STUDY OF REPRODUCTION OF SOME ARBOVIRUSES AT A RAISED TEMPERATURE AS A GENETIC MARKER

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The study of genetic markers of various viruses and determination of their interrelationship provide the most complete picture of the properties of virus strains and variants. By determining the sum of the correlating signs for individual variants of the same virus criteria of their attenuation can be established. So far, however, little attention has been paid to the study of individual genetic markers for arboviruses, and especially for viruses of the tick-borne encephalitis (TBE) complex and their variants with attenuated neurovirulence.

The object of the present investigation was to study the reproductive activity at 42° the rct_{42} marker of certain viruses of the TBE complex and individual variants differing in their degree of neuropathogenicity for albino mice when administered by the peripheral route (the mNsc marker).

EXPERIMENTAL METHOD

Strains Pan, Khab-9, Khab-17 of TBE virus and strain Tp-21 of Malayan Langat virus were used in the investigation. These strains were subjected to passage through the brain in albino mice and used as a 10% suspension of the animals' brain. Lines of strains Pan, Khab-9, and Tp-21 were also studied after prolonged passage in chick embryonic tissue culture at a lowered (28-26°) and raised (40-42°) temperature. Some lines of these strains went through more than 100-150 passages at a lowered temperature and more than 250-300 passages at a raised temperature. A series of clones isolated by the plaque method from a population of a Tp-21 reference strain (clones 194, 237, 241) and clone 114 from Pan strain after prolonged passage in chick embryo tissue culture at 26° were also studied. These clones possessed stabilized low indices of neuropathogenicity for albino mice (mNsc variants) as shown previously [3].

The rct_{42} marker was studied by Karpovich's modification of the plaque method [2], developed for viruses of the TBE complex. The virus was adsorbed in the tissue culture for 1 h at 37°. After covering with agarized nutrient mixture the flasks containing the infected cultures of one batch were incubated at 42° for 3 days, then at 37° for 24 h, i.e., until the results of the experiment were read. The infected cultures of the other control batch were incubated at 37° for 4 days.

To study the rct_{42} marker, a series of experiments was carried out to determine reproductive activity at 42 and 37° during the first cycle of development of the virus in chick embryo tissue cultures preliminarily washed cultures were infected with the corresponding virus in a dose of 30 PFU per cell. After adsorption, the cultures were washed three times to remove virus and incubated at 42 and 37° under a liquid nutrient medium for 18 h. The intracellular virus was liberated by freezing and thawing the tissue cultures three times, after which it was titrated by the plaque method. The experimental results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

The experiments showed that when the infected tissue cultures were incubated under agar at 40° for 1-3 days there was no difference in the reproductive activity of virulent and attenuated variants of the TBE virus complex compared with this index during cultivation at 37°. Cultivation at 42° revealed definite differences between the studied strains and variants of the viruses. The maximal difference in reproductive

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Table 1. Pathogenicity of Strains of Viruses of the TBE Complex and Their Variants for Albino Mice (mNic and mNsc markers) and Their Reproductive Activity at 37° and 42° (rct_{42} marker)

Strain	Titer of virus ($\log LD_{50}$/ml)		Titer of virus (PFU/ml; M ± m)		P
	ic	sc	37°	42°	
Pan*	9,71	6,8	7,99±0,30	8,19±0,01	>0,05
Pan line 42 †	7,0	3,98	5,28±0,425	3,59±0,38	>0,05
Pan line 26 †	6,58	2,57	5,72±0,26	2,89±0,40	>0,05
Clone 114 *	7,25	1,69	5,37±0,29	2,40±0,43	0,03
Khab-9*	9,09	8,5	7,97±0,28	7,83±0,34	
Khab-9 line 26 †	7,24	4,79	6,12±0,26	4,48±0,36	>0,05
Khab-9 line 26 †	7,84	5,27	5,30±0,25	2,61±0,42	
Khab-17 *	9,9	8,1	7,71±0,22	7,51±0,13	
Tp-21 *	8,376	3,2	7,03±0,24	2,28±0,46	0,03
Tp-21 line 42 †	6,87	3,64	6,02±0,37	4,47±0,49	>0,05
Tp-21 line 26 †	4,85	3,65	5,48±0,35	2,56±0,35	0,03
Clone 194 *	7,84	1,07	5,45±0,35	1,63±0,29	0,01
Clone 237 *	6,17	0,94	5,77±0,27	3,80±0,41	0,03
Clone 241 *	6,99	1,15	5,60±0,205	3,89±0,36	0,05

*Reference strain after cerebral passage through albino mice.
†Line of this strain after prolonged passage at 42 and 26°.

activity of the attenuated variants at 37 and 42° was determined after incubation for three days. Strains of TBE virus highly pathogenic for albino mice – Pan and Khab-17 – propagated at the same intensity at 37° and 42° for 1-3 days, whereas strain Tp-21 with low virulence according to the mNsc marker, the line of this strain after prolonged passage at 26° (line 26), and also clones 194 and Pan-144 possessed particularly low reproductive activity during incubation for 3 days at 42°. Results of the study of the rct_{42} marker for a number of strains of the TBE virus complex and their variants are shown in the table, in which this marker is also compared with the mNsc and mNic markers – pathogenicity for albino mice infected by the subcutaneous and cerebral routes. A definite correlation was discovered between the rct_{42} and the mNsc markers. For instance, highly virulent strains – Pan, Khab-9, Khab-17 (mNsc and variants), with titers of above 6.0 for mice infected by the peripheral route, expressed in $\log LD_{50}$/ml, were rct_{42}^{+}. Conversely, the mNsc variants, with titers of 3.5 and less for peripheral infection, expressed in $\log LD_{50}$/ml, almost failed to reproduce at 42° while possessing a high degree of reproduction at 37° (rct_{42}^{-} variants). The population of original strain Tp-21, the population of the same strain after passage at 26°, clones 194, 237, and 241, and also clone Pan-114, were of this type.

To determine the character of the rct_{42} marker, experiments were carried out in which the reproductive activity of strains Pan and Tp-21 and their variants – clones 114 and 194 – was investigated during the first cycle of development, based on data in the literature for 18 h [1]. The results showed that strain Tp-21 and clones 114 and 194, of low pathogenicity, reproduced less actively at 42° than at 37°. These results indicate true depression of reproduction of a number of strains at 42°, rather than the inactivating action of the raised temperature on the virus.

These experimental results show that the rct_{42} marker is a genetic characteristic definitely related to the degree of neuropathogenicity of the strains of the TBE virus complex and their variants which were studied. For instance, variants of viruses with low neurovirulence in accordance with the mNsc characteristic possessed lower reproductive activity at 42° (population of strain Tp-21, clones 114, 194, 237, 241).

It has been shown [4] that rct_{40} variants of poliomyelitis virus at 40° are able to complete the first cycle of development, but not the first and subsequent cycles of reproduction. By studying reproduction of viruses of the TBE complex and of certain of their variants at 37 and 42°, it was found that rct_{42}^{-} variants have much lower reproductive activity even in the first cycle of reproduction. Since the conditions of adsorption of these variants by the tissue culture cells were identical (37°, exposure for 1 h), it must be pre-

sumed that the lower reproduction in the first reproductive cycle at 42° was dependent on partial depression of the intracellular stages of propagation of these variants.

The rct_{42} marker, among other genetic markers of viruses of the TBE complex, is thus valuable for identifying individual strains of this group of viruses and it can be used in some cases to judge the degree of attenuation of natural and artificially obtained variants.

LITERATURE CITED

1. A. A. Avakyan, A. D. Al'tshtein, and Yang Ju-hsi, in the book: Poliomyelitis, Nonpoliomyelitis Enteroviruses and Tick-Borne Encephalitis, Moscow (1961), p. 228.
2. L. G. Karpovich, Acta virol., 10, 356 (1966).
3. E. N. Levkovich, L. G. Karpovich, and N. V. Loginova, in the book: Tick-Borne Encephalitis, Kemerovo Tick-Bite Fever, Hemorrhagic Fevers, and Other Arbovirus Infections, Moscow (1964), p. 52.
4. A. Lwoff and M. Lwoff, Nature, 192, 113 (1961).