Preparation of Noninfectious Arbovirus Antigens by Sucrose–Acetone Extraction of Gamma-Irradiated Tissue (35223)

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Serological studies with arboviruses are frequently carried out with antigens prepared from infectious tissue by emulsifying in sucrose solution, precipitating with cold acetone, drying the precipitate, and extracting the resulting powder with saline. These extracts, derived from infected brain or liver, are termed sucrose–acetone (SA) extracted antigens (1). With many arboviruses, such preparations are active in both hemagglutination (HA) and complement fixation (CF) reactions.

Preparation of SA antigens for agents infectious for man involves potentially hazardous operations that must be carried out with appropriate precautions. In addition, the infectivity of the final preparations imposes limitations on the conditions under which they may be used. Attempts have been made to prepare noninfectious serologic antigens by treatment of the homogenates or extracts with beta-propiolactone or ethylene oxide (2–5). Reports from our and other laboratories have shown the usefulness of gamma irradiation for inactivation of various infectious agents with retension of labile immunizing and serologic antigens (6–9). The applicability of irradiation to preparation of noninfectious serological antigens for representative arboviruses was therefore investigated.

Materials and Methods. Viruses. The viruses used in this study are listed in Table I. The Parton strain of St. Louis encephalitis was obtained from Dr. A. W. Chappell, National Communicable Disease Center and the others from Dr. W. P. Allen, Fort Detrick. The viruses were propagated by intracerebral (ic) inoculation of 2- to 4-day-old Swiss Webster mice of the Bagg strain.

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Infected tissue. Two- to 4-day-old suckling mice were injected ic with 0.02 ml of a 10^{-3} dilution of virus. Moribund mice were sacrificed and the brain tissue was harvested by aspiration into two rubber-stoppered vaccine bottles kept in a Dry Ice–alcohol bath. For group C viruses, livers were removed aseptically with sterile forceps. The tissues were stored at −70°.

Irradiation. One bottle of each pair of tissue samples was exposed to 6.5×10^6 R from a cobalt-60 source. Preliminary experiments had established this dose as sufficient to destroy all viable virus. The samples were irradiated while packed in Dry Ice and remained frozen until both the nonirradiated and irradiated samples were thawed and extracted. Tissues were irradiated at the National Bureau of Standards through the courtesy of Daniel W. Brown. The irradiated tissues were tested for residual virus by ic inoculation of a 10% tissue suspension and final antigen into suckling mice. In no case was viable virus detected.

Antigen extraction. Nonirradiated and irradiated samples of tissue were extracted by the sucrose–acetone procedure of Clarke and Casals (1). When sonication was employed to restore HA activity, the suspension of powder was sonicated in the cold, using a Branson Model W 140D sonifier at 55 W for two periods of 3 min with a 2-min interval between. The suspensions were refrigerated overnight at 4°, sonicated for one 3-min period, and centrifuged for 1 hr at 10,500*g*. Each supernatant was distributed in glass ampoules which were sealed and stored at −70°.

Serologic titrations. HA and hemagglutination-inhibition (HI) tests were performed according to Clarke and Casals (1) using microtiter equipment (10). The hyperimmune

TABLE I. Effect of Irradiation of Tissues of Arbovirus-Infected Mice on Serological Activity of Sucrose–Acetone Extracts.

Group	Virus	Strain	Passage level	Seed stock $MICLD_{50}$	Hemagglutination titer				Complement fixation titer	
					Nonsonicated		Sonicated			
					Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated
A	Eastern equine encephalitis	SC-7	SM_{12}	10.3	5120	160			128	16
	Western equine encephalitis	McMillan	SM_6	8.9	10,240	640			64	64
	Chikungunya	BAN	SM_5	9.4[a]	160	<10	160	80	16	8
	Semliki Forest	Original	SM_4	10.2	320	80	640	640	32	32
	Middelburg	Unknown	SM_4	8.4[a]			320	320		
	Sindbis	AR339	SM_{27}	9.4[a]	10,240	1280			32	4
B	Dengue-2	New Guinea	SM_4	6.0	640	20	1280	80	64	32
	Modoc	MO 2622	SM_4	9.7	1280	160			64	16
	West Nile	Egypt 101	Unknown	7.4	640	80		1280	64	32
	Japanese encephalitis	Nakayama	SM_{39}	8.3	2560	160			1024	1024
	Rio Bravo	HA 119	SM_{10}	10.2[a]	10,240	640			64	32
	St. Louis encephalitis	Hubbard	SM_{101}	10.2[a]	5120	5120			128	256
		Parton	SM_4	9.0	2560	640			256	256
	Yellow fever	17D	Vaccine	6.0	1280	<10		160	16	32
	Powassan	Byer	SM_7	9.8	2560	160			⩾1024	⩾1024
	Zika	M4946	SM_{147}	⩾8.5	2560	320			8	8
	Ilheus	Unknown	Unknown	8.9	10,240	2560			128	128
	Langat	TP-21	Unknown	8.2	10,240	2560			⩾1024	⩾1024
C	Oriboca	Unknown	SM_{13}	6.7[a]			5120	1280		
	Caraparu	An3994	SM_{12}	7.7[a]			1280	1280	64	32
Bunyamwera										
	Bunyamwera	Original	SM_{40}	9.5[a]	1280	40		640	8	8
	Germiston	SAAR 1050	SM_{11}	9.2[a]	<10	<10	<10	<10	16	16

[a] Titrated ic in suckling mice; others were titrated in weanling mice.

mouse asctic fluids (HIAF) were adsorbed with kaolin and goose erythrocytes. Complement fixation tests were performed according to the LBCF procedure (11).

Hyperimmune mouse ascitic fluid. HIAF used in the HI and CF tests were prepared according to the method of Tikasingh *et al.* (12).

Results. Sucrose–acetone (SA) extracts were prepared from irradiated and nonirradiated tissues of mice infected with 22 representative arboviruses. Brain tissues were used for groups A, B, and Bunyamwera viruses, and liver for group C. Most of the extracts were tested for hemagglutination (HA) activity and for antigenicity in the complement fixation (CF) test (Table I). In general, CF activity of the extracts was not changed significantly by prior irradiation of the tissues, and anticomplementary activity was not observed. Such differences as were encountered between irradiated and nonirradiated extracts probably were no greater than would be noted in duplicate extracts prepared by the same procedure.

HA titers of the irradiated extracts tended to be somewhat lower than HA titers of untreated extracts, although in most cases the titers following irradiation were adequate for serological studies. With some of the viruses, however, HA activity was reduced to a low or undetectable level. Ardoin *et al.* (13) reported that sonication increased the HA titers of extracts of certain arboviruses that otherwise contained low HA activity. Accordingly, extractions were repeated with those viruses for which irradiated extracts had yielded HA titers of 1:80 or less, and sonication was carried out during extraction with saline.

Irradiated extracts with acceptable HA titers were prepared in this way for Chikungunya, Semliki Forest, dengue-2, West Nile, yellow fever, and Bunyamwera. Middleburg, Oriboca, and Caraparu, for which extracts had not been prepared without sonication, also yielded satisfactory extracts. In those cases in which comparisons were possible, it appeared that sonication tended to overcome the loss of HA activity that accompanied irradiation, but did not increase the HA titers of nonirradiated extracts. Sonication did not yield active HA extracts for Germiston.

Standard and irradiated antigens for 13 of the viruses were retested for HA titer following storage for 1 to 1.5 years at —70°. All standard SA antigens showed little or no loss of HA titer. Among the irradiated antigens the following maintained HA titers without significant change: Semliki Forest, dengue-2, Modoc, West Nile, Japanese encephalitis, St. Louis encephalitis, Powassan, Zika, Ilheus, Langat, and Bunyamwera. However, hemagglutinating activities of the irradiated antigens for Western equine encephalitis and yellow fever were lost during this period of storage.

The specificity and sensitivity of the irradiated SA antigens were compared with those of standard antigens in the hemagglutination-inhibition (HI) titration of representative antisera. No cross reactions were noted between any of these antigens and heterologous antisera, and sera from animals immunized with normal mouse brain gave no HI with either the irradiated or the standard antigens. With homologous antisera, the irradiated and nonirradiated antigens gave similar HI titers, except in the case of the West Nile system (Table II). In this instance, the sensitivity of the test was significantly less with irradiated antigen.

Discussion. It is evident that the irradiation procedure represents a practical method for preparation of noninfectious antigens for a representative group of arboviruses. Sucrose–acetone extracts of the irradiated tissue were essentially as active in complement fixation titrations as extracts of untreated tissue, and in most cases contained useful titers of hemagglutinin without further treatment. No change in procedure for antigen extraction was necessary and the treatment left no anticomplementary activity or other residua to interfere in serological procedures. In those cases in which irradiation reduced significantly the hemagglutination titers, the effect could be overcome to a considerable extent by brief sonication during the extraction with saline. Evidently irradiation tends to produce aggregation of hemagglutinin, as it has been reported to do with certain enzymes (14).

TABLE II. Comparison of HI Titers Using Standard and Irradiated Homologous SA Antigens.

Antiserum or hyperimmune ascitic fluid	HI titers[a]	
	Standard antigen	Irradiated antigen
Modoc, lot 1	80	40
2	20	20
St. Louis encephalitis (Parton)	320	320
St. Louis encephalitis (Hubbard)	320	320
Japanese encephalitis	40	20
Powassan	10	20
West Nile	160	10
Zika	40	80
Ilheus	80	80
Dengue-2	10	10
Semliki Forest	40	40
Langat	10	10
Bunyamwera	10	10
Oriboca	40	40

[a] Antigen contained 4–8 HA units.

Irradiation of the frozen tissue before it is extracted renders it noninfectious and eliminates the hazards inherent in grinding or blending infectious tissue. Thus effective antigens that can be used in serological procedures without hazard can be prepared in any laboratory equipped for infection and autopsy of suckling mice or other appropriate experimental animal.

The noninfectious antigens should prove useful for epidemiological or diagnostic studies in public health or hospital laboratories. The general applicability of the irradiation method to all of the viruses that were studied suggests that it may also be applicable to highly dangerous agents for which noninfectious antigens would be particularly desirable.

Summary. Gamma irradiation of arbovirus-infected suckling mouse tissue provided a means of inactivating the virus in frozen tissue and allowed safe and convenient preparations of arbovirus serologic antigens by the sucrose–acetone extraction procedure. In most cases the irradiated tissues yielded satisfactory hemagglutination (HA) and complement fixing antigens following extraction. With those viruses for which HA activity was reduced by irradiation, sonication during extraction of the dried acetone–insoluble precipitate with saline restored HA titers to levels approaching those obtained with unirradiated tissue. Irradiation avoids the hazards of extraction of infectious tissue, and the extracts may be used for serological studies in laboratories not equipped for manipulation of infectious agents.

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