# THE EFFECT OF SHORTWAVE LENGTH ULTRAVIOLET RAYS ON THE INFECTIOSITY AND ANTIGENIC PROPERTIES OF THE VIRUSES OF RUSSIAN TICK-BORNE AND JAPANESE ENCEPHALITIS

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(Received November 16, 1956. Presented by Prof. N. N. Zhukov-Verezhnikov, Active Member of the AMS USSR )

We conducted experiments to investigate the effect of ultraviolet rays on the infectiosity and antigenic properties of tick-borne encephalitis.

As is known from the data in the literature, there are different physical and chemical agents in use to eliminate the infectiosity of neuroviruses which preserve the antigenic properties of the viruses.

One of the most widely used inactivators is formalin, which is used to prepare various neurovirus vaccines. In the great majority of cases, however, the use of formalin in a 1:500 concentration causes the titer of antigens to decrease and their anticomplementicity to increase [1, 2, 4, 5].

The possibility of eliminating the infectiousness of viruses while retaining their antigenic properties is mentioned [3, 6] in individual works studying neurovirus inactivation caused by ultraviolet rays 2537 A long.

However, the data given in these works on the time of inactivation are contradictory, and there is no exact data on the relation between the antigenic and infectious properties, which data is necessary to obtain highly active antigens from a dose of ultraviolet radiation. There is almost no data on the virus of tick-borne encephalitis.

## EXPERIMENTAL METHODS

For radiation, we used: a BK bactericidal lamp giving ultraviolet radiation, 85% of which fell within shortwave length ultraviolet rays (SUV) with a wave length of 2, 597 A, and a portable quartz mercury lamp (PQM-4) radiating the whole spectrum of ultraviolet rays, with all the shortwave length ultraviolet rays intercepted by an ultraviolet-transmitting glass filter, so that the maximum radiation consisted of longwave length ultraviolet rays (LUV) with a wave length of 2,970-3,020 A.

Virus-containing suspensions of different concentrations (1 and 10%), from the brain tissue of mouse and chicken embryos infected with the virus of tick-borne or Japanese encephalitis, prepared in a physiological solution of sodium chloride, were subjected to radiation. In a series of experiments, the allantoic fluid from chicken embryos infected with the virus of tick-borne encephalitis was used.

The virus-containing material was centrifuged for 30 minutes at 3,000 revolutions per minute and put in a Petri dish or byuks* so that the suspension to be irradiated was 1 mm thick.

The lamp burners were placed above the suspension to be radiated so that the radiant energy fell at right

* Transliterated.

angles to the suspension. Radiation intensity was 200-210 microwatts per 1 $cm^2$ in all of the experiments. Shortwave length radiation was done at a distance of 25 cm, longwave, at a distance of 17 cm. The radiation lasted 5, 10, 15, 25, 30 and 40 minutes.

The suspension's loss of infectiosity was determined by intracerebrally injecting it simultaneously into 5-6 mice in a dose of 0.03 ml. If there were no clinical signs of sickness in the primarily infected mice, three of them were killed, and their brains passaged to fresh mice.

## EXPERIMENTAL RESULTS

We obtained the following results from the experiments testing the inactivation of the tick-borne encephalitis ("Sofyin" strain) virus contained in the various tissue suspensions which were radiated with SUV rays 200-210 microwatts per $cm^2$ in intensity.

The virus contained in the 10% suspension of mouse brain tissue was completely inactivated after 15-minute or longer radiation. The mice whose brains were infected with the irradiated suspensions did not get sick, and the virus was not evident in the first passage to the mice (Table 1).

TABLE 1

Effect of SUV Radiation (200-210 microwatts per 1 $cm^2$) on the Infectiosity (Inactivation) of the Virus of Russian Tick-Borne Encephalitis

| Material | Radiation time (in min) | Inactivation control | | | | Inactivation results |
|---|---|---|---|---|---|---|
| | | Number of mice | | | | |
| | | Infected | Died | Passaged | Died | |
| Centrifugate of 10% suspension from brain of infected mice | 15 | 5 | 0 | 5 | 0 | + |
| | 20 | 5 | 0 | 5 | 0 | + |
| | 30 | 5 | 0 | 5 | 0 | + |
| | 40 | 5 | 0 | 5 | 0 | + |
| The same . . . . . | Not irradiated | 5 | 5 | × | × | — |
| Centrifugate of 10% suspension from brain of infected chicken embryos . . . . | 5 | 6 | 6 | × | × | — |
| | 10 | 6 | 6 | × | × | — |
| | 15 | 6 | 2 | × | × | ± |
| | 20 | 6 | 0 | 6 | 0 | + |
| | 25 | 6 | 0 | 6 | 0 | + |
| | 30 | 6 | 0 | 6 | 0 | + |
| The same . . . . . | Not irradiated | 6 | 6 | × | × | — |

Symbols: + positive, — negative, x not investigated

When the 10% suspension of chicken embryo brain tissue with a virus titer of $10^{-6}$ was irradiated, complete inactivation of the tick-borne encephalitis virus was obtained after 20-, 25- and 30 minute radiation. When the 10% suspension was irradiated for 15 minutes, only partial inactivation of the virus was obtained, and with 5- and 10- minute radiation, the virus was not inactivated (Table 1).

The virus contained in the undiluted allantoic fluid of the chicken embryos was partially inactivated after 20- and 40- minute irradiation, but not inactivated by 10-minute radiation (Table 2).

Irradiation of the allantoic fluid in a dilution of 1:5, however, caused complete inactivation of the virus after 10-, 20- and 40-minute exposures (Table 2).

The next experiments were done with the Japanese encephalitis virus, contained in a 1% suspension of mouse brain tissue. Shortwave length (SUV) and longwave length (LUV) rays were used for the radiation (Table 3).

TABLE 2

The Effect of SUV rays (200-210 microwatts per 1 $cm^2$) on the Infectiosity of the Tick-borne Encephalitis Virus Contained in Allantoic Fluid

| Material | Radiation time (in min) | Inactivation control | | | | Inactivation result |
|---|---|---|---|---|---|---|
| | | Number of mice | | | | |
| | | Infected | Died | Passaged | Died | |
| Undiluted allantoic fluid | 10 | 5 | 5 | × | × | — |
| | 20 | 5 | 2 | × | × | ± |
| | 40 | 5 | 1 | × | × | ± |
| Allantoic fluid diluted 1:5 | 10 | 5 | 0 | 5 | 0 | + |
| | 20 | 5 | 0 | 5 | 0 | + |
| | 40 | 5 | 0 | 5 | 0 | + |
| The same | Not irradiated | 5 | 5 | — | — | |

Note: Symbols mean the same as in Table 1.

The experiments showed that the inactivating effect of the SUV rays extends to the virus of Japanese encephalitis. Irradiation of the 1% suspension from the brain tissue of infected mice caused inactivation of the virus after 5, 10 and 15 minutes of radiation.

TABLE 3

The Effect of Radiation with Ultraviolet Rays of Various Wave Lengths of the Infectiosity of the Japanese Encephalitis Virus

| Material | Rays | Radiation time (in min) | Inactivation control | | | | Inactivation results |
|---|---|---|---|---|---|---|---|
| | | | Number of mice | | | | |
| | | | Infected | Died | Passaged | Died | |
| Centrifugate of 1% suspension from brain of infected mice | SUV | 5 | 4 | 0 | 4 | 0 | + |
| | | 10 | 4 | 0 | 4 | 0 | + |
| | | 15 | 4 | 0 | 4 | 0 | + |
| | LUV | 5 | 4 | 4 | × | × | — |
| | | 10 | 4 | 4 | × | × | — |
| | | 20 | 4 | 4 | × | × | — |
| | | 30 | 4 | 4 | × | × | — |
| The same | | Not irradiated | 4 | 4 | × | × | — |

Note: Symbols mean the same as in Table 1.

Parallel experiments were also conducted, irradiating a similar suspension with LUV rays of the same radiant energy intensity — 200-210 microwatts per 1 $cm^2$; these rays did not inactivate the virus after 5, 10, 20 or 30 minutes of radiation (Table 3).

Therefore, irradiation with shortwave length ultraviolet rays causes inactivation of the tick-borne and Japanese encephalitis viruses, while irradiation with longwave length ultraviolet rays of the same intensity does not. With radiation by shortwave length ultraviolet rays of unvarying intensity, inactivation depends on the duration of the irradiation, i.e., on the dose of radiant energy, and also on the concentration of the tissue components in the virus-containing material undergoing irradiation.

The antigenic properties of the virus were tested by a complement fixation reaction with specific (experimental) or convalescent serums.

Allantoic fluid was used in the experiment without additional processing, and a thermolysed antigen was

by the method of cold agglutination.

To find out how antigenic properties change in a virus irradiated with SUV rays but not inactivated, we examined undiluted, virus-containing allantoic fluid which had been irradiated for 5, 10 and 20 minutes. The results of the reaction showed that the antigenic properties of the tick-borne encephalitis virus do not change when the infectiosity of the virus is preserved, but change slightly due to 20-minute irradiation, which causes partial inactivation of the virus (Table 4).

TABLE 4

Complement Fixation Reaction with Allantoic Antigensof the Tick-Borne Encephalitis Virus Irradiated by SUV rays

| Complement dose | Radiation time (in min) | Antigens | Serum and dilution | | | | | | | | | Antigen control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Specific No. 1 (exptl.) | | | Specific No. 2 (experimental) | | | Normal | | | |
| | | | 1:4 | 1:8 | 1:16 | 1:4 | 1:8 | 1:16 | 1:4 | 1:8 | 1:16 | |
| 2.0 | 5 | Specific | 4 | 3 | 2 | 2 | 2 | — | — | — | — | — |
| | 10 | " | 4 | 4 | 3 | 3 | 3 | — | — | — | — | — |
| | 20 | " | 3 | 3 | 2 | — | — | — | — | — | — | — |
| | 0 | " | 4 | 4 | 3 | 2 | 2 | + | — | — | — | — |
| | 0 | Normal allantois | — | — | — | — | — | — | — | — | — | — |
| | 0 | Control serum | — | — | — | — | — | — | — | — | — | × |

Symbols: specific antigen — allantoic fluid of chicken embryos infected by virus of tick-borne encephalitis; 4, 3, 2 +) degree of hemolysis inhibition; x) complement fixation reaction not studied; —)complete hemolysis; 0) not irradiated.

TABLE 5

Antigens of Tick-Borne Encephalitis Inactivated by Ultraviolet Rays Tested in Complement Fixation Reaction

| Radiation time (in minutes) | Antigen in dilution | Serum in dilution of 1:8 | | | | | | | | | | Antigen control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Specific | | | | | Normal | | | | | | | |
| | | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:10 | 1:20 | 1:40 |
| 5 | Specific 10% | 4 | 4 | 4 | 2 | — | — | — | — | — | — | — | — | — |
| 10 | The same | 4 | 4 | 4 | + | — | — | — | — | — | — | — | — | — |
| 15 | " | 4 | 4 | 4 | ± | — | — | — | — | — | — | — | — | — |
| 20 | " | 4 | 4 | 3 | ± | — | — | — | — | — | — | — | — | — |
| 30 | " | 4 | 4 | 2 | ± | — | — | — | — | — | — | — | — | — |
| 0 | " | 4 | 4 | 4 | 2 | — | — | — | — | — | — | — | — | — |
| 0 | Normal | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 0 | Control serum | — | — | — | — | — | — | — | — | — | — | — | — | — |

Symbols: Same as in Table 4.

In order to more exactly determine how much the antigenic properties of the virus decreased in relation to the degree of virus inactivation, we conducted further experiments with a 10% suspension of chicken embryo brain tissue, which was irradiated 5, 10, 15, 20 and 30 minutes and then used to prepared thermolysed antigens.

The control antigens were prepared from a nonirradiated virus-containing suspension (positive control antigen) and from a 10% brain tissue suspension from uninfected chicken embryos (normal antigen).

These antigens were titrated in a complement fixation reaction with a specific or a normal serum, used in a dilution of 1:8. The results obtained are shown in Table 5.

The results of these experiments showed that the titers in the complement fixation reaction of the antigens prepared from the completely inactivated suspension of the tick-borne encephalitis virus (20-30 minute radiation) were twice as low (1:40) as those of the noninactivated antigens prepared from the same initial suspension (titer of nonirradiated antigen – 1:80).

Therefore, when the tick-borne encephalitis virus is inactivated by SUV rays, the virus retains its antigenic (complement agglutinizing) properties even after the complete loss of its infectiosity (Table 1 and 5). In this case the titer of antigens is twice as low as the titers of natural antigens prepared from the same initial suspension (Table 5). However, increasing the SUV rays to more than the minimal dose (increasing radiation time), i.e., a stronger dose than is needed to completely eliminate the infectiosity of the virus, causes a greater decrease in the antigenic (complement agglutinizing) properties of the tick-borne encephalitis virus.

## SUMMARY

Irradiation for 5-20 minutes by shortwave lengths of 2597 A of 1-10% brain suspensions infected with Russian tick-borne or Japanese encephalitis virus resulted in complete inactivation of the virus. The same suspensions irradiated by longwave lengths of 2970-3020 A retained their infectiosity even after 30 minutes of irradiation. In complement fixation tests, virus antigens inactivated by shortwave lengths irradiation reacted positively with specific serum.

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