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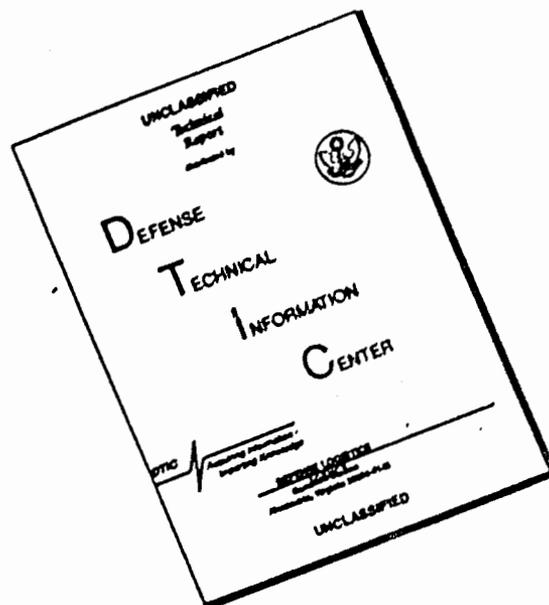
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## Anatomy of Venezuelan Equine Encephalomyelitis Virus

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Virions of Venezuelan equine encephalomyelitis virus purified by gradient centrifugation procedure were disrupted with Tween and ether and then centrifuged in equilibrium cesium chloride density gradient. Fine structures of the resulting fractions of hemagglutinin (HA) and ribonucleoprotein (RNP) were examined in the electron microscope. The HA had a shape of a hollow cylinder 55 - 60 Å long and 45 - 50 Å in diameter. RNP looked like a strand 15 - 17 Å thick.

It is assumed that RNP is packed within the virion in such a way that loops of the external part of the nucleoid interact with each other like capsomers in the cubic type of symmetry thereby determining the quasi-icosahedral form of nucleoids.

The morphogenesis of the virus of Venezuelan encephalomyelitis (equine) - the VEE virus - (2), and several physical properties of separate components of the virus (1) were described earlier. The method of balanced centrifuging in a density gradient of cesium chloride (CsCl), used in the last work turned out to be convenient for the receipt of fractions of highly purified virus and separation of the subviral structure, the building of which can be studied by using the electron microscope.

In this work we introduce information about the relatively fine structure of hemagglutinin (HA) and ribonucleoproteins (RNP) of the VEE virus and we discuss the architecture of virions.

## Methods and Materials

Virus and cells. All the experiments were conducted with the SPF strain of the VEE virus which was grown in a primary culture of chick fibroblasts. The biological properties of the virus and the cultivation methods were described earlier (5).

## Purification and fractionalization of the virus in a balanced density gradient of cesium chloride

The virus yield was gathered in 18 to 20 hours after the cells were infected. The culture liquid underwent preliminary centrifuging at 5000 g for 20 minutes, and the virus was separated out in a carbon rotor 8 X 50 centrifuge MSE superspeed-50 at 80,000 g for 2 hours and then purified (1). The virus was resuspended in 0,01 M of tris-HCl buffer pH 7,4 with 0,33% of crystalline bovine serum albumin. The material in a volume of 0,5 ml. was superposed on a linear gradient CsCl (4,5 ml) with a thickness of from 1,15 to 1,50 g/cm<sup>3</sup> and was centrifuged in a bucket-rotor 3X5 centrifuge Spinco L2 at 45,000 revs/min for 3 hours. The virus formed a clear and visible stripe, clear to the naked eye. The layer of gradient over the strip was carefully drawn off with a pasteur pipet after which we carefully gathered the virus-containing layer.

For receiving the HA and the RNP, the virus suspension was mixed with three volumes of ether and 5 mg/ml of tween-80, shaken intensely for 20

minutes, and then centrifuged at 2000 g for 10 minutes. The upper layer was removed, the water phase of the material gathered and freed from the ether by blowing through with nitrogen. This material was superposed on a density gradient of cesium chloride and centrifuged as described above. The material which had HA activity was distributed diffusely in the upper part of the gradient, and the RNP - formed a clear stripe in the middle part of the gradient. The refraction indicator of the fractions of the density gradient was determined on the refractometer and then the density calculated.

#### Electron microscope

The fractions of the gradient were immediately put on a formvar backing, stained with 1 - 3% water solution of Uranyl acetate or 2% of Phospho-tungstenic acid and were studied in an electron microscope JEM-7 with an instrumental enlargement of X50,000, X70,000 and X120,000. In several experiments we used ultrafine cuts of the infected cells. The methods of cultivation of the virus and the preparation of ultra fine cuts were described earlier (2).

#### Results

**Virions.** The VEE virions after balanced centrifuging in a density gradient of cesium chloride were distributed in a zone with a thickness of  $1,25 \text{ g/cm}^3$ . In the electron microscope, the negatively contrast stained material in this zone could be seen to contain virions of spherical or polygonal form with an outer diameter of 35 - 50 m $\mu$  (sketch 1, a). In several cases it was possible to see the inner structure of the virions: a casing of a thickness of 8 - 10 m $\mu$  and a nucleoid with a diameter of 25 - 30 m $\mu$  (sketch 1, a; see sketch r, a, b, c). Sometimes we met polygenomic virions which contained two and more nucleoids under one general casing (sketch 4, d).

**Hemagglutinins (HA).** After the destruction of the virus with tween and ether and the separating out of the separate components of the virus by the method of balanced centrifuging, the HA occupied a diffuse zone with a density of from  $1,15$  to  $1,18 \text{ g/cm}^3$ . In sketch 1, b is shown a high step of purification of an HA preparation in a density gradient of cesium chloride. Under great magnification it is possible to distinguish the supramolecular structure HA particles (sketch 2). Its outer appearance reminds one of a cylinder with a length of 55 - 60  $\text{\AA}$  and a diameter of 45 - 50  $\text{\AA}$ . From the side (projection parallel to axis of the cylinder) the particles have the appearance of hollow tubes, the walls of which are built of two (sketch 2d) or three (sketch 2, a, b) granules. From the end (projection perpendicular axis of the cylinder) they look like a hollow multifacet (sketch 2, f, g). On the basis of the electron microphotography (sketch 2, a, b, d, f, h) the proffered model was built of the HA (sketch 2, c, d, h). An analysis of the microphotography partially (sketch 2, i, j) and in full (sketch 2, m) of the "uncontorted" structures of HA allows the proposition that they are formed from rods 350 - 400  $\text{\AA}$  in length and about 20  $\text{\AA}$  in thickness, as is indicated on the proposed models (sketches 2, c, e, h, k, m). The cylindrical and partially contorted structures of HA are clearer on the surface of the virion in peripheral (sketch 2, n) and central (sketch 2, o q) areas. About 1/3 of the HA particles are "sunken" in the lipid part of the casing of the virions (see sketch 2, n) as is shown in the model (sketch 2, r, s).

The free HA in concentrated preparations aggregates in structures upon end to end contact (see sketch 2,d), side to side contact (sketch 3, a,b) and end to side (sketch 3,c), the models of which are shown in sketch 3, e, f. Such aggregates and paracrystalline forms of HA are clear as well on the surfaces of virions (sketch 3, d).

The architecture of the nucleoid. The nucleoids inside the virions (sketch a,c) or in the cytoplasm of the infected cells before inclusion in the virions have penta- or hexagonal shapes which is characteristic for the icosahedron.

A more detailed study of the surface and inner structure of the nucleoids reveals in the central part of the nucleoid a cavity of a 40 - 80 Å. Eight threaded strands of a thickness of 15 Å, appearing in the nucleoid, form a spiral-shaped structure (sketch 4, h-m), the outer part of whose loops touch the surface of the nucleoid (sketch 4, i, l), (sketch 5, a - c).

The spindle-like (or filament-like) component of the nucleoid can be freed from the virion which spontaneously breaks down during storage, during treatment of the virus with a weakly alkaline solution (pH 8,5), or as a result of osmotic shock. In sketch 6, is shown a picture of the breakdown of the virion and the separation of the filamentose inner component.

Ribonucleoproteid. The RNP of the virus, received as described above, had a buoyant density of 1,42 - 1,43 g/cm<sup>3</sup>. In the electron microphotographs of material from this zone, it is possible to see an accumulation of spindle-shaped structures. During dilution of the material or in that case when for the receipt of fractions we used a small quantity of virus, there appeared separate strands (sketch 7, a, b). These strands had a diameter of about 15 - 17 Å and a maximum length of 6000 Å, although this last is not an absolute limit. The strands, as shown in sketch 7, are made up of very fine granules, distributed in a bead-like way on the thread. The RNP strands separated out of the broken-down virions with tween ether, are morphologically identical to those freed from the virions (see sketch 6) by other actions and they remind one of the eight-stranded structures in the nucleoids of the virions.

#### Discussion

The figures allow a definition of several peculiarities of the structure of subviral components and to list several considerations relative to the anatomy of the VEE virions.

Centrifuging the virions and the subvirus components in density gradient of cesium chloride insures a high degree of purification of the virus and the division of the broken down virus into two components: HA and RNP which differ from the buoyant density (1): it is possible to make a detailed study of the structure of purified and concentrated subvirus components using the methods of the electron microscope.

It was shown that the inner component of the virions - RNP - had the appearance of a strand with a periodic structure. Inside the virion, the RNP strand, apparently, has a spiral packing. An analysis of electron microscope data permits proposing a model of the RNP packing inside the

virion. It is supposed that the tops of the loops of the deformed RNP spirals interact one with another similar to capsomers in a cubic type of symmetry (sketch 8). In such a case, the upper parts of the loops form a three-cornered facet on the surface of the virion (sketch 8, b, d), bordering the quasi-icosahedral structure (see sketch 5, sketch 9). The most probable type of packing of the RNP strands is presented in sketch 8, although it is possible that there is another way of packing (sketch 8, a, b). Such an interpretation of the data received corresponds more to the propositions of Simpson and Hauser (1) than to the recently proposed model of Horzinek and Mussgay (3).

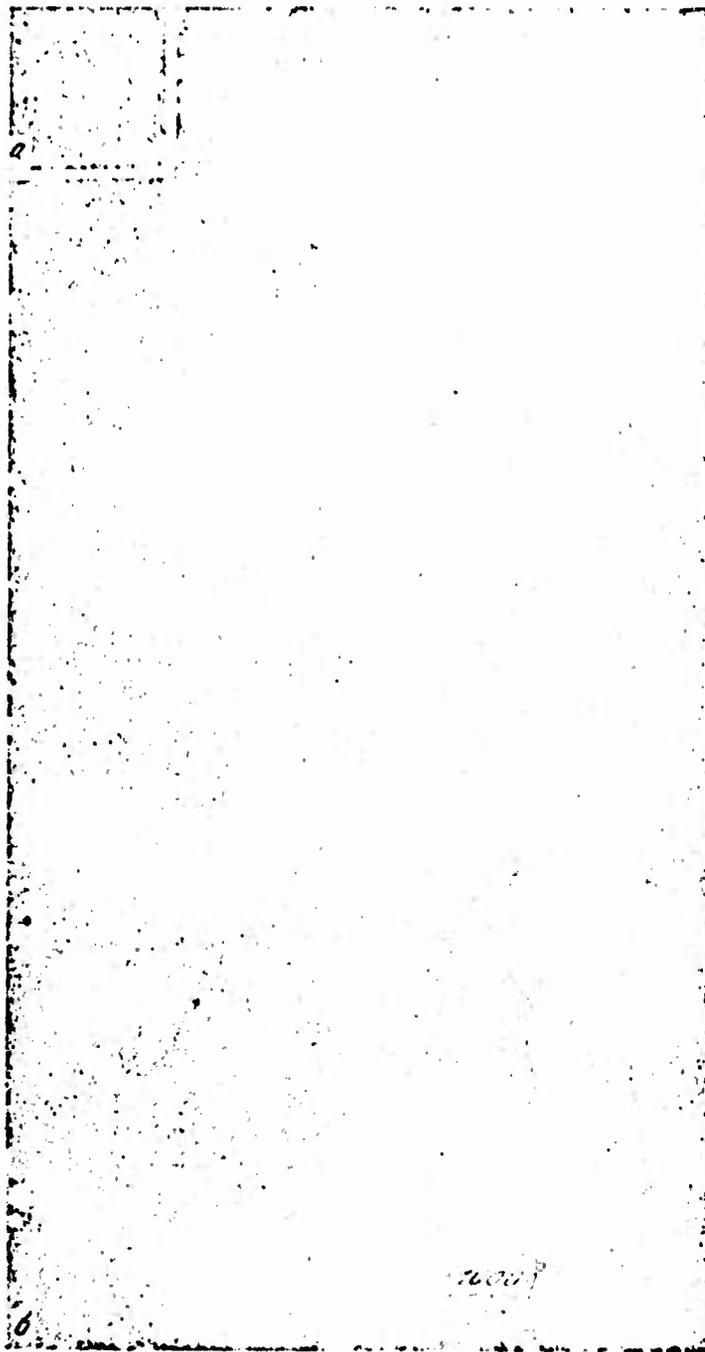
The proposed model of the RNP packing in the nucleoid has the purpose of explaining by what manner the RNP spirals can insure the icosahedral form of the nucleoid. According to this hypothesis, the free parts of the RNP loops which appear on the surface of the nucleoid, interact as separate capsomers. However they differ from capsomers in that in the first place, RNP, not protein is represented, and in the second place, they are not separate structures, but parts of the strand. The tops of the nucleoid in this case is formed by 5 loops, and the side facets by 6 loops. The general quantity of quasi-capsomers on the nucleoid surface is 120.

HA is represented by spindle-like formations which can have the appearance of a cylinder with a height of 55 - 60A, or a structure of 120 - 140 A in length. One third of the HA is sunk in basal membrane surrounding the nucleoid and consisting of lipids and proteins.

The virion model, constructed on the basis of the data introduced and on the basis of discussions, is shown in sketch 9.

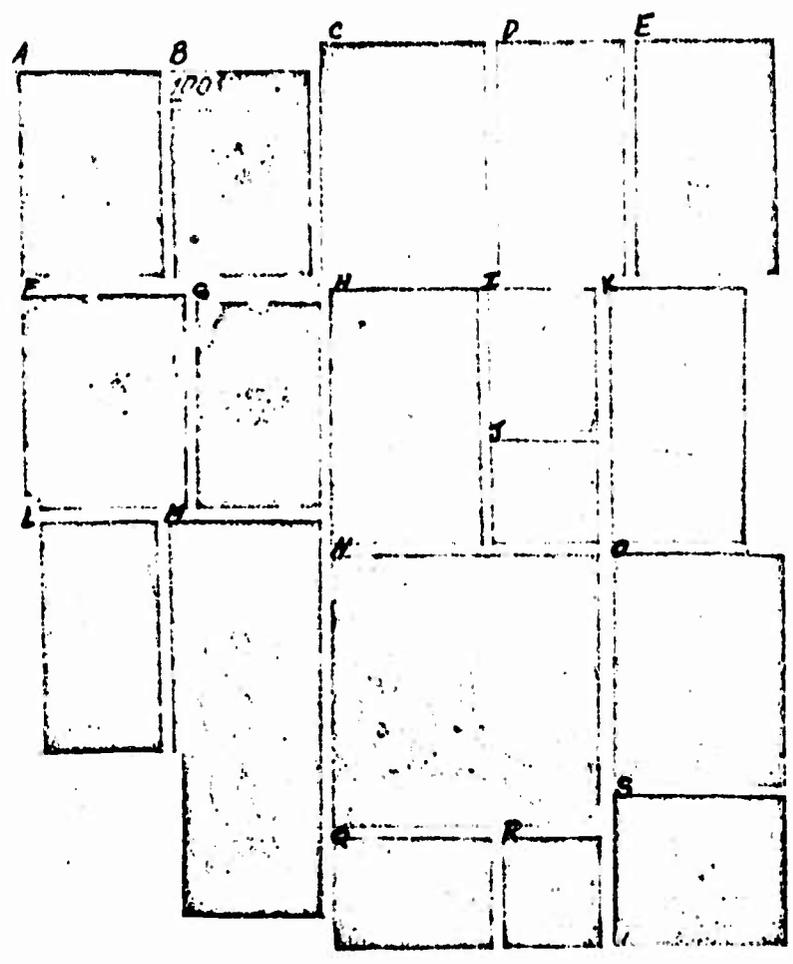
#### Literature

1. Uryvaev L.V., Zdanov V.M., Ershov F.I. and others; *Quest. Vir.*, 1970, No. 3, p. 330.
2. Bykovsky A.F., Ershov F.I., Zhdanov V.M.; *J. Virol.*, 1969, v. 4, p. 496.
3. Horzinek M., Mussgay M.; *Ibid.*, p. 514.
4. Simpson R.W., Hauser R.E.; *Virology*, 1968, v. 34, p. 358.
5. Zhdanov V.M., Ershov F.I., Uryvaev L.V.; *Ibid.*, 1969, v. 38, p. 355.



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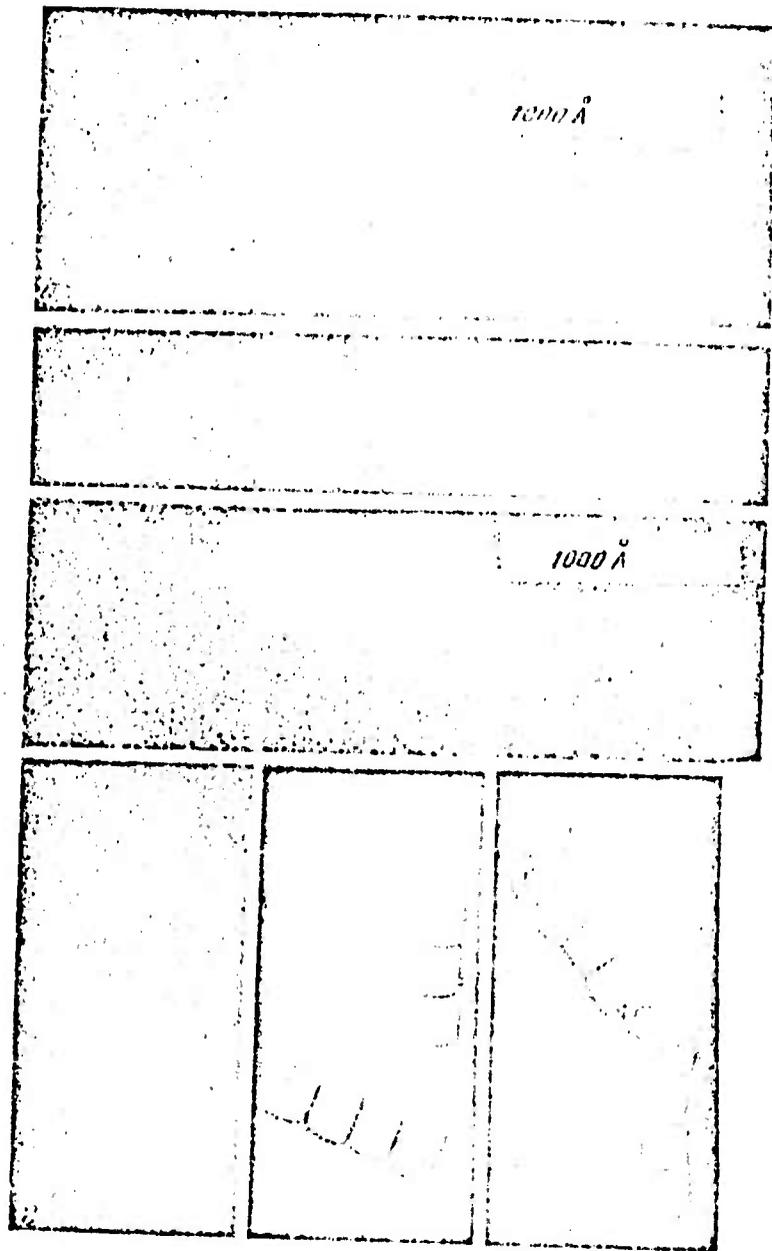
Sketch 1. VEE virions from the zone of cesium chloride gradient with a density of  $1,25 \text{ g/cm}^3$  (a) and material having hemagglutinating activity with a density of  $1,18 \text{ g/cm}^3$  (b).  
Negative staining.



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Sketch 2. Building of VEE hemagglutinin  
 Negative contrast staining a - e; HA particles (side view - abd) and proposed model (b,e); (the separate granules are indicated with arrows); f - h: frontal depiction of HA (f, h) and model (h); contorted structure of HA, side view (i) and at the end (j), model (side view - k); l, m: untwisted structures (l) and model (m); n - peripheral zone of virion casing with HA on surface; side view; arrow indicated untwisted structure of HA; q-s: central zone of the virion, particles of HA in the frontal projection; o,s: model of the untwisted (o) and contracted (s) HA structure.

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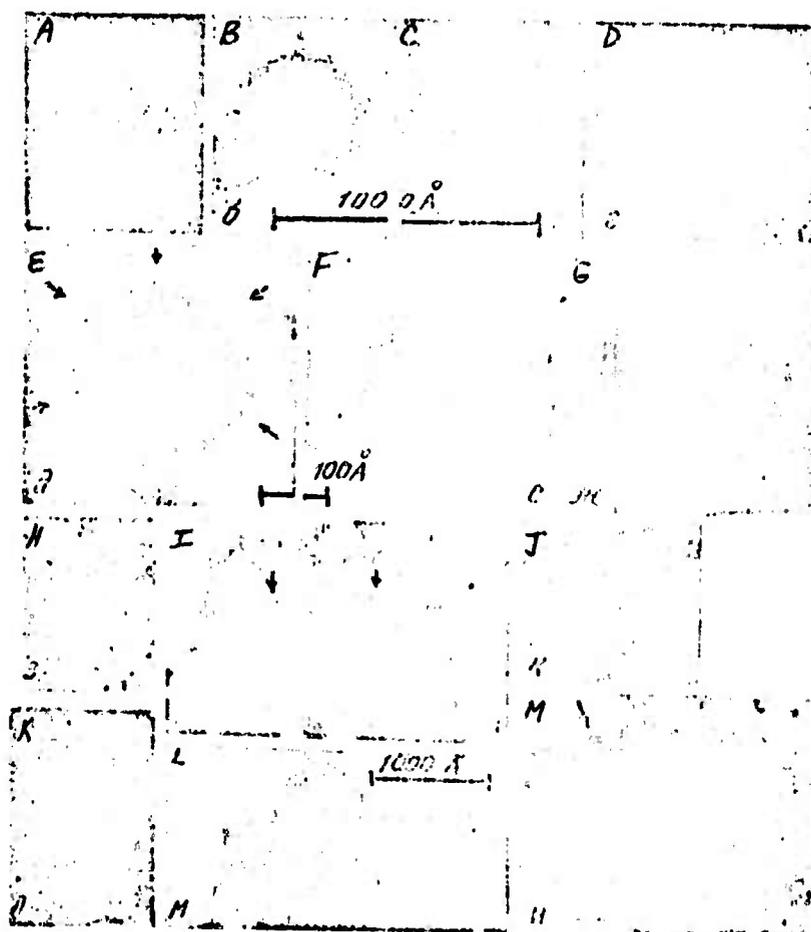


Sketch 3. Aggregation of HA particles.  
 Negative contrast staining. a,b - side to side; c - side to side, end to end, end to side; d - paracrystalline aggregation of HA particles on the virion surface; e,f - model of the aggregation side to side and end to end, and a mixed variant (f).

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## Sketch 4. Nucleoid structure

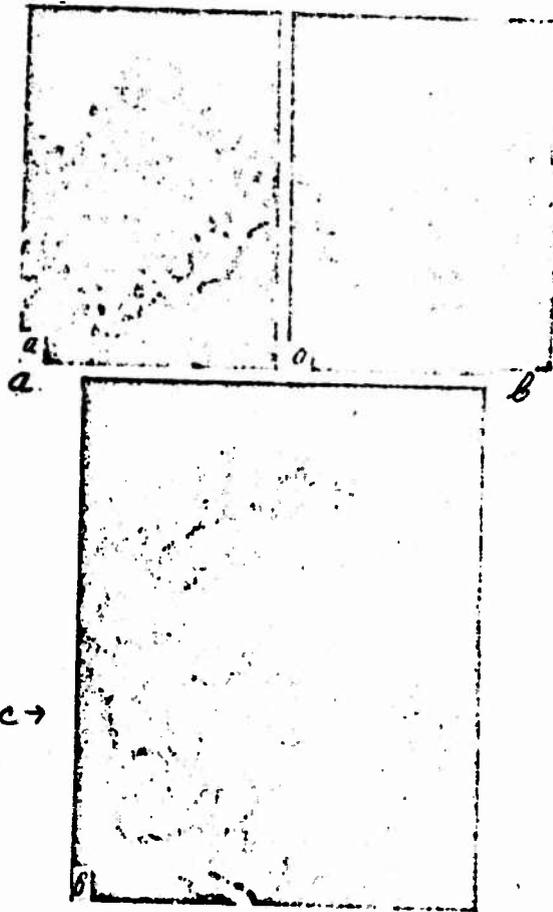
a, b, c - virions with icosahedral nucleoids, negatively stained (a), positively contrasted (b) and an ultra fine cutting (c); d - virion with two nucleoids, negatively contrasted; e, f, g - intracellular nucleoids with hexagonal (e) and pentagonal forms, ultrafine cutting; h-m - eight-stranded spiral - like threads with a thickness of  $15 \text{ \AA}$ , in the whole virion (h, i, j) and separate parts (i, l); h - k - ultrafine cutting perpendicular to the surface of the virion; l - m - ultrafine cutting, tangential to the surface of the virion. The arrows show the loops of the eight stranded threads.



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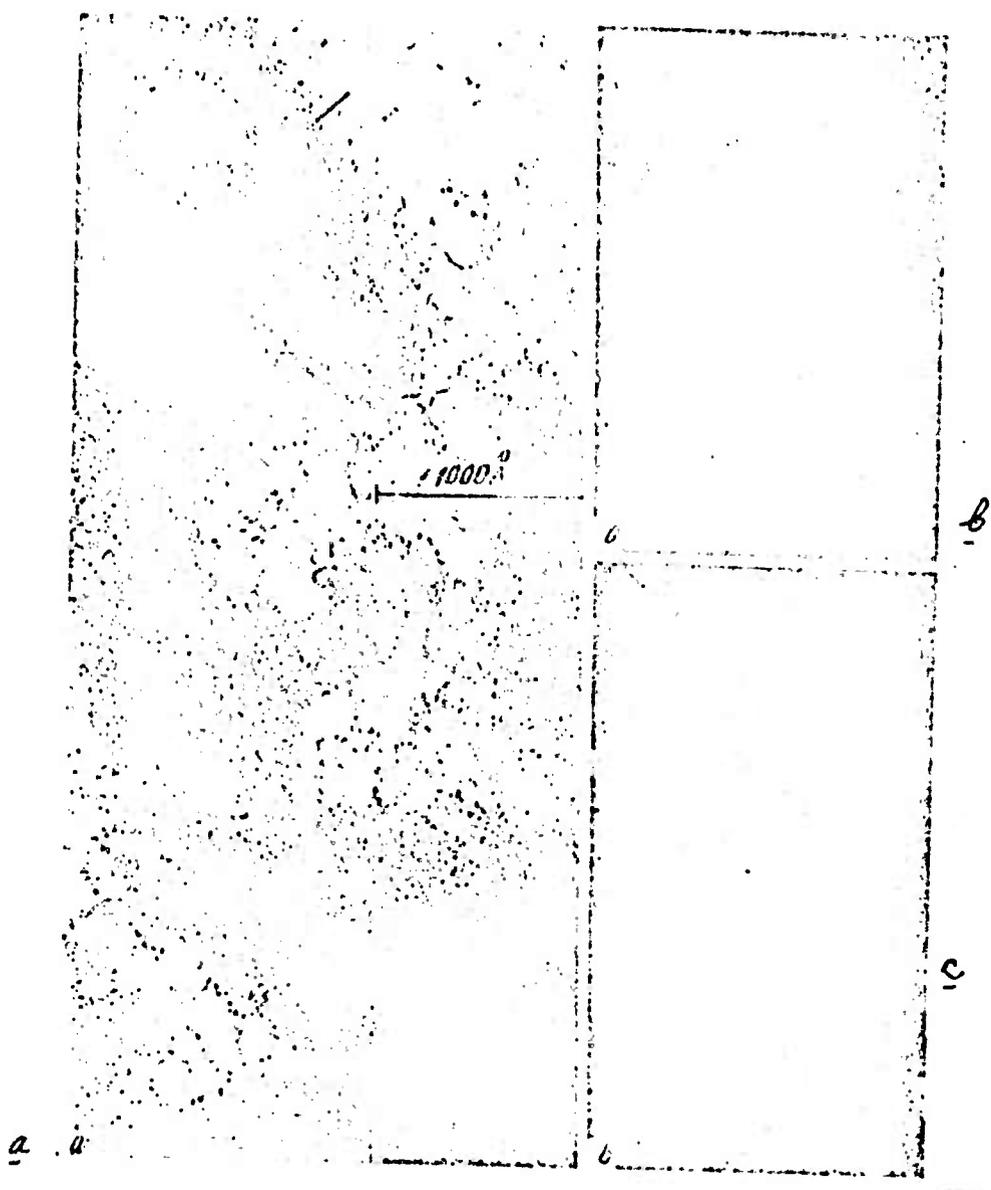
Sketch 5. Surface of the nucleoid.

Negative coloring of uranyl-acetate (a, b) and phosphorotungsten acid (c). The loops (a, b) or outer parts of the loops have a diameter of 15 - 17 Å, as expressed on the surface of the virions (shown by the arrows).



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Sketch 6. Liberation of the inner component of the VEE virions after treatment with a weak alkaline solution (pH 8,5). Negative contrasting; a, b - separation of RNP from the virions; below - partially untwisted (a) and contorted (b) RNP (shown by arrows); c - area of the untwisted RNP.

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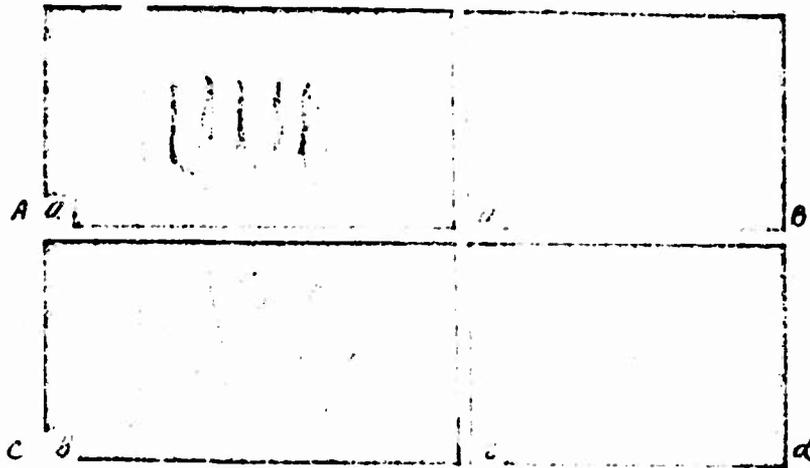
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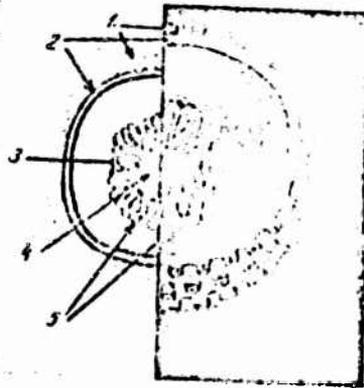
Sketch 7. Ribonucleoprotein of the VEE virus.  
 Negative contrast. a - uncoiled thread of RNP (arrows);  
 b - fragment of untwisted RNP.

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Sketch 8. Proposed model of the RNP packing of the inside of the virion. View from the side (a, c) and in a frontal projection (b, d);- a, b - sinusoidal type of packing; c, d - spiral type of packing



Sketch 9. Model of VEE virion. Ultrafine cutting (left) and negative contrast (right). 1 - HA; 2 - basal membrane; 3 - nucleoid; 4 - strip of the inside of the nucleoid; 5 - top of the loops emerging on the surface of the nucleoid.



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