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An Improved Benzene Extracted Complement Fixing Antigen for Certain Neurotropic Viruses.*

CARLOS ESPANA† AND W. McD. HAMMON.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.

DeBoer and Cox[1,2] have recently described benzene-extracted mouse-brain and chick embryo complement fixing antigens for the diagnosis of neurotropic virus infections. The advantage claimed is that these antigens do not react with syphilitic sera that have been inactivated at only 60°C. We have now prepared antigens according to this new technic for Western and Eastern equine, St. Louis, Japanese B and Hammon-Reeves California viruses. Procedures were repeated several times for most viruses until several lots had been prepared. However, although the antigens completely fulfilled the criteria claimed by the authors, and represent an important advance, their sensitivity was slightly less than that of those we were accustomed to using (prepared by either the technic of Casals[3] or by a modification of that of Havens *et al.*,[4] centrifuging 10 per cent mouse-brain at 16,000 r.p.m.). Furthermore, the preparation described was prolonged (requiring about 4 days), and in our opinion unnecessarily dangerous (transferring and pulverizing powdered antigen from filter paper). We therefore undertook several experimental modifications of this technic. A more complete paper with extensive protocols is being published separately.

Preparation of Antigens. Mice are inoculated intracerebrally with a 10^{-2} or 10^{-3} dilution of virus. The mice, when moribund, are anaesthetized, bled to death and the brains removed. The brains are then ground in a Waring blendor in pyrogen-free, fractionally distilled water to make a 20% suspension. After storage for 3 to 4 hours at 5°C, 25 ml amounts are rapidly shell frozen in 250 ml Pyrex bottles and then lyophilized. The dried tissue is then extracted with benzene for 1 hour at room temperature by adding a volume of benzene equivalent to twice the original aqueous suspension. The benzene is rapidly removed by filtration through a Gooch crucible filter under high vacuum. Two further benzene extractions are performed in the same filter, allowing 30 minutes at room temperature each time before applying vacuum. The extracted tissue is then transferred under a hood by inverting the Gooch filter over a wide mouth stemless funnel placed in the neck of a 250 ml Pyrex bottle and then tapping the crucible filter gently till the dried powder falls free. The remaining solvent is removed by applying negative pressure to the bottle. Saline is next added in the amount of the original volume and rehydration permitted to occur overnight at 5°C, then after centrifugation for 30 minutes at 10,000 r.p.m. the supernatant is removed and Merthiolate is added to a final dilution of 1:10,000. The antigen is ready for immediate use, but for prolonged storage or shipment it is again lyophilized. The antigen is complete within 36 hours after killing the mice.

Characteristics of the Antigen. All tests have been made using essentially the technic described by Casals.[3] The liquid antigen,

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1 DeBoer, C. J., and Cox, H. R., *J. Bact.*, 1946, **51**, 613.

2 *Ibid.*, *J. Immunol.*, 1947, **55**, 193.

3 Casals, J., *J. Exp. Med.*, 1944, **79**, 341.

4 Havens, W. P., Jr., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, **77**, 139.

prior to the final lyophilization has remained unchanged in all its characteristics when stored at 5°C for at least 6 months. The final, dry product goes into complete solution rapidly and requires no centrifugation before use. Its titer is the same as before lyophilization, and if any particular lot of antigen has a low titer, by adding only half the original volume of water to the powder the titer can be doubled. The reconstituted antigens have been tested after 3 months storage in the liquid state and have shown no decrease in titer or tendency to become anticomplementary.

These antigens when first prepared have infective titers of 10^{-3} or 10^{-4}. After a few weeks in the liquid state at 5°C they become practically nonvirulent.

No human serum, syphilitic or otherwise, among approximately 500 that we have tested has given a non-specific response after inactivation for 20 minutes at 60° C.

These antigens should be diluted further for routine use, for when concentrated, relatively low serum titers are obtained. The optimal dilution is determined by performing a combined, serial, two-fold immune guinea pig serum, and two-fold antigen titration. The highest dilution of antigen giving the highest serum titer is selected for use. This is usually a dilution of 1:4 to 1:16 of the antigen (1.25 to 5% brain) and represents about 8 to 16 antigen units. With guinea pig immune sera, antigens titer (in serial 2-fold antigen dilutions) from 1:64 or 1:128 (Western equine and California virus) to 1:256 or 1:512 (Japanese B, St. Louis and Eastern equine). At the optimal dilutions no overlapping can be demonstrated between any of our antigens and any heterologous hyperimmune sera, not even between Japanese B and St. Louis, although when an excess of antigen is used such overlapping is observed.

Human convalescent sera for Western equine infections have titered as high as 1:64 (original serum dilution) and for St. Louis and Japanese B (sera collected by W. M. H. from 1945 Okinawa epidemic) up to 1:512. Human convalescent sera give higher titers with these antigens than with any others we have ever prepared and there has been greater uniformity of titer between repeated preparations with the same virus. Moreover, these are the only antigens that have not given us occasional non-specific reactions with sera from febrile patients with other infections.

These antigens all show a tendency to protect complement during overnight incubation at 5°C and thus avoid certain difficulties shown by some other antigens when used with sera that are very slightly anticomplementary, though not detectably so in the anticomplementary control.

Summary. A time saving and safer procedure for preparing benzene extracted brain antigens is described. The resultant antigen is more sensitive and more specific than others previously tested by us. In its final form it is a stable, lyophilized product that can be easily shipped and can be used without centrifugation after rehydration.