Use of Glutaraldehyde-Fixed Goose Erythrocytes in Arbovirus Serology

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Goose erythrocytes preserved with glutaraldehyde were compared with fresh cells in hemagglutination and hemagglutination-inhibition tests for arbovirus antigens and antibodies. The glutaraldehyde-fixed cells were as sensitive and specific as the fresh erythrocytes and were stable at 4°C for 6 months.

Cox and Pirtle (6) used erythrocytes preserved with Formalin in hemagglutination (HA) tests for influenza virus and reported that the fixed cells resisted lysis and retained HA sensitivity for a year. Formalinized erythrocytes have also been used reliably for titration of antibodies to *Pasteurella multocida* (4), *Yersinia pestis* protein fraction IA (3), and various arboviruses (8). A brief-exposure glutaraldehyde (Ga) fixation procedure for erythrocytes was initially described by Bing et al. (2), who reported the fixed erythrocytes to be superior to Formalin-treated cells for detection of antibody and stable for at least 6 months.

Our use of Ga-fixed goose erythrocytes for arbovirus HA and hemagglutination-inhibition (HI) testing was stimulated by previous use of Ga-fixed sheep erythrocytes in the passive HA test for plague (9). Ga fixation proved to be superior to Formalin fixation in that antibody titrations were more reproducible, the cells could be stored for longer periods of time, and cells coated with antigen reacted more specifically and yielded higher antibody titers (9).

This report describes the use of Ga-fixed male goose erythrocytes in arbovirus HA and HI tests. Sensitivity, specificity of reactivity, and stability of the preserved erythrocytes were compared to those of fresh goose cells.

MATERIALS AND METHODS

Arbovirus antigens and mouse ascitic fluids were obtained from the Arbovirus Reference Branch, Center for Disease Control, Fort Collins, Colo. Ascitic fluids were acetone extracted and adsorbed with fresh goose erythrocytes before they were used in the HI tests (5). A microtiter procedure adapted from that of Clarke and Casals was used for HA and HI tests, with 8 HA units of antigen titrated with fresh erythrocytes (5).

Male goose erythrocytes in Alsever solution (Colorado Serum Co., Denver, Colo.) were prepared for HA and HI testing according to standard techniques (5). An equal volume of fresh erythrocytes was fixed

in Ga, using techniques modified from Csizmas (7). The erythrocytes were collected by centrifugation for 5 min at 2,000 rpm at room temperature and washed six times in physiological saline, using centrifugation. A 10% (vol/vol) suspension of packed cells was prepared in saline and placed in a large flask. Dialysis tubing, containing a volume of Ga equal to one-fourth of the volume of the cell suspension, was placed in the flask with the cells and subjected to dialysis with constant agitation for 4 h at room temperature. The tubing was then punctured to add its contents to the erythrocyte suspension, and the mixture was stirred overnight at room temperature. The cells were poured through cheesecloth to remove clots, washed six times, suspended to 25% (vol/vol) in borate-buffered saline, and adjusted to the proper concentration for use (5). The suspensions of both fresh and fixed erythrocytes were standardized for arbovirus HA and HI tests to contain 16,000 cells/mm³ (1).

RESULTS AND DISCUSSION

Goose erythrocytes preserved with Ga appeared somewhat smaller than fresh cells and were more opaque, and the nucleus was no longer visible. The treatment made the cells more adherent, since vigorous shaking was required to resuspend them evenly. In negative HA or HI reactions at 26°C, the fixed cells usually settled in a doughnut-shaped ring rather than into the compact button formed by fresh cells. Nevertheless, these reactions were readily differentiated from those manifesting partial HA or positive HI reactions.

The relative sensitivity of Ga-fixed and fresh erythrocytes in HA reactions was determined for representative alphaviruses, flaviviruses, and bunyaviruses. In most instances, two different antigen preparations were used for each virus as well as antigen preparations from different strains of the same virus. Alphavirus HA reactions had similar pH optima with both types of erythrocyte preparation (Table 1). HA titers were generally two- to fourfold higher when Ga-fixed cells were used. Flavivirus HA

TABLE 1. HA titers and optimum pH range exhibited by arbovirus antigens tested with fresh and Ga-preserved male goose erythrocytes

Antigen	Strain	Lot no.	HA titer			
			Fresh RBCs ^a		Ga-fixed RBCs	
			HA	pH	HA	pH
Chikungunya	IPDA/CS13	1	320	6.2	1,280	6.2
Chikungunya	IPDA/CS13	2	320	6.2	1,280	6.0
Eastern equine encephalitis	NJ60		320	6.0-6.2	640	6.0-6.2
Eastern equine encephalitis	NJ0		2,560	6.4	10,240	6.0-6.4
Sindbis	AR 1055	1	640	6.0	2,560	6.0-6.2
Sindbis	AR 1055	2	640	6.0-6.2	2,560	6.0-6.2
Venezuelan equine encephalitis	Three Rivers		320	6.0-6.2	640	6.0
Venezuelan equine encephalitis	GJ9-1BJ		1,280	6.0	1,280	6.0
Western equine encephalitis	Fleming		640	6.2	2,560	6.0
Western equine encephalitis	McMillan		2,560	6.2	≥10,240	6.0-6.2
Dengue type 2	TR 1751	1	1,280	6.2	2,560	6.6
Dengue type 2	TR 1751	2	640	6.4	160	6.8-7.0
Japanese encephalitis	G 8924	1	1,280	6.6	2,560	6.4-6.6
Japanese encephalitis	G 8924	2	640	6.8	320	6.0-7.0
Murray Valley encephalitis	11A	1	320	6.4-6.8	640	6.8
Murray Valley encephalitis	11A	2	640	6.4	640	6.0-6.8
St. Louis encephalitis	TBH-28		2,560	6.4-6.6	2,560	6.6
St. Louis encephalitis	Parton		640	6.4-6.8	640	7.0
West Nile	AR 248	1	160	6.4-6.8	640	6.0-6.2
West Nile	AR 248	2	80	6.4-6.6	320	6.0
Yellow fever	Asibi	1	1,280	6.0-6.2	1,280	6.0-6.6
Yellow fever	Asibi	2	5,120	6.0-6.4	2,560	6.0-6.2
Oriboca	BeAn 17		320	6.0	320	6.0
Madrid	40308		40	6.0-6.2	80	6.0

^a RBCs, Erythrocytes.

reactions were comparable for both erythrocyte preparations, although the pH optima were different. The two bunyavirus antigens appeared to behave similarly whether tested with Ga-fixed or fresh goose cells.

Further tests were performed to determine the effect of incubation temperature on the stability and sensitivity of the Ga-fixed and fresh cells during HA titrations. One virus representative of the alphaviruses, flaviviruses, and bunyaviruses was tested at incubation temperatures of 4, 26, and 37°C. HA titers of Western equine encephalitis, St. Louis encephalitis, and Madrid virus with fixed and fresh cells were equivalent at each of the incubation temperatures.

The sensitivity of fixed erythrocytes in the HI reactions was evaluated and compared with that of fresh erythrocytes. HA antigens and immune reagents for Western equine encephalitis, St. Louis encephalitis, yellow fever, and Oriboca viruses and HA antigens only for Japanese encephalitis and dengue type 2 viruses were used in these experiments. Table 2 shows that equally specific HI reactions were obtained with the fixed cells. As expected, intergroup heterologous HI reactions were not observed with the antisera to Western equine encephalitis, St. Louis encephalitis, or Oriboca viruses.

TABLE 2. Comparison of the sensitivity and specificity of fresh and Ga-fixed male goose erythrocytes in HI tests for arboviruses^a

HA antigen (8 HA units)	Antibody	HI titer	
		Fresh RBCs	Ga-fixed RBCs
WEE	WEE	2,560	2,560
	SLE	<10	<10
	ORI	<10	<10
SLE	WEE	<10	<10
	SLE	1,280	2,560
	YF	80	40
	ORI	<10	<10
Den-2	SLE	80	80
	YF	20	20
JE	SLE	40	40
	YF	40	20
YF	SLE	80	40
	YF	320	160
ORI	WEE	<10	<10
	SLE	<10	<10
	ORI	640	1,280

^a Abbreviations: WEE, Western equine encephalitis; SLE, St. Louis encephalitis; Den-2, dengue type 2; JE, Japanese encephalitis; YF, yellow fever; ORI, Oriboca; RBCs, erythrocytes.

Intragroup HI cross-reactions among the flaviviruses—St. Louis encephalitis, dengue type 2, Japanese encephalitis, and yellow fever—occurred as expected. Homologous HI titers were comparable with both cell preparations and were distinct from the observed heterologous HI reactions.

The availability of stable, preserved goose erythrocytes would facilitate arbovirus HA and HI testing in areas where such testing is required and where fresh goose erythrocytes are not readily available. The use of the preserved cells could also provide a standardized reagent, thus reducing the frequency of fresh cell collection and eliminating the necessity of weekly or biweekly processing and standardization.

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