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INFLUENCE OF CHEMICAL AND PHYSICAL MUTAGENIC FACTORS ON THE VIRUS
OF TICK-BORNE ENCEPHALITIS

Following is the translation of an article by G. D. Zasukhina and Ya. Zavada, published in the Russian-language monograph Genetics of Microorganisms, Medgiz, 1963, pages 200-207.

In recent years works have appeared on the study of induced mutability in animal viruses. Mutants, differing in some features, were obtained in the poliomyelitis virus under the influence of nitrous acid, proflavine, and 5-bromuracil, in the virus of foot and mouth disease under the influence of proflavine, and in certain other viruses (Dulbecco, Vogt, Li, 1958; Boeye's, 1959; Brown, Steward, 1960).

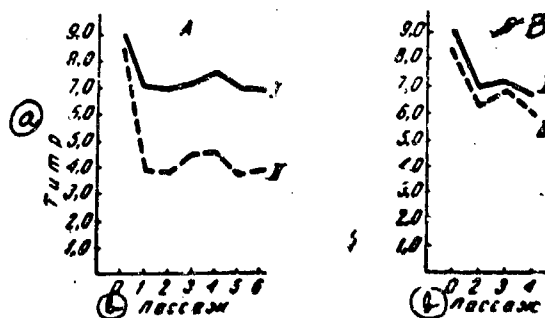


Fig. 1. Influence of 5-bromuracil on the virus of tick-borne encephalitis.

A - test; B - control; I - titer of virus following intracerebral infection; II - titer of virus following intraperitoneal infection. Key: (a) titer; (b) passage.

Of particular interest is the study of the experimental mutability on a model of the virus of tick-borne encephalitis, the genetic properties of which are highly stable and there are practically no variants of the virus with conclusive avirulence or with any other hereditarily fixed features, and these stable properties are well-known to all who work with this virus. Work is complicated by the fact that there are no clear and regularly reproducible plaques, and besides this up until now no characteristic markers, capable of being studied in vitro, have been found.

We studied the possibility of obtaining variants of the tick-borne encephalitis virus under the influence of certain chemical and physical agents. In the experiments we used azouridine, azouracil, and 5-bromuracil from the class of mutagenic substances, analogs of metabolites, formaldehyde, acting in the manner of "impact poisons," urethan, which takes part in the synthesis of nucleotides, and proflavine, which disrupts the replication of nucleic acids and its bonds with protein.

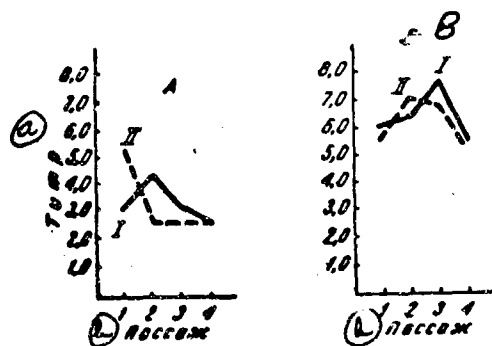


Fig. 2. Influence of formaldehyde on the virus of tick-borne encephalitis.

A - experiment; B - control; I - titers following intracerebral infection; II - titers following intraperitoneal infection.

Key: (a) titer; (b) passage.

The virus in the vegetative stage was subjected to a single treatment with mutagenic substance in a tissue culture, after which it went through 3-6 passages in tissue culture of one or three types. In the last passage the end dilution of virus was used. Virulence of the virus was tested in each passage in tests of titration in the brain and peripherally - on white mice.

After the influence of 5-bromuracil (Fig. 1) on virus in a culture of SOTs cells and passage of the virus in cultures of sheep kidney cells, beginning with the 1st passage a sharp lowering (by 3 log) was observed in the peripheral activity of virus following infection of white mice. This change was preserved for 6 passages. Change of infestation properties of the virus was also expressed in an increase of the incubation period of the disease in mice following peripheral infection. While in the control (Table 1) the average indices of the incubation period of the disease in mice following peripheral infection was 7-8 days, in the test it was 11-12 days.

Analogous data on the lowering of peripheral activity were obtained in the tests with formaldehyde. Here a sharp lowering was observed not only of peripheral but also of cerebral activity of the virus (Fig. 2). While in the control the peripheral and intracerebral titers of virus were equal to 6-7 log, in the test the titers of virus

following intracerebral and peripheral infection were lowered and were less than 3 log. Consequently a single exposure of virus to formaldehyde and passaging of the virus in a highly sensitive cell system preserve a lowering of virulence of virus for white mice.

Table 1

Average indices of incubation period of disease in mice after exposure of virus (strain PAN) to 5-bromuracil and formaldehyde

a) Мутагенный фактор	b) Пассажи					
	1-я	2-я	3-я	4-я	5-я	6-я
c) 5-бромурацил	7.0±0.2	6.0±0.3	4.6±1.1	5.7±0.9	5.5±0.9	6.1±0.8
	12.3±1.1	10.4±1.2	10.2±1.4	10.0±0.4	11.1±0.8	12.0±1.3
d) Формальдегид	7.2±0.3	7.0±0.5	6.8±1.0			
	8.3±0.4	8.2±0.3	7.9±0.8			
e) Контроль	6.5±1.1	6.5±0.5	6.3±1.0			
	7.1±0.5	6.0±0.3	7.5±0.3			

Note: Numerator - incubation period of disease (in days) during intracerebral infection of mice, denominator - during peripheral infection of mice.

Key: (a) Mutagenic factor; (b) Passages; (c) 5-bromuracil; (d) Formaldehyde; (e) Control.

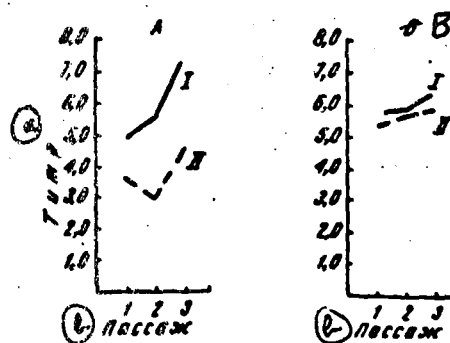


Figure 3. Influence of urethan on the virus of tick-borne encephalitis. A - test; B - control; I - titers following intracerebral infection; II - titers following intraperitoneal infection. Key: (a) titer; (b) passage.

Urethan also caused a lowering of peripheral activity of virus which was intensified with passaging and reached differences in titers by 3 log (Fig. 3). Combined action on the virus by proflavine and urethan, which affect various phases and replications of the virus

particle, was also expressed in a lowering of peripheral activity of virus with passaging (Fig. 4.). However, in contrast to 5-bromuracil this was manifested positively only with the 3rd passage, and in the 4th passage the difference in peripheral and intracerebral titers of virus reached 4 log. Such a type of action is also inherent to proflavine alone (Fig. 5), when, beginning with the 3rd passage, the peripheral activity of the virus drops to 3 log and in the 4th passage the difference in titers also reaches 4 log. Both in the case of combined action and under the influence of one substance the control viruses had approximately the same titers during peripheral and intracerebral infection of mice.

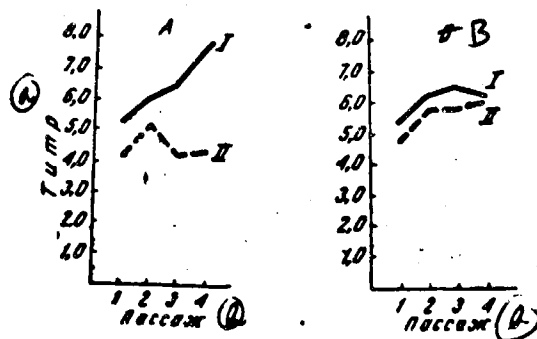


Figure 4. Influence of proflavine and urethan on the virus of tick-borne encephalitis.

A - test; B - control; I - titers following intracerebral infection; II - titers following intraperitoneal infection.

Key: (a) titer; (b) passage.

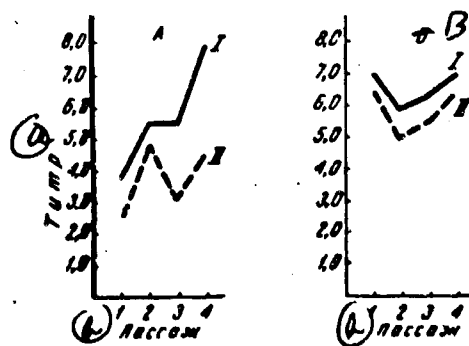


Figure 5. Influence of proflavine on the virus of tick-borne encephalitis.

A - test; B - control; I - titers following intracerebral infection; II - titers following intraperitoneal infection.

Key: (a) titer; (b) passage.

Thus the influence of certain mutagenic factors on the vegetative virus of tick-borne encephalitis either sharply lowers the virulence of the causative agent (tests with formaldehyde), or immediately lowers the peripheral activity of the virus (tests with

5-bromuracil), or the peripheral activity of the virus is lowered with passaging (tests with proflavine). Subject to further study is the problem of the reversibility of these properties, and also the fixing of these features and obtaining of pure lines of virus with reduced virulence.

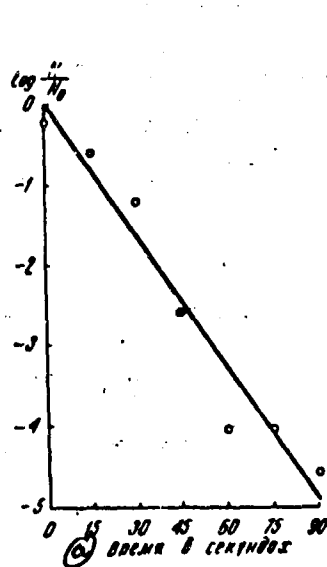


Fig. 6. Results of titration of control virus of tick-borne encephalitis and virus which was irradiated by ultraviolet rays (2nd passage).

Fig. 7. Dependence of titers of virus of tick-borne encephalitis on the duration of exposure to ultraviolet rays.

Key: (a) Time in seconds.

In addition to the influence of chemical mutagenic factors, the virus of tick-borne encephalitis was subjected to ultraviolet irradiation.

In our experiments the vegetative virus of tick-borne encephalitis during infection with a calculation of several viral particles per 1 cell in a tissue system of a chick embryo was subjected to ultraviolet irradiation with a strength of 400,000 erg/cm². Extracellular virus was inactivated in 90 seconds by 5 log; this indicates that during an exposure of 10 minutes, which was used in our experiments, the titer of the virus would be lowered by 33 log. It is apparent from Fig. 6 that the titer of virus is found in an exponential dependence on dose of irradiation. It is clear from Fig. 7 that intracellular virus is inactivated considerably more slowly than

infectious extracellular virus. This is probably explained by the multiple reactivation of intracellular virus. After a single irradiation the virus of tick-borne encephalitis in our tests was revealed in tissue cultures during titration on white mice, and in addition to this a new property of the virus was manifested - it caused a cytopathogenic effect in tissue cultures of chick embryo. This cytopathogenic effect was passed in tissue cultures and was specific following its neutralization by homologous serum. The results of titration of control and irradiated viruses of the 2nd passage are presented in Table 2.

Table 2

Results of the titration of control and ultraviolet-irradiated virus of tick-borne encephalitis (2nd passage)

(a) Штамм вируса	log PFU/ml	log ID _{50 ic} /ml	log ID _{50 ip} /ml	log TCD ₅₀ /ml
(b) ПАН	5.4	6.2	3.7	—
(c) ПАН после ультрафиолетового облучения	5.7	6.2	5.1	4.5
(d) СОФ'ИН	5.4	5.0	4.4	—
(e) СОФ'ИН после ультрафиолетового облучения	6.0	7.0	4.7	5.35

Note. — - absence of cytopathogenic action of virus.

Key: (a) Strain of virus; (b) PAN; (c) PAN after ultraviolet irradiation; (d) SOF'IN; (e) SOF'IN after ultraviolet irradiation.

It is clear from the material presented that the property of cytopathogenic activity of virus is not connected with a lowering of virulence. An analogous experiment was set up jointly with Doctor Stanček on L cells. Virus of the 5th passage after irradiation in a comparison with control had the cytopathogenic activity, but the incubation period of disease in mice during intracerebral infection for the control virus was 5.5 days, and for irradiated - 8 days. From these first results it is clear that under the stated conditions there is a possibility of multiple reactivation of the tick-borne encephalitis virus, and also the obtaining of different variants of this virus. Though from certain tests it follows that a convincing lowering of virulence of virus is obtained, we were not able to obtain a virus which was virulent for mice. Promising in this direction is either the isolation of a large number of clones of virus with a simultaneous study of their virulence or the multistage influence of mutagenic factors on the virus.

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