The Use of Tween-80–Ether for Preparation of Haemagglutinins of Arboviruses

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In 1958, Clarke & Casals [a] described techniques particularly suitable for the preparation of haemagglutination and haemagglutination-inhibition antigens of arboviruses. Since then, these methods have been used successfully in many laboratories. Recently Mussgay,[b] Mussgay et al.[c] and Mussgay & Rott [d] reported an increase of haemagglutinin activity after treatment of Sindbis virus with polyoxyethylene sorbitan monooleate (Tween 80). It was therefore of interest to test the Tween 80 treatment on a variety of arboviruses for the preparation of haemagglutinins, as suggested by Mussgay et al.[c]

Materials and methods

Viruses. Details of the 22 arboviruses tested are given in the accompanying table. Brain passages were carried out in 2-3-day-old white mice (NMRI strain). The 10% brain suspensions were stored in ampoules at about −60°C. The tissue-culture passages were carried out with BHK21 cells (passage levels 190-194) grown in 30-ml disposable Falcon plastic bottles. The original growth medium of Stoker & MacPherson [e] was used for the formation of the cell sheets. A special serum-free maintenance medium developed by Bergold & Mazzali [f] served for the multiplication of viruses.

Preparation of haemagglutinins. The Tween-80–ether method of Mussgay et al.[c] was followed exactly: 1 g of mouse brain or liver, 9 ml of chilled (4°C) borate buffer saline (BBS) at pH 9.0, 10 ml of chilled diethyl ether and 0.2 ml of Tween 80 are mixed in a chilled Omni-Mixer for 5 min at 25 000 rev/min. The mixture is centrifuged for 10 min at 4000 rev/min at about 4°C. The intermediate portion, containing the antigen, is aspirated out. Nitrogen is used to remove the residual ether. It was found that this was not necessary if the material is lyophilized immediately.

When serum was used as starting material the proportions were 2 volumes of serum, 8 volumes of BBS and 10 volumes of ether. If tissue cultures were used as starting material, the buffer solution was omitted, and the antigen was mixed directly with an equal volume of ether.

The sucrose-acetone method was carried out as described by Clarke & Casals.[a]

Haemagglutination test. The microtechnique using disposable trays [g] was carried out with BBS of pH 9.0 plus 5% bovine albumin as a diluent. Goose red cells [h] were employed, washed 3 times in 0.85% NaCl and suspended in BBS/bovine albumin at a final concentration of 2%. The incubation period was 1 hour at 37°C.

Results

The results obtained with the Tween-80–ether and sucrose-acetone methods are compared in the accompanying table. The haemagglutinin of Mayaro, Venezuelan equine encephalitis (VEE), Western equine encephalitis (WEE) and Eastern equine encephalitis (EEE) viruses of group A generally gave somewhat better HA titres when prepared by the Tween-80–ether method than when prepared by the sucrose-acetone method or (in the case of VEE) by the β-propiolactone method. In our laboratory the sucrose-acetone, ether-acetone, and protamine methods always gave negative results for Una antigen. With the Tween-80–ether treatment, a titre of 1:640 could be obtained. The antigens of group B—Bussuquara, dengue 1, dengue 2, St Louis encephalitis (SLE), Ilheus and yellow fever (IVIC 1)[i]—showed somewhat better titres when prepared by the Tween-

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[b] Mussgay, M. (1966) Paper presented to the Third Venezuelan Congress of Public Health, Caracas, 20 March-27 March 1966.

[c] Mussgay, M., Fadda, G. & Peralta, M. (1967) *Nature (Lond.)*, **213**, 304-305.

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[e] Stoker, M. & MacPherson, I. (1964) *Nature (Lond.)*, **203**, 1355-1357.

[f] Bergold, G. H. & Mazzali, R., to be published.

[g] Sever, J. L. (1962) *J. Immunol.*, **88**, 320-329.

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[i] Bergold, G. H. & Weibel, J. (1962) *Virology*, **17**, 554-562.

HA TITRES OF ARBOVIRUS ANTIGENS PREPARED BY THE SUCROSE-ACETONE AND TWEEN-80-ETHER METHODS

Group	Antigen	Strain designation	Previous passages	Our passages	Sucrose-acetone method		Tween-80-ether method	
					HA titre	pH	HA titre	pH
A	Mayaro	TRVL 15537	12	2	1:160	6.3	1:1 280	6.2
	WEE	Rio 80391	7	2	1:1 280	6.3	1:1 280	6.2
	EEE	Agr. Res. Serv.		2	1:1 280	6.4	1:5 120	6.5
	VEE	IVIC PHO 231S		2	1:180 [a]	6.0	1:160	6.0
	Una	Bogotá 43332	7	3	< 1:10 [b]	6.0-7.0	1:640	6.5
B	Bussuquara	Bogotá 41922	25	2	1:320	6.6	1:1 280	6.5-6.6
	Dengue 1	Finlay V-65	82	6	1:20	6.4	1:160	6.4-6.5
	Dengue 2	Finlay V-64	56	6	1:160	6.3-6.4	1:640	6.3
	SLE	Cali GR-12634-35	11	1	1:640	6.8	1:1 280	6.6
	Ilheus	Finlay V-59	34	4	1:1 280	6.8	1:1 280	6.6
	YF 17-D (Colombia)	Finlay vaccine strain		7	1:80	6.3	NT [c]	
	YF 17-D (England)	Burroughs Wellcome		5	NT [c]		1:160	6.5
	YF IVIC 1	IVIC PA2S		6	1:320	6.4	1:1 280	6.3
C	Caraparú T	TRVL 34053	7	3	1:80 [d]	6.2	1:320 [e]	5.8
					< 1:10 [d]	5.8-6.5	1:80 [e]	5.8
	Oriboca	TRVL 58436	14	2	1:40 [d]	6.0	1:160 [e]	6.0
Bunyamwera	Cache Valley	TRVL 20659	3	3	< 1:10	6.0-7.0	< 1:10	6.0-7.2
	Guaroa	Bogotá 35211	39	3	< 1:10	6.0-7.0	< 1:10	6.0-6.8
Guama	Guama	TRVL 33579	8	2	< 1:10	6.0-7.0	< 1:10	6.0-7.0
	Catu	TRVL 32046	3	2	< 1:10	6.0-7.0	< 1:10	6.0-7.0
	Bimiti	TRVL 8362	9	2	< 1:10	6.0-7.0	< 1:10	6.0-7.0
Simbu	Oropouche	TRVL 9760	12	2	< 1:10	6.0-7.0	< 1:10	6.0-7.2
Ungrouped	Manzanilla	TRVL 3587	20	2	< 1:10	6.0-7.0	< 1:10	6.0-7.0

[a] Prepared by the β-propiolactone method.
[b] The same result was obtained with antigen prepared by the ether-acetone method or by the protamine method.
[c] NT = not tested.
[d] From serum treated with ether-acetone.
[e] From serum.

80–ether method than when prepared by the traditional method.

The haemagglutinins of Caraparú virus were prepared from two serum pools. As shown in the table, the Tween-80–ether method gives higher titres than the ether-acetone method. The Oriboca virus, also of group C, shows a higher HA titre with the Tween-80–ether method than with the ether-acetone method. The livers, brains and spleens of mice inoculated with Caraparú virus were examined; only the livers gave haemagglutinins with the Tween-80–ether method, at the very low titre of 1:10.

The other viruses studied, Cache Valley, Guaroa, Guamá, Catú, Bimiti, Oropouche and Manzanilla, did not show any haemagglutinin activity either with the Tween-80–ether or with the conventional method.

Of the 22 viruses studied, 6 (Mayaro, WEE, Una, yellow fever (YF) 17-D (Colombia), Oriboca and Cache Valley) were grown in BHK21, but neither method produced HA from the cultures obtained.

As shown in the table, the haemagglutinins prepared by the Tween-80–ether method had more or less the same pH specificity as those prepared by conventional methods.

Some of the antigens prepared with Tween-80–ether lost almost half of their activity after 3 weeks' storage at −60°C. However, Caraparú and Bussuquara are very stable. Mayaro virus gave the same titre for 2 months after lyophilization, whereas the titre of the unlyophilized material dropped to 1:10.

This study revealed that with brain a 4-fold greater volume of haemagglutinin is obtained with the Tween-80–ether treatment than with the conventional sucrose-acetone extraction.

Discussion

The loss of activity of haemagglutinin prepared by the Tween-80–ether method even when stored at −60°C is probably due to the residual ether, which is very difficult to remove by bubbling a stream of nitrogen through the mixture. However, the ether is completely eliminated by lyophilization of the material. Immediate lyophilization of the prepared haemagglutinins is therefore recommended to maintain high titres, as was demonstrated with Mayaro virus.

The Tween-80–ether method for the preparation of haemagglutinins is very useful, because it reduces the time of treatment to 30 min-45 min for each antigen instead of the 26 hours required by the conventional treatment. This method, possibly with slight modifications, shows promise in the study of arboviruses since it is more sensitive, more efficient, cheaper and quicker than previously described techniques.

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The Use of Discriminating Concentrations in Genetical Studies on DDT-Resistance in Larval *Aedes aegypti* L.

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It has been common experience in genetical studies on DDT-resistance in *Aedes aegypti* that the hybrid (R/+) phenotype overlaps with the resistant (R/R) and susceptible (+/+) phenotypes, so that a satisfactory discriminating concentration cannot be found to separate them. Brown & Abedi [a] showed that 0.05 ppm and 2.0 ppm at 24 hours' exposure were the most satisfactory concentrations for separating phenotypes, but even so considerable overlap occurred between concentration/mortality lines.[b] Klassen & Brown [c] were also unable to separate R/R and R/+ phenotypes satisfactorily but they found that 0.25 ppm DDT over a period of 2.5 hours killed all susceptible (+/+) larvae and only 5% of the heterozygotes (R/+), in the Trinidad and Isla Verde strains. Pillai & Brown [d] selected the Trinidad DDT-resistant strain with a 1:1 mixture of DDT and WARF Antiresistant (*N*, *N*-dibutyl-*p*-chlorobenzenesulfonamide) until it became highly resistant to the mixture, and much more highly resistant than it had been before to DDT alone. When this mixture-resistant (and highly DDT-resistant) strain was crossed to susceptible the hybrid was intermediate when tested with DDT but did not overlap with susceptible when a discriminating concentration of 0.04 ppm was applied for 24 hours.

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