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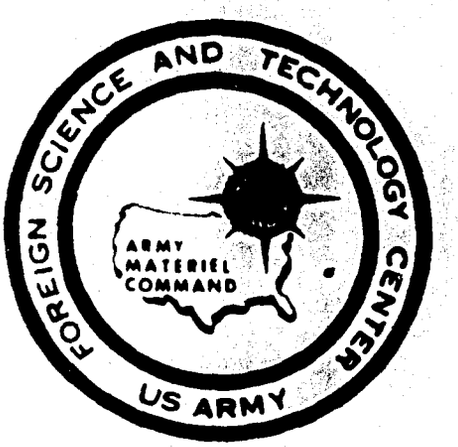

DRY, NON-INFECTIOUS, HEMAGGLUTINATING ANTIGENS OF SIX GROUP A ARBOVIRUSES GROWN IN TISSUE CULTURE

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DRY, NONINFECTIOUS, HEMAGGLUTINATING ANTIGENS OF SIX GROUP A ARBOVIRUSES GROWN IN TISSUE CULTURE

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Several authors have shown that hemagglutinins form in growth on tissue cultures of many representatives of group A arboviruses [1-8]. These hemagglutinins have proven highly stable at 37°, which has made possible a reliable method of thermal inactivation of infectious properties without altering the capacity to agglutinate erythrocytes.

In this paper, results of studies performed on various conditions used in drying noninfectious hemagglutinating antigens are presented and experimental features of dry preparations are given.

Materials and Methods

Western (WEE) and Eastern (EEE) equine encephalomyelitis, Sindbis, Semliki forest, Chikungunya, and Middelburg viruses were used.

The WEE, EEE, Sindbis, and Semliki forest viruses were grown in monolayer cultures of chick embryo fibroblasts.. Transplanted cells of kidney epithelium of hamster embryo (VNK-21) were used for accumulation of Chikungunya and Middelburg viruses.

Monolayer cultures were grown in Ru liter separating flasks. Prior to infection, the growth medium was decanted, the monolayer was washed with Hank's solution, after which one ml of virus-containing suspension of mice brain at 1:100 dilution was added to it. The medium No. 199 without serum was used as the maintenance medium.

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The infected separating flasks were kept in a thermostat for 7 days in the study on Chikungunya and Middelburg viruses, and for 9 days in the study on the WEE, EEE, and Sindbis viruses. Exposure to 32° for 7 days proved optimal for the Semliki forest virus. Under these conditions, as we have shown earlier, maximum accumulation of hemagglutinins and total cessation of infectivity occur [22].

The prepared antigens were dried by the chamber method in the volume after preliminary freezing at temperatures from -60 to 70°. For the first 3 to 4 hours of drying in chamber the temperature was kept at -45 to -35°, then it was gradually raised, and by the 11-12th hour it was raised to 0-1°, and in 24-30 hours -- 20-24°. The drying lasted 28-30 hours at a vacuum of 20-80 microns.

Saccharose and gelatin (final concentration, respectively, 10 and 1 percent), saccharose (final concentration 10 percent), and protamine-sulfate (0.6 mg/ml) were used as the protective mixture.

The hemagglutination reaction [HR] was established with 0.5 percent suspension goose erythrocytes at room temperature.

Results

Experimental results with WEE, Sindbis, and Middelburg viruses showed that when they are dried without protective mixture the activity of noninfected hemagglutinating antigens is reduced by 16 times (Table 1). When protamine-sulfate was added, the hemagglutinating activity stopped altogether. In the presence of saccharose maintenance of one-half to one-eighth the original titer was noted.

TABLE 1

Activity of Noninfectious Hemagglutinating Antigens Dried in the Presence of Various Protective Mixtures

(A) Antigen	(B) Titer of hemagglutinins				
	(C) original	(D) without filler	(E) with filler		
			(F) protamine-sulfate	(G) saccharose	saccharose & gelatin (H)
(I)	256	16	0	32	256
(J)	256	16	0	32	256
(K)	128	8	0	64	128

LEGEND: A - antigen
B - hemagglutination titer
C - original
D - without filler
E - with filler
F - protamine-sulfate
G - saccharose
H - saccharose & gelatin
I - WEE
J - Sindbis
K - Middelburg

TABLE 2

Stability of Liquid and Dry Noninfectious Hemagglutinating Antigens of Group A Arboviruses at 4 and at 37°

(A) Антиген	(B) Физическое состояние	(C) Титр гемагглютинации после хранения									
		(D) исходный	(E) при 37°					(F) при 4°			
			(G) срок хранения (в днях)								
			15	20	25	30	90	180	1	3	6	12
ЗЭЛ (H)	(N) Жидкий	1:256	1:256	1:64	1:16	1:4	—	—	1:256	1:256	1:128	1:128
	(O) Сухой	1:256				1:256	1:256	1:128	1:256	1:256	1:256	1:256
ВЭЛ (I)	Жидкий	1:64							1:64	1:32	1:32	
	Сухой	1:64							1:64	1:64	1:64	
Синдбис (J)	Жидкий	1:128	1:128	1:32	1:8	0			1:128	1:128	1:128	1:128
	Сухой	1:128				1:128	1:128	1:64	1:128	1:128	1:128	1:128
Леса Семлики (K)	Жидкий	1:64							1:64	1:64	1:16	
	Сухой	1:64							1:64	1:64	1:32	
Чикунгунья (L)	Жидкий	1:64	1:32	1:8	1:2	0			1:64	1:64	1:64	1:64
	Сухой	1:64				1:64	1:64	1:32	1:64	1:64	1:64	
Миддельбург (M)	Жидкий	1:128	1:64	1:16	1:2	0			1:128	1:128	1:128	
	Сухой					1:128	1:128	1:64	1:128	1:128	1:64	

LEGEND: A - antigen
B - physical condition
C - hemagglutination titer after storage
D - original
E - at 37°
F - at 4°
G - length of storage (in days)
H - WEE
I - EEE
J - Sindbis
K - Semliki forest
L - Chikungunya
M - Middelburg
N - liquid
O - dry

The mixture of saccharose and gelatin gave the most pronounced protective effect. It made it possible to get dry antigens without variation in the initial activity in the HR.

Several series of antigens of each viruses were obtained with this protective mixture.

Table 2 lists results in the determination of the hemagglutinating activity of liquid and dry antigens during storage and at 4 and 37°. It was found that liquid antigens of most of the viruses studied are highly stable at 4°. Titers of the antigens -- WEE, EEE, Sindbis, and Chikungunya — remained practically unchanged for the course of the year (the

course of the observation period).

The Middelburg antigen retained all its activity for 9 months (observation period). Hemagglutinins of Semliki forest virus showed less stability. After 6 months of storage, a fourfold reduction was noted. At 37° all antigens tested quickly lose the ability to agglutinate erythrocytes. In 15 days, the antigen of Semliki forest virus becomes inactive, and in 30 days -- the antigens of Sindbis, Chikungunya, and Middelburg become inactive. In a month, the titer of hemagglutinins of WEE virus was reduced by 64 times.

Dry antigens show much stability not only at 4, but also at 37°. Under thermostat conditions their activity remained unchanged for 3 months in experiments with all the viruses. After 6 months, using the models of the viruses WEE, Sindbis, Middelburg, and Chikungunya, only a twofold titer reduction was observed.

Conclusions

1. An optimal regime for lyophilization of noninfectious hemagglutinating antigens of group A arboviruses grown on tissue cultures has been developed.

2. The high stability of dry antigens at 37° for a three-month period has been demonstrated.

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13. ABSTRACT

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Western eqine encephalomyelitis virus						
Eastern equine encephalomyelitis virus						
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Chikungunya virus						
Middelburg virus						
Dry antigen						
Noninfectious antigen						
Hemagglutinating antigen						
Lyophyization						
Group A arboviruses						