LOW-TEMPERATURE THRESHOLDS OF REPRODUCTION OF SOME GROUP A ARBOVIRUSES IN TISSUE CULTURE

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(The authors determine the lower reproduction thresholds of Group A Arboviruses (Sindbis*, eastern, western and Venezuelan equine encephalomyelitis) in tissue culture prepared from chicken embryo fibroblasts; they also study the effect of superthreshold temperatures on the yield of these viruses. For viruses whose areal includes part of the temperate zone, the lower temperature threshold of reproduction is lower (for the Sindbis virus, 15°C, and for the eastern and western types, 17°) than for viruses distributed in the equatorial, subequatorial and tropical zones (the threshold for the Venezuelan virus is 19°). By raising the incubation temperature by 1°, the yield of viruses was increased: by 1.05±0.025 lg for the Sindbis, by 1.1±0.036 lg for the eastern, by 1.26±0.12 lg for the western, and by 1.62±0.27 lg for the Venezuelan type).

As is well known, the arboviruses, since they are transmitted by mosquitoes are limited to certain climatic zones. Some of them appear predominantly in the equatorial and subtropical zones (only a few species of this ecological group may be found in the southern portion of the temperate zone [13]. The present authors earlier advanced the idea that one of the important factors limiting the distribution of natural foci of the mosquito-transmitted arboviruses is the lower temperature threshold of reproduction of the viruses in carriers [4, 5, 8-9]. Reeves and Hammon [15] have reported that in California the virus of western equine encephalomyelitis (WEE) is found in Culex tarsalis if the mean daily temperature reaches 26.5° or higher. These authors conclude that for effective transmission of the

*Adapted from the Russian.

virus by mosquitoes, a minimal threshold of continuous temperature is required.

The present authors present the results of a study of the lower temperature thresholds of reproduction, using a tissue culture of chicken embryo fibroblasts. A number of Group A arboviruses were studied which are found in various climatic zones of the world.

Material and methods. Strains of several viruses were used: those of the eastern and western equine encephalomyelitis, and the Sindbis, all supplied by the D. I. Ivanovskiy Institute of Virology, USSR Academy of Medical Sciences. These were cultured in a culture of chicken embryo fibroblasts. The test cultures were infected by viruses in the amount of 100 biol. poison units each. The volume of the innoculate was 0.1 ml. The growth medium (hydrolysate of lactalbumin with 3% normal ox serum) was separated from the cultures before introduction of the virus. Adsorption of the virus proceeded for 1½ hours at the temperature of the test. Following removal of the unadsorbed virus, a supportive medium was added (medium No 199 with 1% of normal ox serum), and incubation followed at the given temperature.

Tests for the amount of virus present were made 3 hours following infection ("zero point"), and again after 96 hours, with the exception of temperatures 27° (after 72 hours) and 36° (after 48 hours), when cell degeneration began. In every test, the culture liquid from 3 cultures was combined. Titration of the virus was conducted by the

plate method, on chicken embryo culture. The lower reproduction threshold was taken as the temperature at which the virus titer

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Correlation Link between Temperature of Incubation and Accumulation of Virus (biol. sept. units per 0.1 ml)

Temp. (°)	Sindbis	WEEv	EEEv	VEEv
14	0	0	0	0
15	2.4	0	0	0
16	3.3	0	0	0
17	4.8	1.4	1.9	0
18	5.3	3.6	4.5	0
19	6.0	3.0	4.0	2.9
20	7.2	4.3	4.6	4.3
21	8.1	5.9	6.9	5.9
22	9.1	7.1	7.4	7.5
23	10.2	9.3	7.6	8.3
R= a	0,992±0,05	0,965±0,23	0,983±0,075	0,986±0,18
P= b	99,9	99,9	99,9	99,9
R= c	1,05±0,025	1,20±0,12	1,1±0,036	1,62±0,27

NOTE: "0" indicates that the viruses are not reproducing

a - the coefficient of correlation, r, is defined as follows:

$r = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 (y-\bar{y})^2}}$, where $\sum (x-\bar{x})(y-\bar{y})$ denotes the sum of deviations of temperature x and virus yield y, from the mean values of x and y, respectively.

b - error of the coefficient of correlation m is defined by the formula

$$m = \sqrt{\frac{1-r_{xy}^2}{n-2}}$$

c - reliability of the coefficient of correlation P (in %) is defined as

$$t = \frac{r_{xy}}{m_r}$$

where t is supplied from a table; for n = 2, t will correspond to the reliability of the presence of a link (P).

d - the coefficient of regression R is defined as

$$R = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2}.$$

e - the error of the coefficient of regression m is defined as

$$m = \sqrt{\frac{1 - r_{xy}^2}{n - 2}} \cdot \frac{\sum y}{\sum x}.$$

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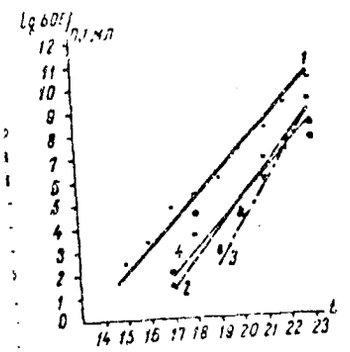
in platelet-forming units per 0.1 ml in the final test increased over the "zero point" by not less than a factor of 3. Apart from low-temperature thresholds of reproduction, the effect of superthreshold temperatures on virus yield was determined. The tests were run at temperatures of 14-23, 27 and 36°C. Temperature skips at the time of the tests did not exceed ± 0.1°.

In statistical processing of the test results, the coefficients of correlation (r) and regression (R) were determined by methods described by I. P. Ashmarin [1], I. Beyli [2] and V. Yu. Urbakh [11]. Increase in virus yield with a 1° increase in temperature was determined graphically, in connection with the regression equation.

Results. As is evident from the table, rproduction of the Sandbis virus be-

gins with 15°; that of the Venezuelan equine encephalomyelitis, and the western and eastern types of equine encephalomyelitis virus, at 17°, 17° and 19°, respectively. For all the viruses, up to a level of 23° there was established a high degree of direct correlation between temperature rise and virus accumulation (See Table).



Yield of viruses at superthreshold temperatures.

1 - Sindbis virus; 2 - western equine encephalomyelitis; 3 - eastern equine encephalomyelitis; 4 - Venezuelan equine encephalomyelitis

Increase in the incubation temperature beyond 23° did not increase the yield of viruses. Increase in yield with rise of incubation temperature of 1°, is indicated in the sketch on the basis of the regression equation.

In incubating infected cultures at 23° or below, no cytopathic changes whatever were observed in the culture cells at any time during the experiments. At 27°, the cultures degenerated on the third day, and at 37° on the second day; while the accumulation of virus at the height of the cytopathic effect, as a rule, was not greater than that at 23°. At 27 and 36°, the content of Sindbis virus in the liquid

phase of degenerated cells amounted to 0.1 ml (10.3 and 9.6 lg BSU); that of the Venezuelan equine encephalomyelitis virus to 8.5 and 8.9 lg BSU; that of the western equine encephalomyelitis virus to 9.3 and 10.0 lg BSU; and that of the eastern equine encephalomyelitis virus to 9.1 and 9.3 lg BSU.

Discussion. Part of our object was to establish the minimal temperature at which reproduction of the viruses studied begins in a chicken embryo culture, and to determine as well the increase in virus yield with a 1° rise in temperature. The data collected show that the reproduction of the arboviruses studied begins at various different temperatures, while the temperature thresholds of reproduction correspond to an advance of the areal of this or that arbovirus in a polar direction. Actually, the highest temperature threshold of virus reproduction was that for the eastern equine encephalomyelitis virus, the areal of which dies not extend beyond the limits of the equatorial and subequatorial zones of South and Central America [15] (mean temperatures of $\approx 27^{\circ}$ the year round). Flare-ups and isolated cases of illness from this virus are met with also in the tropical zone of the American continent as far as and including southern Florida (mean summer temperature of $\approx 24^{\circ}$).

The lower temperature threshold of reproduction of the Venezuelan and western equine encephalomyelitis was 2 [illegible] than for the eastern e. e. virus. Areals of the first two extend into the southern part of the temperate zone of North America. According to data of Hess et al. [13], the area of flare-ups brought about by the western equine encephalomyelitis virus is limited to regions with temperature of $\geq 21^{\circ}$. As our own analysis showed, the areals of these viruses are limited by the isotherm $\Sigma_{T>10^{\circ}}$ [9]. The lowest of the reproduction thresholds is that of the Sindbis virus, whose areal embraces practically the whole of the African

continent, the Near East, and southeastern Asia [6]. In the USSR this virus has shown up in Azerbaydzhan [10]. The use of serological research methods have revealed the existence of foci on the Volga Delta [3]. In view of the high ecological activity of the Sindbis virus, in particular its ability to reproduce at temperatures up to 15°, one may assume that this representative of the ecological group of arboviruses, transmitted by mosquitoes, is quite capable of penetrating far within the bounds of the temperate zone.

As regards increase in the reproduction of the viruses studied as a result of increased temperature, one can say that increase in virus content in the liquid phase of cultures with rising temperature (by 1°) is described by the equation of linear regression. Increasing temperature by 1° (up to 23°) leads to increase in the virus yield of 1.05 1.62 lg BSU units per 0.1 ml. In future tests it will be of interest to clarify the question of the degree to which this increase is associated with increase in virus yield per individual cycle of development, and to what degree it is associated with a possible contraction of the cycle.

Of great interest is the fact that with superthreshold temperatures in the infected cultures, despite a large virus accumulation, no degenerative changes were observed. The Group A arboviruses studied are among those agents which cause marked destructive action on cells cultured in vitro. Therefore, the observed absence of cytopathic changes supports the conclusion that at low temperatures the character of interrelationships of the virus with the cells changes substantially.

Thus, analysis of the results indicates a link between the dissemination of a number of mosquito-transmitted arboviruses toward the poles and their ability to reproduce at relatively low temperatures. The data obtained may help in compiling

prognoses for the arboviruses studied, in connection with specific territories. However, in constructing prognoses, of particular importance are data on the relationship between the external incubation period of the viruses in carriers, and temperature. This particular subject, and also the reproduction thresholds of viruses grown on various cell cultures, including the cells of the Arthropoda, will be dealt with in a future communication.

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The low temperature thresholds of reproduction were studied in chick embryo fibroblast cultures for some arboviruses occurring in the equatorial subequatorial climatic zones (Venezuelan equine encephalomyelitis virus) and in subtropic and temperate climatic zones (Western and Eastern equine encephalomyelitis, Sindbis viruses). Reproduction of Venezuelan equine encephalomyelitis virus was found to take place starting from 19°C, of Western and Eastern equine encephalomyelitis from 15°C, Sindbis virus from 15°C. The yield of viruses upon increase of the temperature by 1°C increased for Sindbis virus by 1.05±0.025 lg, for W.E.E. by 1.26±0.12 lg for E.E.E. by 1.1±0.36 lg and for V.E.E. by 1.62±0.27 lg.