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PHYSICO-CHEMICAL PROPERTIES OF ESTERO REAL VIRUS

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Summary. — Estero Real (ER) virus can pass through the Millipore membrane filter of 0.22 μm pore size; it is sensitive to heating, sodium deoxycholate (SDC) and ether treatments. It replicates to the highest titres in a slightly alkaline medium. Actinomycin D (Act. D) does not prevent its multiplication in cell culture. The presence of heamagglutinin was ascertained.

Key words: Estero Real virus; filtrability; pH stability; heat inactivation; sensitivity to SDC and ether; influence of AD; haemagglutinin

Introduction

In addition to the biology of ER virus (Málková *et al.*, 1987), its physico-chemical properties have been investigated. As assumed, ER virus is an arbovirus (Málková *et al.*, 1985); the physico-chemical studies were focussed to the properties of these viruses.

Materials and Methods

Virus. The strain K 329 was used. The virus suspension was prepared in mice as described (Málková *et al.*, 1987).

Filtration. Lyophilized mouse brain supernatant prepared in PBS was centrifuged at 3000 rev/min for 20 min and then filtered through Millipore membrane filters of 0.45 and 0.22 μm pore size. Virus detection was done in 2-day-old suckling mice inoculated by intracerebral (i.c.) route.

Stability. Citrate buffer of pH 4.6, phosphate buffer of pH 6.0, 7.1 and 8.0, glycine buffer of pH 9.7, 10.4 and 12.4 were examined. One part of virus supernatant and nine parts of buffer were mixed and incubated at 4 °C for 24 hr. Before virus titration in suckling mice the pH of each sample was adjusted to 7.2—7.4.

Heat inactivation. One ml volumes of supernatant of a 10% suspension prepared in PBS were distributed in thin walled tubes and heated for 30 and 60 min at 37, 45, 56 and 60 °C. The attempts were made in water bath "cyclotherm". Control was kept at 4 °C. After heating, the tubes were stored in an ice bath until i.c. inoculation to suckling mice.

Susceptibility to SDC and ether. Lyophilized 20% mouse brain suspension was used. Equal volumes of 0.1, 0.2 or 0.02% SDC solution and virus suspension were mixed and incubated at 37 °C for 60 min (Theiler, 1957). The susceptibility to ether was tested at 4 °C for 24 hr (Lennette, Schmidt *et al.*, 1969). All mixtures were inoculated into mice immediately after treatment.

Influence of Act. D. CV-1 cell monolayers in Leighton tubes were inoculated with 0.2 ml of mouse brain suspension. After incubation at 37 °C for 60 min the inoculum was removed, the cell culture washed 3-times in PBS and further incubated at 37 °C with medium containing Act D (Calbiochem) in a concentration of 0.2 μg/ml. Higher concentration (2.0 μg/ml) was not used because of its cytotoxic effect. The virus multiplication was checked by 24—96 hr p.i. on suckling

Table 1. Filtration attempts with Estero Real virus (strain K 329)

Experiment no.	Filter (Millipore membrane)		
	0.45 μm	0.22 μm	Control
1	> 5.9*	> 5.4	6.37
2	> 6.0	5.16	7.0

* mouse log LD_{50}/0.01 ml

mice. In parallel, the multiplication of Ťahyňa and pseudorabies viruses was assayed. Pseudorabies virus was inoculated into chick embryo cells (CEC) in the presence of 0.05 μg/ml of Act D (because of higher susceptibility of CEC to the cytotoxic effect of the drug).

Haemagglutinating properties of ER virus were examined with antigens prepared by sucrose and aceton extraction (Clarke and Casals, 1958) and sonication (Kolman and Meergansová, 1972) using goose and/or sheep erythrocytes. The reaction with 0.25% goose erythrocytes was followed at pH 5.7 – 7.3, with sheep erythrocytes at pH 6.0 – 6.9 both at 37 °C.

Results

As can be seen from Table 1, the ER virus passed through Millipore filter of pore size 0.22 μm in diameter without significant loss of its titre.

The results demonstrated on Fig. 1 show that the virus was completely inactivated in acid medium at pH 4.6 and in alkaline medium at pH 12.4. Maximum virus titres of 4.4 and 4.38 log LD_{50} occured at pH 7.1 and 8.0. In acid medium at pH 6.0 the titres were 3.65 log LD_{50}, in alkaline medium at pH 9.7 3.0 log LD_{50} and at pH 10.4 2.5 log LD_{50}/0.01 ml, respectively.

As seen from Table 2, the temperature of 37 °C/60 min did not significantly influence the virus titre in comparison with the control temperature of 4 °C. The decrease in titre was evident after heating to 45 °C. Complete inactivation of the virus occured after 30 min incubation at 56 °C.

The results in Table 3 demonstrate that SDC concentrations of 0.2 and 0.1% inactivated the virus completely, 0.02% SDC concentration decreased the virus titre significantly. Ether treatment decreased the virus titre by

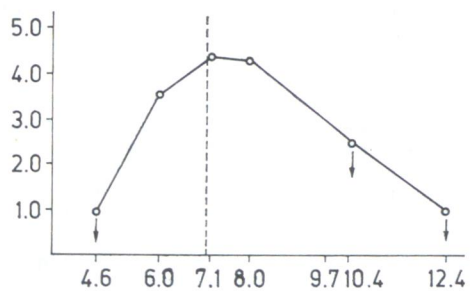

Fig. 1.
Effect of pH on Estero Real virus (strain K 329) at 4 °C (for 24 hr) Abscissa: pH values; ordinate log LD_{50}

Table 2. Influence of temperature on Estero Real virus (strain K 329)

°C Temperature	Incubation time	
	30 min	60 min
4	4.83*	4.15
37	ND	4.00
45	3.43	2.61
56	neg	neg
60	neg	neg

* mouse log LD_{50}/0.01 ml

about 2 log LD_{50}. Differences in the control titre in both experiments were caused by different stocks of virus suspensions.

Act D did not influence the multiplication of the virus in cells, similarly as it was in case of RNA virus Ťahyňa (Table 4).

The ER antigen agglutinated goose erythrocytes at pH 6.8—7.3. The highest titres — though the dilution was 1 : 40 only — were achieved at pH 7.0. The attempts to agglutinate sheep erythrocytes were negative.

Discussion

As the ER virus was isolated from ticks, the study of its physico-chemical properties was directed to the characteristic of arboviruses. It was demonstrated that ER virus was a small RNA virus, which grows to highest titres in slight alkaline medium; it was sensitive to heating, SDC and ether and it has a haemagglutinin. According to these results and owing to some biological properties (Málková *et al.*, 1987) including the histological changes in the central nervous system in mice (encephalitis) as well as multiplication of the virus in the cytoplasm of cells, the virus possesses the characteristics typical for arboviruses. Nevertheless, further laboratory experiments, such as multiplication in and transmission of virus by the vector can answer the question of relevance of this virus to arboviruses. In addition, electron microscopic

Table 3. Resistance of Estero Real virus to sodium deoxycholate and ether (strain K 329)

Experiment no.	Sodium deoxycholate				Ether	
	0.2%	0.1%	0.02%	Control	20%	Control
1	neg	neg	1.79*	3.22	4.16	6.61
2	neg	neg	ND	4.49	4.31	6.28

* mouse log LD_{50}/0.01 ml

Table 4. Influence of actinomycin D on multiplication of Estero Real virus (strain K 329)

Virus	MOI	Act.D (μg/ml)	Virus titre (mouse log LD_{50}/0.01 ml) at hr p.i.			
			24	48	72	96
K 329	> 1.0	0.2	> 2.5	> 4.5	4.1	4.51
		0*	3.4	5.4	4.36	2.88
	0.01	0.2	< 1.0	2.15	> 3.0	2.62
		0	> 1.33	> 3.5	3.56	1.68
Ťahyňa	> 1.0	0.2	4.65	6.1	> 7.5	2.69
(RNA virus)		0	3.83	7.5	> 7.0	1.57
Pseudorabies	0.1	0.05	neg	neg	neg.	ND
(DNA virus)		0	6.5	> 6.5	complete CPE	ND

* Medium not containing Act. D

studies should be made to characterize the morphology of viral particle for further classification. In the case of ER virus this is of fundamental importance, because no serological relationship with known arboviruses was ascertained (Málková *et al.*, 1985).

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