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BIOLOGICAL CHARACTERIZATION OF UNA VIRUS  
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Interest in the study of arboviruses has increased recently in connection with their isolation in many countries of the world. The present study was concerned with biological characteristics and development of optimal methods of introducing and preserving Una arbovirus belonging to Group A according to Casals' classification. In the course of the study Una virus was found to produce CPE in VNK-21 tissue culture and plaques in chick embryo fibroblasts. The clinical pattern of the disease in white mice weighing 6-7 grams inoculated with Una virus intracerebrally is characterized by an acute development of the disease and paralysis of hind legs developing in 4-5 days after inoculation. The highest titers of the virus are found in the blood in 6 and 48 hours and in the brain in 72 hours after inoculation. Una virus may be preserved by lyophilization of the brain suspension with and without stabilizers in a vacuum-drying apparatus. Among stabilizers studied 5% peptone was found to be the best.

Increased interest in the study of arboviruses in recent years is connected with their isolation in many countries of the world [1, 5]. We made a study of biological characteristics, and developed the optimal methods of introducing and preserving Una arbovirus which belongs to group A of arboviruses according to Casals' classification [6]. There is no detailed description of the properties of this virus in literature.

Material and Methods

Work was conducted with Una virus obtained from the State Collection

of Viruses at the Institute of Virology imeni D. I. Ivanovskiy, Academy of Medical Sciences USSR. Una virus was isolated in 1959 from mosquitoes *Psorophora ferox* in Brazil [6] and passed on to the Institute of Virology in 1964. A strain which underwent 11 subinoculations into 1 to 4-day mice was taken for the work [3]. The titer of the virus was determined on white mice weighing 6-7 grams with intracerebral administration and calculated according to Read and Mench. It was equal to  $10^{4.6}$ . A 10% suspension of the brain of suckling mice in physiological solution served as the source of the virus.

The following cultures were tested for the ability to produce cytopathogenic effect under the influence of Una virus: chick fibroblasts (KF), epithelium of the kidney of a newborn hamster (VNK-21), human embryo kidneys (PECh), the heart of the monkey cynomolgus (SOTs) and NER-2. The tissue cytopathogenic dose was calculated according to Read and Mench.

The plaque-forming ability of the virus was determined in the cultures of KF and VNK-21 according to Porterfield's [7] and Karpovich' [3] method.

The reproduction characteristic of Una arbovirus in the organism of white mice weighing 6-7 grams was studied in dynamics; the blood and brain tissue of sick mice were titrated.

To determine the optimal method of preserving Una virus we subjected to lyophilization a virus-containing cerebral suspension of suckling mice clarified by centrifuging at 3,000 rpm for 20 minutes. The virus-containing material was placed in ampules with a capacity of 6 milliliters at the rate of 0.5 milliliter each without and with stabilizers: a) 10% saccharose solution + 1% gelatin solution, b) 20% nonfat cow milk, c) 5% peptone. Virus-containing material was stored at different temperatures: 33° (T); 20-24° (K); 4° (F); -20° (Z).

Drying was done by means of lyophilization from a frozen state in a vacuum-drying collecting apparatus with a gypsum moisture absorber for 18 hours at -18-20° with a gradual raising of the temperature to room temperature. Final drying was done at room temperature for 4-6 hours [4]. The titer of the virus was determined before and after drying and in the period of storage at different temperatures.

## Results and Discussion

In Table 1 are given the results of determination of cytopathogenic effect and plaque-forming capacity on different tissue cultures.

As may be seen from this table, Una virus produces cytopathogenic effect only in VNK-21 culture. In doing so, it starts to appear on the second day after inoculation of the culture in the form of large granulation of the cells and disturbance of the intactness of the cellular layer. This

Table 1

Cytopathogenic Effect and Plaque-Formity Capacity  
of Una Virus in Different Tissue Cultures

Культура ткани	Цитопати- ческий эффект	Бляшкообразующая способность вируса	
		метод Пор- терфилда	метод Кар- пович
ВНК-21 VNK-21	+	—	0
КФ КФ	—	+	+
ПЭЧ ПЭЧ	—	0	0
HEp-2 HEp-2	—	0	0
СОЦ СОЦ	—	0	0

Key to Table 1

- 1) Tissue culture;
- 2) Cytopathogenic effect;
- 3) Plaque-forming capacity of the virus;
- 4) Porterfield's method;
- 5) Karpovich' method.
- 6) VNK-21 tissue culture is not suitable for the determination of plaque-forming capacity of the virus since cells degenerate in 24 hours under agar coating.

pattern did not change after 4 days of keeping the culture at 33°. In the VNK-21 culture  $CFD_{50}$  was equal to  $10^{-6.0}$ - $10^{-6.6}$ . The plaque-forming dose in the culture of chick fibroblasts amounts to  $5 \cdot 10^5$  PFU/milliliter.

Una virus had the capacity to form plaques only in the KF culture. They were seen on the second day after inoculation. Their number and exterior appearance did not change with the further keeping of the culture at 33°. The plaques were round with a diameter of 1-1.5 mm, with uneven edges. In exterior appearance the plaques formed on KF are alike when using different coatings; they are less transparent only in coating according to Karpovich.

It may be seen from Table 2 that in the blood of the mice virus appeared only in 6 hours after inoculation. Una virus is characterized by 2 rises of titers in the blood in 6 hours ( $10^{-2.2}$ ) and in 48 hours ( $10^{-2.3}$ ). In the brain tissue virus was found in 3 hours after inoculation; its quantity gradually increased. The highest titer of the virus was observed in 72 hours ( $10^{-4.3}$ ); then it gradually decreased. On the 7th day the titer was  $10^{-1}$ .

Table 2

## Dynamics of Una Virus Accumulation in the Blood and Brain Tissue of Infected Mice

Источник вируса	4 Срок после заражения (в часах)								
	3	6	9	24	48	72	94	142	166
Мозг	0,8*	1,6	1,7	2,8	2,2	4,3	3,7	2,2	1
Кровь	0	2,2	1,3	2	2,3	0,9	1,2	0,5	0,5

5 Примечание. Цифры в таблице — lg титров вируса.

## Key to Table 2

- 1) The source of the virus;
- 2) Brain;
- 3) Blood;
- 4) The period after inoculation (in hours);
- 5) NOTE. Figures in the table are logarithms of the titers of the virus.

Table 3

## Variations in Virus Titers in Drying and in Storing Lyophilized Suspensions at Different Temperatures

Способ консервации	До сушки	После сушки	7 Срок хранения									
			1 месяц		2 месяца		3 месяца		7 месяцев			
			F				F					
			T	K	Ф	Z	T	K	T	K	Ф	Z
1) Вирус на физиологическом растворе без стабилизатора	4,8	5,1	1,7	1	3,5	3	1	1	1	1,7	2,6	3
2) Вирус + 10% сахарозы + 1% желатин	4,3	4,7	2,8	3,6	2,5	3,7	1	3	1	2,7	3	4,1
3) Вирус + 20% молока	4,6	5,4	4	6	4,5	4,3	3,8	4,3	1	3,5	4,4	3,1
4) Вирус + 5% пептона	4,2	5	3,5	5,5	4,3	4,6	2	4,3	2	4,3	4,1	4,3

10 Примечание. Цифры в таблице — lg титров вируса.

## Key to Table 3

- 1) Method of preservation;
- 2) Virus in physiological solution without a stabilizer;
- 3) Virus + 10% of saccharose + 1% of gelatin;
- 4) Virus + 20% of milk;
- 5) Virus + 5% of peptone;
- 6) Before drying;

[Key continued on next page]

Key to Table 3 (continued)

- 7) After drying;
- 8) Period of storage;
- 9) Method of storage;
- 10) NOTE. Figures in the table are logarithms of the titers of the virus.

During the observation of sick mice we noted that they are much less lively than the healthy mice, that their hair is often ruffled, paralysis of the hind legs were observed on the 4th or 5th day after inoculation. Most of the animals died on the 4th day after inoculation.

In Table 3 is shown the variation of virus titers in the course of drying and storing lyophilized suspensions at different temperatures. As may be seen, virus-containing suspension both with and without stabilizers withstands well the process of drying under the conditions selected. A sharp lowering of the titers was noted when storing Una virus dried out without stabilizers during 1 month at a high and room temperature. The best stabilizer is peptone in a final concentration of 5%. Peptone and also 20% milk retarded the lowering of the titer of the virus in the course of its storage at 4° during the period of observation. Virus stored at -20° without and with stabilizers also retained its titers well in the period of observation.

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