Inactivated Complement Fixing Antigen from Venezuelan Equine Encephalitis Virus Grown in Tissue Culture[1] (35820)

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Arbovirus complement fixing (CF) antigens are customarily prepared from infected suckling mouse brains or from infected tissue culture fluids. Mouse brain antigens are usually obtained by sucrose acetone extraction because these preparations are potent, free of host materials, and free from anticomplementary (AC) activity. Furthermore, these preparations can be used for both hemagglutination-inhibition (HI) and CF tests (1). These antigens are not inactivated, and their preparation and use for viruses pathogenic for man requires caution and an appreciation of the restrictive conditions under which they may be used. Antigens derived from tissue culture have generally been of low potency, anticomplementary, and also infectious.

Ionizing radiation has been used to prepare noninfectious diagnostic CF antigens for influenza, mumps, herpes simplex, and smallpox viruses from suspensions of infected chicken embryos (2). A CF antigen for western equine encephalitis virus was also obtained by irradiation of infected allantoic fluids (3). Irradiation of Venezuelan equine encephalitis (VEE) virus tissue culture suspensions with gamma rays was shown to be applicable for the production of an HI antigen suitable for serology (4). In the present study, gamma radiation was utilized to prepare noninfective VEE CF antigens of high potency, stability, and freedom from AC activity.

Methods. The Trinidad strain of VEE virus was used (5). Details concerning the tissue culture system, and the virus growth and purification procedure have been described previously (6, 7). Assays for virus infectivity were performed by plaque titration in 24-hr chick embryo monolayer tissue cultures (CETC) or by titration of lethality in 10- to 12-g weaning mice. The LD_{50} values were calculated by the method of Reed and Muench (8). CF titrations were performed by microtiter methods (9, 10).

Purified VEE virus preparations were inactivated by irradiation at the National Bureau of Standards through the courtesy of Daniel W. Brown. Suspensions of virus were exposed to gamma radiation from a cobalt-60 source, at a dose rate of 7×10^4 R/min. The single radiation dose of 6×10^6 R was selected for inactivation because prior studies had determined that similar VEE virus preparations were inactivated at that dose without loss of serological reactivity (6). The virus preparations were irradiated in the frozen state in Wheaton glass serum bottles fitted with flange-type rubber stoppers. Prior to inactivation, the virus preparations were diluted 1:4 in borate saline. Following irradiation all preparations were safety-tested for potential residual virus infectivity as described previously (6, 7). In all cases VEE virus infectivity was inactivated at the dose of 6×10^6 R. In contrast, the *in vitro* CF antigenicity was fully retained. The dose of gamma radiation required to destroy the CF

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TABLE I. CF Titration of Immune Sera with Inactivated Tissue Culture VEE Virus Antigens.[a]

Antigen preparations	Titer of antiserum				Normal rabbit
	Horse	Rabbit B	Rabbit C	Rabbit D	
SMB-BPL[b]	256	64	128	64	<4
D4H	128	64	64	32	<4
D5H	64	32	32	64	<4
D6S	256	64	64	32	<4
D6H	256	16	64	32	<4
D7S	256	32	32	64	<4
D7H	256	32	64	64	<4

[a] National Center for Disease Control Laboratory Branch CF (NCDC-LBCF) procedure.
[b] Control antigen of 20% infected suckling mouse brain suspension inactivated with 0.3% BPL.

activity of these preparations was not determined.

Results and Discussion. Six gamma-irradiated preparations from 4 separate DEAE column purification experiments were examined for use as complement fixing antigens after storage at 5° for various intervals up to 12 weeks. Block titrations with a standard hyperimmune equine serum indicated similar CF antigen optimal dilutions for all 6 preparations, and a complete absence of any antigen anticomplementary activity. Standard CF tests with these antigens and several immune rabbit and an equine sera produced serum titers similar to those obtained with control CF antigens prepared by beta-propiolactone (BPL) inactivation of infected suckling mouse brains (Table I). No anticomplementary activity was observed in tests with the inactivated antigens and no false reactions were obtained in systems utilizing normal serum.

The stability of these purified and inactivated CF preparations was investigated. Complement fixation tests were conducted after storage for 8 to 9 months at 5° with the same horse serum used previously and at the same antigen optimal dilution. The BPL-inactivated brain preparation was also tested. Results are given in Table II. The 4 inactivated tissue culture preparations tested were stable. For all preparations, the optimal antigen dilutions determined were identical to those obtained before storage. Antiserum titers obtained with 3 of the 4 preparations were identical with, or only one 2-fold dilution less than, the titers initially determined. The control mouse brain preparations also produced serum titers one 2-fold dilution lower. Only 1 tissue culture preparation was markedly weaker (D6S), and even in that case the antiserum showed a relatively strong titer. As observed initially, no anticomplementary activity was noted for the tissue culture antigens, even after this prolonged storage.

Infected tissue culture fluids, purified and concentrated prior to inactivation by ionizing radiation, appear suitable as a source of VEE virus CF antigen for routine laboratory CF tests. These preparations possess high pre- and postirradiation CF titer, exhibit no anticomplementary activity, and the inactivated preparations are very stable at 5°.

TABLE II. Stability of Inactivated Tissue Culture VEE Virus CF Antigens.

Antigen preparation[a]	Interval (month)	CF titer of horse antiserum[b]	
		Original	Final
SMB-BPL[c]	9	256	128
D6S	9	256	64
D6H	9	256	256
D7S	8	256	128
D7H	8	256	128

[a] The optimal antigen dilution used for each antigen was identical in both the original and final test.
[b] NCDC-LBCF procedure.
[c] Control suckling mouse brain preparation inactivated with 0.3% BPL.

Preparation from a tissue culture source makes readily available suitable antigen in large volume. Furthermore, this procedure may prove useful for preparing inactivated CF antigens of other viruses pathogenic for man.

Summary. Noninfectious complement fixing antigens of Venezuelan equine encephalitis virus were prepared by chromatography of infected tissue culture fluids and subsequent inactivation of the purified product by exposure to ionizing radiation. The product was highly active, stable, and free of anticomplementary activity.

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