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## TECHNICAL TRANSLATION

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ENGLISH TITLE: Some Results of Investigating "Marburg Virus" (Rhabdovirus simiae)

FOREIGN TITLE:

AUTHOR:

Z. V. Shevtsova

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## NOTICE

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In 1967 in West Germany and Yugoslavia appeared a previously unknown infectious disease, which received the name of "Marburg" (Cercopithic hemorrhagic fever). It was characterized by fever, exanthema, hemorrhaging. destruction of the liver, kidneys and intestines, blood changes and a high degree of lethality; there were seven deaths out of 31 cases, (2,3,11,14,34). The infection appeared after contact with the blood and organs of the green monkey <u>Cercopithecus acthiops</u> (6 7), but there was no opportunity to observe the course of spontaneous infection in the animal. The monkeys were used for preparing cell cultures after they had been brought from Uganda, until the appearance of clinical symptoms. Only experimental reproduction of the disease in green monkeys and rhesus mankeys was described in detail (4, 5, 20, 21).

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In the study of the etiology of this infection, in addition to ferman scientists there was participation by representatives of various countries (England, U.S.A., USSR, Yugoslavia, Austria and others). In material obtained from sick or dead persons, attempts to find some type of microorganism were made, but no bacteria, prozoa or rickettsia were found (15, 19, 23).

The agent of Marburg disease was isolated from guinea pigs in 1967 almost simultaneously in several laboratories (1, 15, 19, 23, Stoykovich and Bordzhoski cit. 1). In guinea pigs infected with primary material

(blood and organs of sick and dead humans), temperature rose after four to ten days of incubation. The febrile period lasted for six days, after which the animals recovered. With passaging the incubation period decreased to three days, and on the eighth or ninth day and animals died. Therapy with tetracycline antibiotics was ineffective (23). The concentration of agent by the third passage reached  $10^6$  infectious doses per milliliter. In the dead or killed guinea pigs a characteristic morphological changes were discovered: hemorrhages and infection of the lymph nodes and reticulo-endothelial system. The greatest damage was observed in the liver, spleen, and lungs. In the liver along with degerative changes in the sells of the parenchyma, cytoplasmic inclusions and extracellular distribution of granules of verious sizes were discovered (19, 22). Their specificity was verified by the immunofluorescent method (19, 22) with the use of sera from immunized guinea pigs and humans who had recovered from the disease. A direct relationship was established between the number of passages, degree of damage to the organs, concentration of agent and increase in amount of specific antigen revealed by the immunofluorescent method. The largest amount of agent was observed in the liver, and then (in diminishing quantities) - in the spleen, ovaries, abdominal cavity exudatse and lungs (22) The etiological connection between human infection and experimental infection of guinea pigs was shown in neutralization tests as well as in the immunofluorescent reation. Newborn hamsters displayed insensitivity to innoculation with material from ill humans, but became sick after intake of material which had been passaged more than five times in guinea pigs (19).

In two-day old and adult white mice signs of infection were not observes upon the introduction of material from humans or material passaged in guinea pigs (in the brain and abdominal cavity) (19), however in the

brains of newborn mice there appeared latent reproduction of virus (8, 13). Material which had undergone 1, 3, 7, and 12 passages in newborn white mice caused in guinea pigs characteristic nonlethal pathology. Upon further passaging in guinea pigs the pathogenicity of the virus for the given species of animal was re-established. The identity of the virus, isolated from mice, was shown in neutralization tests of guinea pigt using serum from humans who had recovered from the disease (13). The virus did not multiply in chick embryoes (19).

In experiments with 18-cell cultures it was establiched that replication of the virus in most of these was not accompanied by cytopathogenic changes: of the first cultures of green monkey kidney cells, rhesus monkey kidneys, human amnion, chick fibroblasts, guinea pig fibroblasts and embrion or in cultures of Sots cells, Vero, and LLC-MK<sub>2</sub> (18, 19, 23, 9, 10). Most sensitive to virus were primary and subinoculated (GMK-AH-1 and Vero) cells of green monkey kidney, and also cell lines from kidneys of newborn ham**p**sters (BHK-21). The multiplication of virus im primary tripsinized green monkey cells and in Vero cells was not accompanied by visible changes, but was verified by the immunofluorescent method (18). In GMK-AN-1 cells latent replication of virus ( $10^6 \text{ LD}^{50}/\text{ml}$ ) was indicated by means of infecting guinea pigs (9). A cytopathogenic effect was noted after three passages of virus in these cells (10).

Information on agent replication in BNK-21 cells is presented in a number of reports. Some authors reported only virus multiplication in guinea pigs (9, 23), whereas in other experiments the multiplication was accompanied by cytopathogenic changes (10, 25, 26). The time required for the appearance of changes depended on the nature of the virus-containing material (25, 26). After innoculation with material obtained from sick monkeys,

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inclusions and an explicit cytopathogenic effect appeared carlier than after introduction of material, obtained from guinea pigs the emination of two-clones of BHK-21 cells revealed that clone CCL-10 was more sensitive than W12(10). In cells of clone CCL-10 after three passages there appeared a marked cytopathic effect, and the virus titer after seven passages reached  $10^{6.5}$ CPC<sup>50</sup>/ml. Data on the replication of viruses in HeLa and L cells is contradictory (9, 15, 18, 23). In MA-104 and CB<sup>44</sup> cell lines the agent did not multiply (26).

The virus is sensitive to ether and sodium deoxycholate (10, 13, 71, 19); heating to 56 degrees for 30 minutes significantly lowered the infectious titer (10), and the virions contain RNA (10). The diameter of the particles was determined by filtration through membrane filters: 450 to 1,000 (15) and 340 to 790 millimicrons (23); according to electron microscopy data, the length of the particles was 10,000 Å (10, 13, 15,16, 19, 27). The virus multiples in Aedeasegypti mosquitoes. After two passages in mosquitoes indentification was made by means of infecting guinea pigs and the immunofluorescent method (12).

Sera were taken from convalescent humans and guinea pigs and also from immunized guinea pigs for serological study (13, 17, 19). Antibody titers in the complement-fixation reaction were 1:32 to 1:64 (8, 19, 23). In human sera the antibody titer rose from second to the twenty-first to twentyeighth day of illenss (8, 19). Immunesera were used for determining viralantigen in organs and tissues of humans and guinea pigs (18, 22). Data obtained in the complement --fixation and immunofluorescent reactions agreed as a rule; some of the sera with antibody titer in complement--fixation reaction of 1:64 displayed weak virus neutralizing properties. This agrees with the observation of Shop, who studied representatives of the

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Takaribe group (8).

To establish the serological relationship between the agent studied and any known viruses, many investigations were carried out with the participation of researchers in a number of countries (Enders-Ruckle, Casals, Clark, Downs, Johnson, Gear, McIntosh, Kunz, de Roever-Bonnet and co-authors) (18). Human and guinea pig immune sera were used in complement--fixation and hemagglutination inhibition reactions with viruses of the following groups: vesicular stomatitis, herpes, ornithoses, cytomegaly, influenza, measles, ubella, mumps, enteroviruses, arbolvirus groups A, B, and C and numerous ungrouped arboviruses isolated in Africa, Asia, America and Australia, but serological relationship between the Marburg agent and the viruses examined was not discovered (18).

Determinative data was obtained by means of electron-microscopic study of material taken from experimentally infected guinea pigs and monkeys (blood, liver, and spleen preparations). Photographs revealed bacilliform particles, in the shape of loops or hooks, often with a bulboid thickening at one end (13, 19, 27). The dimensions of the particles varied significantly, but their average length varied from 7,000 to 10,000 Å and the diameter measured from 500 to 650 Å. More often they were distributed in the cytoplasm, less often extracelluarly (13). Similar structures were found in BHK-21 cells (27) and in ultracentrafuged culture fluid, obtained after infection of these cells (10). After studying the pecularities of particle structure, the researchers make the conclusion that they are similar to Stomatoviridae of Rhabdovieren, but differ from these viruses in particle length and variability (16).

Sensitivity to ether, sodium deoxycholate and temperature; the RNAcontaining structure, its multiplication in the brain of newborn mice, in

cell cultures sensitive to arboviruses and in insects, characterize the agent as an arboyirus. Morphologically it is similar to khabdovieren. However, the results of serological study show that there is no antigen similarity to known arboviruses. Kunz and co-authors have suggested that the "Marburg Virus" be termed the Rhabdovirus simise (12).

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