cc.) resulted, leaving a moderately opalescent supernatant fluid.

The removal of this large amount of sediment by horizontal centrifugation did not materially reduce the infectivity of the supernatant fluid. A method of differential centrifugation was then introduced into the technique for washing the virus; for example, the sediment resulting from high speed centrifugation was resuspended and run at 10,000 R.P.M. for from 6 to 10 minutes in the ultracentrifuge. The slow ultracentrifugation, like the horizontal run, gave a considerable amount of sediment which only slightly reduced the infectivity of the supernatant fluid. Hence, the supernatant suspension from the slow speed centrifugation was saved while the sediment was discarded. Differential centrifugation of this type, with alternate fast and slow runs, always resulted in some loss of virus when the sediment was washed 3 or 4 times. In typical experiments about 0.15 cc. of washed sediment was ultimately obtained from 10 infected spleens. This material, resuspended in 20 cc. of serum-saline solution and run at 10,000 R.P.M. for 6 minutes, gave a faintly opalescent supernatant fluid which was infective in a dilution of 10^{-5} or 10^{-6}. Original splenic suspensions were infective at dilutions of 10^{-7} or occasionally 10^{-8}. Suspensions of washed virus did not form an appreciable amount of sediment when stored for several weeks at 3°C., that is they were stable. Moreover, although the infectivity gradually decreased on storage, one specimen of washed virus with an original titer of 10^{-7} still killed animals when diluted to 10^{-3} after storage for 4 weeks at 3°C.

The appreciable loss in infectivity, which occurred when the above method of differential centrifugation was employed, led us to modify the technique. Ultrasediment was washed 3 or 4 times but centrifugation at 10,000 R.P.M. was omitted until after the ultrasediment had been resuspended for the last time. Suspensions of washed virus obtained by this method had an appearance similar to that of the previous preparations but were as infectious as the original suspensions of infected spleen (Table I).

Results of attempts to demonstrate an agglutination phenomenon with suspensions of washed virus were not sufficiently clear cut to be convincing. Examination of suspensions under dark field illumination revealed many tiny particles of uniform size which appeared smaller than elementary bodies of vaccinia viewed under similar conditions. The explanation of the failure to obtain definite agglutination may depend upon the fact that insufficient virus was present. Thus, Parker and Rivers (19) observed that the concentration of elementary bodies of vaccinia required for visible agglutination was log 8.29 particles per cc. On the basis of Merrill's studies (20), the number of particles per cc. of the virus of lymphocytic choriomeningitis necessary for a visible agglutination reaction would be greater than the number of Paschen bodies. It is at once apparent that the concentration of active virus of lymphocytic choriomeningitis in the final preparations was even less than that required for an agglutination reaction with elementary bodies of vaccinia. Furthermore, only a portion of the particles visible in the dark field could have been bodies of virus, since

preparations made by the same technique from spleens of normal guinea pigs contained at least half as many small, uniform particles as did the preparations made from infected spleens.

Separation of Complement-Fixing Antigen from Virus by Means of Ultracentrifugation

In the preceding experiments it was found that virus can be readily sedimented from suspensions of infected spleen by ultracentrifugation at 30,000 R.P.M. for 20 minutes. The supernatant fluid, freed of virus, fixed complement in the presence of immune serum as well as the uncentrifuged material. On the other hand, particles of virus which had been washed several times were capable of binding complement only to a slight extent. The following experiment is typical of five which gave similar results.

Spleens were removed aseptically from 10 guinea pigs 6 days after infection with the W.E. strain of virus. The organs were weighed, ground in a mortar, and made up to a 10 per cent suspension with buffered saline solution, pH 7.2, containing 2 per cent inactivated normal guinea pig serum. 50 cc. of a supernatant suspension were obtained after centrifugation at 2500 R.P.M. for 20 minutes. 15 cc. of this were set aside for determination of infectivity and complement-fixing titer, both before and after Seitz filtration. The remaining 35 cc. were placed in lusteroid tubes[1] and run at 30,000 R.P.M. for 20 minutes. Floating material was removed with a cotton swab and the clear supernatant fluid was taken up with a capillary pipette; residual particulate material was removed from the supernatant fluid by another run at the same speed; then the fluid was filtered through a Seitz pad and saved for titrations. The sediment from the original ultracentrifugation which contained the virus was thoroughly dispersed in 15 cc. of serum-saline solution and again sedimented. In this manner, the virus was washed 3 times, and then taken up in a volume of 15 cc.; the first 2 wash waters were saved. The virus suspension was next centrifuged at 10,000 R.P.M. for 6 minutes; the faintly opalescent supernatant fluid was considered as the final suspension of virus; the sediment was discarded.

The results of this experiment are summarized in Table I. The suspension of infected spleens after the first horizontal centrifugation gave complete fixation of complement when diluted 1:32, but was anticomplementary in low dilutions; when diluted 10^{-6} it was infectious for all mice inoculated, and killed 1 of 3 mice which received the next higher dilution. A marked reduction in infectivity and a slight but detectable diminution in amount of complement-fixing antigen occurred when the crude suspension was filtered through a Seitz pad. Ultracentrifugation of the crude suspension, followed by Seitz filtration of the supernatant fluid, resulted

[1] Manufactured by the Lusteroid Container Corporation, South Orange, New Jersey.

in a complete loss of infectivity for mice. However, the complement-fixing antigen was still present in the same amount as in the previous filtrate. Decreasing amounts of antigen were present in successive lots of fluid which were used to wash the sedimented virus. The final preparation of washed

TABLE I

Separation of Soluble Antigen from Virus in Infected Splenic Tissue by Centrifugation and Filtration

Material	Infective titer	Approximate dilution of original splenic tissue	Dilution of material					
			1:1	1:2	1:4	1:8	1:16	1:32
Horizontal supernatant unfiltered	10^{-6}	10^{-1}	Anticomplementary			++++	++++	++++
Horizontal supernatant Seitz filtrate	10^{-3}	10^{-1}	++++	++++	++++	++++	+++	−
Ultrasupernatant Seitz filtrate	Negative	10^{-1}	++++	++++	++++	++++	+++	−
1st wash water Seitz filtrate	Not done	10^{-2}	++++	++++	+++			
2nd wash water Seitz filtrate	" "	10^{-2}	+++	−	−			
Final virus suspension washed 3 × (in ½ original volume)	10^{-7}	10^{-6}	+++	+++	±	−	−	−

Guinea pigs were infected with W.E. strain of L-C-M virus.

Original suspending medium and "wash water" consisted of buffered saline solution containing 2 per cent heated normal guinea pig serum.

Preliminary centrifugation was done in horizontal machine at 2500 R.P.M. for 20 minutes; ultracentrifugation at 30,000 R.P.M. for 20 minutes.

Degree of complement fixation is indicated in the usual manner.

virus was as infectious as the original suspension of spleen; it killed all 3 mice which were inoculated with a 10^{-6} dilution and 2 of the 3 mice which received a 10^{-7} dilution. In contrast to the high infective titer the final virus preparation, in a dilution of 1:2, reacted incompletely with complement. Final suspensions of virus were not anticomplementary even in the undiluted state, provided that aggregated particles were removed by a final centrifugation at 10,000 R.P.M. When this procedure was omitted, the

lower dilutions of virus suspension were anticomplementary and the slight fixation displayed by the virus was missed.

The ease with which the complement-fixing antigen can be separated from the infective agent indicates that lymphocytic choriomeningitis belongs to the group of virus infections in which is found a specific soluble antigen separable from the virus. Moreover, as in vaccinia (21) and psittacosis (22), the washed particles of the virus of lymphocytic choriomeningitis are capable of fixing complement to a certain degree.

Distribution of Soluble Antigen in Organs of Infected Animals

Antigen prepared according to the routine method from infected spleens of guinea pigs usually gave complete fixation of complement when 0.2 cc. of a dilution of 1:16 were mixed with hyperimmune serum. However, soluble antigen was occasionally not demonstrable in such preparations in a dilution greater than 1:4; on the other hand, certain lots fixed complement in a dilution of 1:32. The distribution of complement-fixing antigen in a number of tissues and in the blood of infected guinea pigs was next investigated. Antigenic material, prepared according to routine from lungs showing pneumonic consolidation, completely fixed complement in the undiluted state. Moreover, in several experiments, undiluted extract obtained from livers was shown also to fix complement partially. On only two occasions did extracts of infected brains give even partial fixation. Soluble antigen was not present in detectable amounts in extracts of kidney nor was it demonstrable in the serum, although spleens of the same animals contained antigen in large amounts. Furthermore, the lots of pooled serum which were tested for the presence of antigen were infective in dilutions of 10^{-5} and 10^{-6}; the serum was used as antigen after being heated for 15 minutes at 56°C. in order to inactivate complement which might have been present.

The presence of soluble antigen was also demonstrated in extracts prepared from organs of infected mice. The amounts of antigen obtained from spleen and liver were comparable to those which occurred in extracts of these organs of infected guinea pigs. In neither of two experiments was antigen found in demonstrable quantity in preparations from brains of infected mice.

Finally, complement-fixing antigen was demonstrated in an extract of consolidated lung obtained from a monkey dead of lymphocytic choriomeningitis.

One experiment was made with materials from a monkey (*Macacus rhesus*) infected by the subcutaneous route with 10^5 mouse doses of virus of the W.E. strain. The

animal developed fever 48 hours after injection and died on the 12th day. Blood taken on the 11th day was infectious for guinea pigs but contained neither soluble antigen nor complement-fixing antibody in demonstrable amounts. An extensive hemorrhagic pneumonia was found at autopsy performed immediately after death. Consolidated portions of lung were ground and made into a 15 per cent suspension (wet weight). The infective titer of the crude suspension of lung was found to be 10^{-6}. After preparation by the usual centrifugation technique the lung extract fixed complement when diluted 1:16. The spleen was not used because of the presence of tubercles.

TABLE II

Distribution of Soluble Antigen in Material from Animals Infected with Virus of Lymphocytic Choriomeningitis

Species	Source of antigen	Dilution of antigen					
		1:1	1:2	1:4	1:8	1:16	1:32
Guinea pig	Spleen	++++	++++	++++	++++	++	−
	Lung*	++++	++−	−	−	−	−
	Liver	++	−	−	−	−	−
	Brain	++	−	−	−	−	−
	Kidney	−	−	−	−	−	−
	Serum	−	−	−	−	−	−
Mouse	Spleen	++++	++++	+++	±	−	−
	Liver	+++	−	−	−	−	−
	Brain	−	−	−	−	−	−
Monkey	Lung*	++++	++++	++++	++++	++++	−
	Serum	−	−	−	−	−	−

* Only areas showing pneumonic consolidation were employed.

Hyperimmune guinea pig serum was used in a dilution of 1:32.

Animals infected with W.E. strain of virus were sacrificed at the height of the disease. 10 per cent suspensions of fresh organs were subjected to ultracentrifugation and Seitz filtration, except in the case of the monkey lung (see text).

Degree of complement fixation is indicated in the usual manner.

A comparison of the distribution of complement-fixing antigen in material obtained from acutely ill animals is shown in Table II.

Occurrence of Antigen in Guinea Pigs Infected with Different Strains of the Virus

Four strains of the virus of lymphocytic choriomeningitis maintained in this laboratory vary in their pathogenicity for the guinea pig. The W.E. strain, with which most of the present work is concerned, is almost invariably lethal for guinea pigs when inoculated either intracerebrally or

subcutaneously. The brain of a guinea pig moribund after inoculation of this strain is infectious in a dilution of 10^{-7} or 10^{-8} when passed intracerebrally to other guinea pigs. On the other hand, the W.W.S. strain rarely kills guinea pigs when administered even in large amounts by either of these routes. Moreover, it has a low pathogenicity for mice. Two other strains, F.A. and R.E.S., behave in a manner intermediate between the two just mentioned. In view of these facts, it was of interest to determine whether the presence of complement-fixing antigen could be demonstrated in preparations of spleens from animals infected with the 3 less virulent

TABLE III

Occurrence of Soluble Antigen in Spleens of Guinea Pigs Infected with Four Strains of Virus

Guinea pig serum	Strain of virus	Dilution of antigen					
		1:1	1:2	1:4	1:8	1:16	1:32
W.E. hyperimmune (dilution 1:32)	W.E.	++++	++++	++++	++++	+	−
	F.A.	++++	++++	−	−	−	−
	R.E.S.	++++	+++	−	−	−	−
	W.W.S.	++	−	−	−	−	−
W.W.S. immune (dilution 1:32)	W.E.	++++	++++	++++	++++	+++−	−
	F.A.	++++−	++	−	−	−	−
	R.E.S.	++++	−	−	−	−	−
	W.W.S.	±	−	−	−	−	−

10 per cent suspensions of infected splenic tissue were subjected to ultracentrifugation and Seitz filtration.

Degree of complement fixation is indicated in the usual manner.

strains of virus. Furthermore, it was desirable to know whether the soluble antigen exhibited any evidence of strain specificity. Accordingly, splenic extracts were prepared in the usual manner from guinea pigs infected with each of the 4 strains of virus and tested against the serum of a guinea pig immune to the W.W.S. strain and against the pooled hyperimmune serum used throughout the experiments.

From the results shown in Table III, it can be seen that splenic extracts prepared from animals infected with each strain of virus contained soluble antigen. Moreover, no evidence of type specificity was encountered in the 4 strains. However, certain quantitative differences were noted in the preparations, *viz.*, the amount of antigen obtained from spleens of animals infected with the 4 strains corresponded directly to the virulence of each strain. This observation might be interpreted to mean that the soluble

antigen contributes to the virulence of the strain of virus. On the other hand, infection with the most virulent strain (W.E.) results in a higher concentration of demonstrable virus in tissues of animals than does infection with the milder strains. This greater amount of virus might easily account for the presence of more soluble antigen in tissues infected with the virulent strain, irrespective of the origin of the soluble substance, *i.e.*, virus particle or host cell.

Specificity of the Complement-Fixation Reaction

Extracts of normal organs of the guinea pig, mouse, and monkey, prepared according to the routine procedure, did not fix complement in the presence of lymphocytic choriomeningitis hyperimmune serum. Nor did serum from the normal guinea pig, mouse, or monkey react with organ extracts of the homologous species which contained the soluble antigen. Howitt (9) observed no cross-reaction when materials obtained from animals infected with the viruses of lymphocytic choriomeningitis, St. Louis encephalitis, and several strains of equine encephalomyelitis, were employed in complement-fixation tests. Similarly, we have found no evidence of cross-reaction in tests in which the soluble antigen of lymphocytic choriomeningitis was mixed with serum from rabbits hyperimmunized with the virus of vaccinia or of myxomatosis. Furthermore, no detectable reaction occurred when the antigen was used with human serum which contained antibodies against the soluble antigen of influenza.[2] Finally, solutions of antigen, which reacted specifically with antisera for vaccinia, myxomatosis, and influenza, respectively, gave no fixation of complement when added to serum from guinea pigs hyperimmunized with the virus of lymphocytic choriomeningitis. Results of experiments dealing with the specificity of the reaction are shown in Table IV.

Complement-fixing antigens from sources other than virus-infected tissues were also tested. No cross-reactions were observed by Eaton (23) between malarial antiserum and malarial antigen, respectively, and soluble substance of lymphocytic choriomeningitis and hyperimmune guinea pig serum which we supplied. Furthermore, human sera known to give positive Wassermann reactions[3] did not react with the soluble antigen of lymphocytic choriomeningitis; this finding agrees with observations of Lépine, Mollaret, and Sautter (24). In addition, other human sera capable of fixing complement in the presence of gonoccocal antigen[3] gave no reaction with the antigen of lymphocytic choriomeningitis.

[2] Influenzal antigen and antiserum were provided by Dr. Frank L. Horsfall, Jr.

[3] Known positive and negative Wassermann and gonococcal complement-fixing sera were provided by Dr. Archibald McNeil.

TABLE IV

Specificity of Soluble Antigen of Lymphocytic Choriomeningitis

Antiserum	Source of antigen	Dilution of antigen	Dilution of antiserum									
			1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Vaccinia hyperimmune (rabbit)	Vaccinia, dermal filtrate	1:25						++++	++++	++++	+	−
	L-C-M, spleen	1:3						−	−	−	−	−
Myxoma hyperimmune (rabbit)	Myxoma, acute serum	1:25						++++	++++	++++−	++	−
	L-C-M, spleen	1:3						−	−	−	−	−
Influenza convalescent (human)	Influenza, mouse lung	1:10	++++	++++	++++	++++	++++−	±				
	L-C-M, spleen	1:3	−	−	−	−	−	−				
L-C-M hyperimmune (guinea pig)	L-C-M, spleen	1:3						++++	++++	++++	++++	−
	Vaccinia, dermal filtrate	1:25						−	−	−	−	−
	Myxoma, acute serum	1:25						−	−	−	−	−
	Influenza, mouse lung	1:10						−	−	−	−	−

Extracts of tissue prepared from normal animals did not contain specific soluble antigen.
Degree of complement fixation is indicated in the usual manner.

Preparation of Antigen by Methods Other than the Routine Procedure

We have demonstrated the presence of complement-fixing antigen in extracts of infected brains of guinea pigs, prepared according to Howitt's (9) modifications of the technique of Craigie and Tulloch (25), which consists of the extraction of dried brain with ethyl-ether, resuspension of the non-extractable residue in physiological saline solution followed by repeated freezing and thawing in a salt-ice mixture, and removal of coarse particles by centrifugation. At times, however, this procedure did not yield detectable antigen and on no occasion was the serologically active substance present in large amounts.

It seemed likely that in the usual method of preparation of splenic extracts a considerable amount of antigen was lost in sedimented tissue which was discarded after the original horizontal centrifugation. Consequently, horizontal sediments were resuspended in the original volume of fresh serum-saline solution, frozen in a mixture of salt and ice, and subsequently thawed. Alternate freezing and thawing were repeated 5 or 6 times, after which the suspensions were carried through the regular procedure, *i.e.*, horizontal and ultracentrifugation, and Seitz filtration. On two occasions, extracts prepared in such a manner had a titer approximately equivalent to that of corresponding extracts prepared according to routine. However, on one occasion, only a comparatively small amount of antigen was obtained from the reclaimed sediment.

It became of interest to determine the largest amount of complement-fixing antigen which could be extracted from infected spleens and to compare it with the amount present in the regular preparations.

Accordingly, 10 infected spleens were divided longitudinally; one set of halves was ground and suspended in a volume of 30 cc. of diluent and prepared according to routine. The titer of the complement-fixing antigen in this extract was 1:16. The 10 remaining halves of spleens were ground in a mortar, without abrasive, suspended in sufficient saline solution to enable the material to be transferred to a tube, and desiccated *in vacuo* from the frozen state. The dried material, 0.2 gm., was resuspended in 20 cc. of saline solution and repeatedly frozen and thawed. After horizontal centrifugation, the supernatant suspension had a complement-fixing titer of 1:32. Thus, on the basis of dry weight, each gram of spleen yielded 12,000 fixing doses of antigen when treated according to the routine method, while 16,000 fixing doses were extracted by the modified procedure.

It is apparent from the above experiment that extracts of infected spleens, prepared according to the method we have usually employed, contained only a part of the complement-fixing antigen which could be obtained by another method.

DISCUSSION

The present observations indicate that a specific soluble substance separable from the virus of lymphocytic choriomeningitis plays the chief rôle in the complement-fixation reaction which occurs when extracts of infected tissue are mixed with immune sera. The slight fixation displayed by washed virus may be due to adsorbed soluble antigen. In certain virus diseases it has been suggested that the soluble antigen derives from the host instead of the infectious agent (26); the most suggestive example is the soluble substance found in yellow fever (2). On the other hand, the available evidence (27) regarding the soluble antigens of vaccinia indicates that they are intimately associated with the virus and are not derived from the host. At present it is not possible to give an opinion concerning the origin of the complement-fixing substance of lymphocytic choriomeningitis. However, it may be pointed out that, as in vaccinia, the antigen of lymphocytic choriomeningitis parallels in amount the infectious agent and is present irrespective of the species of animal infected, *i.e.*, guinea pig, mouse, or monkey.

Observations on the characteristics of the soluble antigen of this disease will be published later. Solutions of the antigen remain active for long periods of time when stored at ice box temperature, and they do not become anticomplementary. This stability facilitates the use of the soluble antigen in a diagnostic test which we have employed with serum from human beings suffering from lymphocytic choriomeningitis (10). Our results with patients' sera are in agreement with those of Lépine, Mollaret, and Sautter (24) who used for their antigen suspensions of consolidated lung of guinea pigs which undoubtedly contained both specific soluble substance and virus.

SUMMARY AND CONCLUSIONS

The virus of lymphocytic choriomeningitis can be sedimented in the ultracentrifuge and washed repeatedly; the virus retains its activity provided that a small amount of normal serum is present in the diluent.

A soluble substance capable of fixing complement in the presence of immune serum can be separated from the virus. Washed virus fixes complement poorly.

The serologically specific soluble antigen is widely distributed in tissues of infected guinea pigs, mice, and monkeys.

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