Preparation of Specific Complement-Fixing Antigens from the Three Prototypes of Poliomyelitis Viruses.* (19268)

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Specific complement fixing (CF) antigens have been prepared from nerve tissues infected with Lansing poliomyelitis virus by technics involving acetone desiccation(1), methanol precipitation(2), and ultracentrifugation(3). Lansing poliomyelitis virus was adapted to new-born mice, and from this adapted virus, a CF antigen was prepared which appeared to show some antigenic relationship to Brunhilde type antibody contained in monkey and in human antiserum(1). While extensive studies involving neutralization and vaccination-challenge technics indicate that Lansing, Brunhilde and Leon viruses are antigenically distinct(4,5), one might speculate either that the Lansing virus may have been altered by such an adaptation or that the method used in preparing such CF antigens did not yield a specific antigen. Since the technic employed has repeatedly yielded specific CF antigens with other neurotropic viruses (6), the latter supposition might thereby be vitiated. It should be pointed out that the Brunhilde serums used above were not tested for CF properties with Brunhilde antigen. More recently a specific CF antigen was prepared from Lansing infected cotton rat brains by a technic involving acetone desiccation as referred to above(7).

Prior to the use of adjuvants in immunization procedures, it was very difficult to demonstrate CF antibodies in immunized monkeys. It was felt at that time that perhaps monkeys did not produce such antibodies. By mixing the virus inoculum with depot-producing agents, monkeys thus immunized rapidly responded with high titer neutralizing antiserum and rapidly developed cerebral immunity to challenge with homologous virus(8-10). Since specific CF antigens were consistently produced from Lansing infected cotton rat (LCR) tissues by the ultracentrifugation technic(3), it was felt that nerve tissues infected with the Brunhilde and the Leon types of poliomyelitis virus should be processed in the same manner for specific CF antigens.

Methods. Antigens. The method employed has been previously described(3) and will be briefly outlined as follows: Spinal cords of *Macacus mulatta* monkeys or brains of cotton rats *(Sigmodon hispidus)* collected early in the course of symptoms of poliomyelitis were emulsified in cold 0.15 M NaCl by a Waring blendor (6 minutes). The final dilution of cord material was a 20% suspension by weight. The emulsions were clarified at 5000 rpm for 1 hour and 18,000 rpm for 10 minutes. The clear supernatant fluid was mixed with ⅓ volume di-ethyl ether and stored at 4°C for one hour. The mixture was centrifuged at 5000 rpm for 30 minutes and the aqueous portion thereof was stored at −20°C overnight. The thawed and clarified suspension was then centrifuged in the Spinco preparative ultracentrifuge at 144,000 x G for 2 hours. The sedimented pellet was resuspended in 1/10 volume M/5 phosphate buffer (pH 7.2) stored overnight at 4°C, and after centrifugation at 5000 rpm for 30 minutes, the clear supernate was tested as antigen. CF antigens were thus prepared from cotton rat tissues infected with Lansing virus; and from monkey tissues infected with Brunhilde, Wisconsin, and Leon viruses.†

Antiserum. The 3 prototypes of poliomyelitis virus (Lansing, Leon and Brunhilde) were individually mixed with a depot producing adjuvant,‡ and inoculated at weekly intervals into the gastrocnemius muscles of *M. mulatta* monkeys. The monkeys were each bled at

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† Viruses were kindly supplied as follows: Brunhilde and Leon by Dr. David Bodian; Wisconsin by Dr. H. K. Faber.

‡ "Pendil" manufactured by Endo Products, Richmond Hill, N. Y., contains oxycholesterol, peanut oil, and beeswax.

TABLE I. Specificity of Complement-Fixing Antigens Prepared by Ultracentrifugation of the Virus.

Monkey serum	Antigens					
	LCR*	Brunhilde	Wisconsin	Leon	Rabies	SLE
Lansing	4+ 1:40	—	—	—	—	—
Brunhilde	—	4+ 1:5	4+ 1:5	—	—	—
Leon	—	—	—	4+ 1:5↑	—	—
Frederick	—	4+ 1:5	4+ 1:10	—		

* LCR—Lansing virus in cotton rat tissues. Rabies—in cotton rat tissues. SLE—St. Louis encephalitis virus in cotton rat tissues.

weekly intervals and the clear serum was stored at –20°C until used. Antiserum specimens were also kindly supplied by Drs. Jonas Salk (Brunhilde and Leon), C. F. Pait (Leon), and H. A. Howe (Frederick). The serums from Dr. Salk were prepared by the technic employing a depot-producing adjuvant (Arlacel A and Bayol F) as an antibody enhancing agent(9).

C.F. test. The serums were inactivated (1 in 5 dilution) at 61°C for 20 minutes. Two-tenths cc each of serum, antigen, and complement (2 units) were mixed and incubated at 4°C for approximately 18 hours. The amboceptor and 2% sheep red blood cells (0.2 cc each) were added and the mixtures were stored at 37°C for 30 minutes at which time the results were recorded. Serum was titrated in a constant dilution of antigen and antigen was titrated in a constant dilution of serum. All CF tests were set up at least 3 times. Serum, antigen, and complement controls accompanied each test. CF antigens similarly prepared from cotton rat tissues infected with rabies and with St. Louis encephalitis (SLE) viruses served as further controls on the specificity of the antigens.

Results. As indicated in Table I, specific complement fixation was obtained with Lansing antigen and antiserum. Leon antiserum fixed complement with Leon antigen, and Brunhilde antiserum with Brunhilde antigen. Antigen prepared from Wisconsin infected monkey tissue fixed complement with Brunhilde and with Frederick antiserum. The antigens prepared from monkey tissues were not potent beyond 1:1 dilution and often had to be used undiluted. The LCR antigen could often be used at 1:2 and 1:4 dilution. The latter antigen retained its specificity when stored for at least 80 days at –20°C. Not all Brunhilde antigens prepared were suitable as CF antigens; whereas, those prepared from Wisconsin virus were more consistently useful. The water clear antigens were neither anticomplementary nor procomplementary. The average yield of antigen per gram of tissue was 0.5 cc.

When 4 monkeys were immunized with Lansing virus-adjuvant mixtures, they responded within two weeks with specific CF antibodies. Two monkeys without adjuvant very gradually developed detectable low titer CF antibodies after 12 weekly inoculations. When adjuvant-virus was inoculated into the latter monkeys, they responded within 1 week with CF antibody titers of 1:40 and 1:80. The CF antibody levels of all virus-adjuvant inoculated monkeys remained elevated for at least six months without further exposure to antigenic stimulus.

Two monkeys which were inoculated with Brunhilde-adjuvant and two with Leon-adjuvant mixtures did not produce detectable CF antibodies even after nine inoculations. The antigens used to test these serums did fix complement specifically with the Leon and the Brunhilde antiserums that were supplied by Dr. Salk.

Discussion. The specific serological differentiation exhibited by the three prototype viruses is in agreement with the results attained by others through serum neutralization and vaccination-challenge technics(4,5). The close relationship demonstrated for Brunhilde, Wisconsin and Frederick viruses is in accord with the above findings(11). The CF serum titers of the non-Lansing tests (Brunhilde & Leon), while relatively low, were significant. It must be emphasized that the CF antigens prepared from the latter 2 viruses were difficult to attain. That not every at-

tempt was successful might be attributed to the initial low titer of virus in the tissues used or that the virus may have been dissipated while being processed. Specific LCR CF antigen is consistently produced by the technic described. While it was difficult to process consistently satisfactory CF antigens from Brunhilde infected cords, the comparative success with Wisconsin virus has caused us to concentrate our efforts on the latter virus. It is possible that another virus might be superior even to the latter.

We have prepared specific Lansing CF antigens by the acetone-ether technic previously described(6,7) from infected brains of mature cotton rats. Thus far, similar attempts with Leon and Brunhilde-infected monkey tissues have not yielded CF antigens when tested with suitable antiserums.

The adjuvant "Pendil" as used by us did not enhance the CF antibody response of monkeys to Brunhilde and Leon viruses as did the Arlacel A and Bayol F of Salk *et al.*(9). Why "Pendil" worked with LCR and not with Brunhilde and Leon antigens is as yet beyond explanation. The advantage of the depot-producing agent with LCR is apparent and explains the CF difficulties in past experiences with antiserums prepared in monkeys. This is a good example of how suitable CF antigens might be discarded because of unsuitable antiserum, the latter presumably being produced by sound technics.

We have demonstrated specific CF with the three prototype viruses of poliomyelitis. Whether this will have a practical application will depend on future development with the virus and on the serological pattern demonstrated in human serums. Thus far the CF antigens processed from Leon and Brunhilde-type infected tissues were neither easily prepared nor potent when finally attained. We still need a better source of virus than that used for this study.

Summary. Specific complement-fixing antigens were prepared from Lansing-, Brunhilde-, and Leon-infected tissues by sedimentation of the viruses in the high speed centrifuge and using the resuspended sediment as antigen. Specific complement fixing antiserums were produced more rapidly in Lansing immunized monkeys when the virus inoculum was incorporated into a depot-producing adjuvant. It appears that the adjuvant treatment as recommended by Salk is needed for production of Brunhilde and Leon complement fixing antiserum.

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Acute Disseminated Encephalomyelitis Produced in Mice by Brain Proteolipide (Folch-Lees).* (19269)

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The isolation and identification of the encephalitogenic agent present in brain tissue has been sought since experimental disseminated (allergic or isoallergic) encephalomyelitis was first definitely produced(1). Various fractions deriving from extracts of cerebral tissue by lipid or other solvents—substances considered by some investigators to be leci-