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Virion Diameter as a Genetic Marker of the Virus of Venezuelan Equine Encephalitis

by

B. V. Gushchin, Ya. Ya. Tsilinskiy, S. M. Klimenko and D. K. L'vov

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b. V. Gushchin, Ya. Ya. Tsilinskiy, S. M. Klimenko and D. K. L'vov

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13 ABSTRACT

The population of the wild virus of Venezuelan equine encephalitis (VEE) consists of heterogeneous virions; while the clones isolated from this population present a fairly uniform diameter. Actually, the mean diameter of the virions of various clones varies between 72.68 and 86.12 millimicrons. The diameters which are characteristic of clones are retained in the progeny. The diameter of VEE virions can be regarded as a genetic sign, since it characterizes the individual pure lines, and is definitely hereditary.

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VIRION DIAMETER AS A GENETIC MARKER OF THE VIRUS OF VENEZUELAN EQUINE ENCEPHALITIS

/Paper by B. V. Gushchin, Ya. Ya. Tsilinskiy, S. M. Klimenko and D. K. L'vov; Moscow, Voprosy virusologii (Problems of Virology), Russian, No 2, 1970, pp 159-163/

The population of the wild virus of Venezuelan equine encephalitis (VEE) consists of heterogeneous virions; while the clones isolated from this population present a fairly uniform diameter. Actually, the mean diameter of the virions of various clones varies between 72.68 and 86.12 mμ. The diameters which are characteristic of clones are retained in the progeny. The diameter of VEE virions can be regarded as a genetic sign, since it characterizes the individual pure lines, and is definitely hereditary.

As is well known, an external sign such as diameter is a heritable characteristic. As regards animal viruses, however, this thesis is only hypothetical, since in the genetic aspect the diameters of virions have not been studied. Our purpose in the present paper is to set forth experimental data demonstrating that the virion diameter of a certain human and animal virus, namely that of Venezuelan equine encephalitis (VEE), is determined on an hereditary basis.

Materials and Methods Used

We studied the virion diameter in a VEE population, and in the clones isolated from that population. We also followed through the preservation of such diameters in the progeny of pure lines. The virus population was of the wild strain of VEE taken from the collection of the virus museum of the imeni D. I. Ivanovskiy Institute of Virology, USSR Academy of Medical Sciences. Genetically speaking, the wild strain was uneven as regards a number of markers — diameter of plaques, interferogeneity, thermal stability, sensitivity to the inhibiting action of agar polysaccharides, capability of autointerference, and so forth. The clones selected for study differed

among themselves in respect to one or more of these markers. The virus was passed through a culture of large fibroblasts (infecting dose equal to 100 TCD_{50} per test culture), then reseeded after 24 hours.

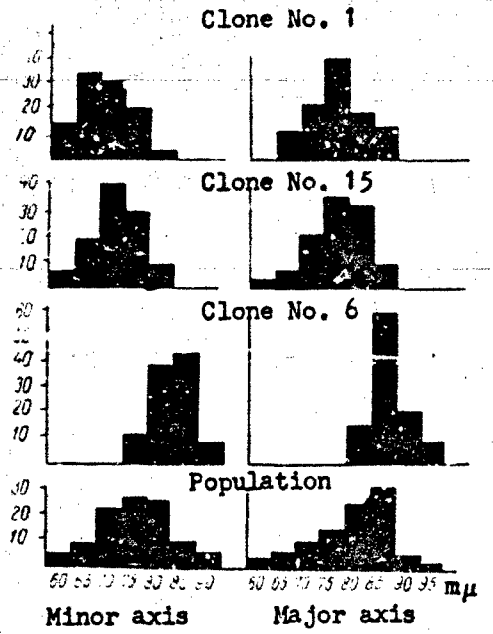

Figure 1. Histograms showing distribution of axial lengths of virions in clones and in population of VEE.

In obtaining virus suspensions for study under the electron microscope, the chicken fibroblast cultures used in the tests, infected with a small dose of the virus (30-100 TCD_{50} per culture), were incubated in a rotating drum at 37°C. To the supporting medium before the test, was added 0.25 mg/ml of glutamine. After 16-20 hours, when the first signs of cytopathologic action by the virus had appeared, the liquid culture medium was collected and freed from cellular detritus by centrifugation at 10,000 rpm for a period of 30 minutes. The virus suspension was then imposed on an electrically treated Formvar-carbon base and contrasted with 2% phosphotungstic acid at pH 7.0, and then studied with the ЭМ-6С electron microscope at magnification of 50,000.

The material studied was photographed by the covered-cadre (field) method, which guaranteed a random selection of virions for subsequent mea-

surement. In each suspension no fewer than 100 virus particles were photographed. The negatives were then placed in the photographic enlarger. The image was projected onto white paper at 4X, the contours of the virions were outlined precisely with a sharp pencil, and then measured with a slide caliper graduated in divisions of 0.05 mm. In the case of those virions which were eliptically shaped, both major and minor axis were measured; in the case of the circular ones, the diameter. Any virions in one or another stage of destruction were ignored. In all cases we measured sufficient number of intact virions so that the size of sample (n) should guarantee a mean value ($\bar{X}$) with maximum error of 3%, and a credible reliability of 99%. A sample of 50-100 virions satisfied this requirement.

In the statistical processing of results we constructed histograms of the distribution of major and minor axes, and calculated the basic parameters of the statistical sequence: weighted mean ($\bar{X}$), dispersion (s^2), mean-square deviation (s), and coefficient of variation (V). In determining the dispersion, we introduced Sheppard's correction, so that the adopted class interval was fairly large, being 5 mμ. Using the test of normality to determine the character of the empirical distributions obtained, we were able to establish that magnitude of the virion axis within clones follows very closely the Gaussian law. On this basis, we determined the reliability of differences between mean values according to the rule of comparison of means for two large samples, selected from the sets with normal distribution. In our calculations we used formulas and auxiliary tables given in the manuals by Bailey [1] and Urbach [2].

In determining virion size, we ignored the so-called giant virions [3], which may be encountered in suspensions of unclonated virus. The The question of the conditions of organization, and the possible nature, of the latter sort of suspension will be the subject of a special paper.

Results; Discussion

The histograms of the distribution of major and minor axis size of the virions which were part of the population studied, differ from the corresponding histograms for the closes isolated from that population (Figure 1). For the population, a gradual drop-off, and elongation of the curve, are characteristic; this reflects the considerable variability into the quantities forming the distribution. By contrast, the histograms for the clones show a sharp drop off, with little elongation, while the majority of variants are closely clustered around the distribution mean. Relative dispersion, as is shown by comparison of the coefficients of variation (Table 1), is about twice as great for the population as for any of the eight clones studied. From this we can conclude that a clone, as distinct from a population, consists of virions which are relatively homogeneous as regards diameter.

TABLE 1

Basic Parameters of Distribution of Sizes of Major and Minor Axes of the Virions of the Population and the Clones of VEE

Virus	Number of varions (n)	Varion size	Size of axis: Minor axis $\bar{X}\pm s$ (in mμ)	Minor axis V(%)	Major axis $\bar{X}\pm s$ (in mμ)	Major axis V(%)
Clone						
No. 15	74	Small	70.88±4.95	6.9	72.68±5.13	6.7
No. 3/5	92		70.11±4.89	6.8	73.11±4.92	6.7
No. 1-1	98	Medium	68.26±4.15	6.0	75.20±5.08	6.7
No. 8	87		74.60±4.03	5.4	59.25±4.65	5.8
No. 12-7	90		70.28±4.39	6.2	81.03±4.58	5.6
No. 12-8	97		78.55±4.03	5.1	82.00±4.77	5.8
No. 5	48	Large	79.89±4.53	5.6	82.18±5.75	7.0
No. 6	89		82.24±3.89	4.7	86.12±4.01	4.7
Population	78		75.06±7.35	9.8	78.58±8.12	10.3

If we approximate the obtained empirical distributions as normal, then we can calculate the limits $\bar{X} \pm 2s$ within which lie 95 percent of the variant. In the population, the dimensions of the minor axis of 95 percent of the virions lie within the range from 60.34 ($\bar{X} - 2s$) up to 89.78 mμ ($\bar{X} + 2s$): in other words, they occupy a range of 29.42 mμ. The corresponding figures for the major axis are 62.34 to 94.83 mμ, or a range of 32.44 mμ. In the case of the clones, by reason of their smaller scatter, the variant of the statistical sequence and the great homogeneity of the represented quantities, the range within which 95 percent of the virion axes may be found is a good deal shorter. Actually, the length of the given range for the clones varies between 15.66 (clone No. 6) and 19.82 mμ (clone No. 15) for the minor axis, and from 16.04 (clone No. 6) to 22.62 mμ (clone No. 5) for the major axis. The ranges embracing 95 percent of each of the separate clones line within an overall range which is characteristic of the population. This conclusion readily follows a comparison of the histograms of the distribution of virion axes in the population and in the different clones (See Figure 1).

Figure 2. Differences in the size of virions for various clones: A - clone No. 6; B - clone No. 3/5. Contrast 2% FVC. 200,000 X.

We determined whether there were differences in the size of the virions of various clones. The mean sizes of the minor and major axes of the eight clones studied, together with all data for determining the reliability of differences between the means, are given in Table 1 above. It is readily seen that the differences between the means, both for the major and for the minor axes of the virions of the clones studied, are statistically reliable, with the exception of clones Nos. 15 and 3/5; the confidence level is 0.02. This justifies the conclusion that all of the clones studied (except Nos. 15 and 3/5) differ among themselves as regards size of virion. Tentatively, we may distinguish three groups of clones: 1) clones with large virions having a major axis greater than 80 mμ; 2) clones with small virions having a major axis smaller than 75 mμ; and 3) clones with medium-size virions having a major axis of 75–80 mμ.

TABLE 2

Hereditary Preservation of Virion Dimensions in VEE Clones

Clone number	Passage	Number of measured virions (n)	Size of axis: minor axis x±s (mμ)	minor axis V(%)	major axis x±s (mμ)	major axis V(%)
5	1st	48	79.89±4.53	5.6	82.18±5.75	7.0
	3rd	56	78.89±4.76	6.0	83.05±4.73	5.6
6	1st	89	82.24±3.89	4.7	86.12±4.01	4.6
	3rd	93	80.15±3.65	3.7	85.83±4.51	5.2
8	1st	87	74.60±4.03	5.4	79.25±4.65	5.8
	3rd	76	73.84±3.92	5.3	78.44±4.86	6.2
12-8	1st	97	78.55±4.03	5.1	82.00±4.77	5.8
	3rd	100	79.20±4.42	5.5	84.90±5.04	5.9

An idea of the clonal differences as regards virion diameter can be obtained from the microphotographs shown in Figure 2 above.

Having established that genetically pure lines of VEE contain relatively homogeneous virions (as regards diameter), and that different lines differ precisely in virion diameter, we attempted to verify whether these characteristic properties are passed on to the descendants. The coefficient of variation characterizing the scatter of magnitudes of the major

and minor virion axes, remains practically unchanged following passing of the virus, for all four clones studied (Table 2). This justifies the conclusion that the relative homogeneity of virion size in genetically pure lines of the virus is maintained from generation to generation. The dimensions of the virions of clones Nos. 5 and 8 remained unchanged after three passages. Differences between diameters, both of the major and the minor axis, in these clones are statistically insignificant before and following passing.

The data relating to clones Nos. 6 and 12-8 are somewhat difficult to interpret. The sizes of the major axes of the virions of clone No. 6 before and after passing are the same; those of the minor axes, however, differ (although only slightly) with a confidence level of 0.05. In the case of clone No. 12-8, on the contrary, it is the size of the minor axes which is preserved, while that of the major axes varies. True, these discrepancies would preclude any categorical affirmation of the inheritability of virion size in the case of clones Nos. 6 and 12-8; but the assumption remains perfectly tenable. Actually, despite the slight variation in one of the two parameters by means of which the estimate of virion size was made, the two clones following passing remained within the same group (those with large virions more than 80 mμ in diameter).

The correlation between the biological properties of the virus and the virion diameter will be the subject of a subsequent special communication.

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