PENETRATION OF THE ŢAHYŇA VIRUS TO VARIOUS ORGANS OF THE AEDES VEXANS MOSQUITO

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Abstract. The paper deals with the penetration of the Ţahyňa virus to various organs of the Aedes vexans mosquito in the period between 3 and 15 days. The virus was recorded in all examined organs: oesophagus with dorsal and ventral diverticules, ventriculus with intestine, lymph, salivary glands, ovaries, Malpighian tubules and ganglia. The highest amount of virus was detected in ventriculus with intestine, the lowest in the Malpighian tubules.

Our previous studies on the multiplication dynamics of the Ţahyňa virus in individual body parts (head, thorax, abdomen, legs) of the Aedes vexans mosquito (DANIELOVÁ 1962) revealed that the dynamics of virus multiplication in individual body parts was different and therefore we tried to obtain a more detailed information on virus penetration directly to individual organs. We examined the salivary glands, oesophagus with ventral and dorsal diverticules, ventriculus with intestine, ovaries, Malpighian tubules, ganglia and lymph for the period of 15 days following ingestion of the virus by the mosquitoes.

MATERIAL AND METHODS

Virus. In two experiments the neural strain "181" was used in 24th and 26th intracerebral passage in mice and in one experiment the extraneurally passed strain "236" was used in 8th passage. The adult Aedes vexans mosquitoes were obtained from the locality at Žitný ostrov in southern Slovakia shortly after hatching. In contrast with the previous studies (DANIELOVÁ 1962) where the temperature had been between 22 °C and 27 °C, this time mosquitoes were kept at laboratory temperature not lower than 25 °C and at relative humidity between 80 – 90 per cent. The mosquitoes became infected by feeding on infectious suspension. In experiments with the strain "181" the mixture was made from 20 per cent of brain suspension, in 10 per cent horse serum saline pH 7.4 with 100 units PNC and STM in 1 ml, defibrinized blood of quinea pig and glucose. In the experiment with the strain "236", which had also been used in the previous studies (DANIELOVÁ 1962), the infectious mixture was made from 3 parts of defibrinized viremic blood of hamsters and 1 part of 25 per cent glucose solution in saline.
The virus in the organs was demonstrated by isolation tests in suckling mice. The brains of the perished mice were examined bacteriologically and also passed through suckling mice.

The dissection of mosquitoes was carried out under sterile conditions and in cooled environment. The mosquitoes were killed on dry ice. First their wings and legs were detached, then their bodies were briefly immersed into 70 per cent ethylalcohol and washed in saline solution pH 7.4. The individual organs were prepared separately in fresh saline solutions. After successive washing in 10 drops of saline the same organ taken from each of 5 mosquitoes was placed in one test tube filled with 0.02 ml 10 per cent horse serum in saline pH 7.4 with 1,000 units ATB in 1 ml. After the appropriate organs were removed, the remaining body parts of each mosquito were placed in separate test tubes. After dissection of all five mosquitoes suspensions were made from individual organs by grinding the latter with a chilled rod in a mortar with 1 ml of 10 per cent horse serum. The suspensions made from organs were centrifuged for 2 minutes at 2,000 r. p. m., the suspensions made from the remaining body parts of mosquito were centrifuged for 5 minutes at the same speed. The supernatant was then inoculated into each of a litter of 2 to 3 day-old suckling mice.

RESULTS

Altogether 3 experiments were conducted. The first one served as a preliminary test for determining the approximate interval of virus penetration to individual organs. In the second experiment the virus penetration to organs was traced in 2 day intervals. Finally, the third experiment served for comparing the penetration of extra-neural strain "236". The results are given in Table 1.

Table 1. Penetration of Ťahyňa Virus to various organs of Aedes vexans mosquito

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Strain and titer of the virus in log L.D₅₀</th>
<th>Incubation period in days</th>
<th>Organs</th>
<th>Remainders of the body of individual mosquitoes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salivary glands</td>
<td>Oesophagus with diverticules</td>
</tr>
<tr>
<td>1 &quot;181&quot; 4.25</td>
<td>3</td>
<td>0/10</td>
<td>6/10</td>
<td>2/9</td>
</tr>
<tr>
<td>2 &quot;181&quot; 5.16</td>
<td>8</td>
<td>0/9</td>
<td>0/7</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0/8</td>
<td>0/10</td>
<td>3/8</td>
</tr>
<tr>
<td>3 &quot;236&quot; 3.50</td>
<td>5</td>
<td>0/10</td>
<td>8/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0/10</td>
<td>6/9</td>
<td>10/10</td>
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</tbody>
</table>

† Number of suckling mice dead/inoculated.
It was ascertained that virus penetrated to salivary glands as early as on 4th day after infection; however, the virus was not always detected there, even though it was present in the lymph and other organs, as evident from 8th day of the second experiment. In this way the lower percentage of transmission rate than that of infection rate can be explained (DANIELOVÁ 1966).

In both parts of the digestive tract virus was detected between 3rd and 15th day after acquisition of virus. In suspensions made from oesophagus with diverticiles a lower amount of virus was present, while on 13th day of the first experiment and on 10th day of the second experiment none was detected there. Consequently, virus multiplication does not always occur in this part. On the contrary, the accumulation and multiplication of virus in the ventriculus with intestine is a pre-requisite for virus penetration to other parts of the body, as indicated by 8th day of the first experiment, when virus was absent in this part of the digestive system as well as in other parts of the body.

The penetration of virus to the lymph was also demonstrated on 4th day and on all following days. The results of the isolation tests from the remaining body parts of individual mosquitoes indicate that virus penetration from the intestine to the lymph does not occur in all mosquitoes.

The penetration of virus to ovaries and Malpighian tubules was demonstrated at the same time: on 8th day of the second experiment, on 5th day of the third experiment following the acquisition of virus by the mosquito. At the shorter interval of the latter experiment the organs were not examined. A low amount of virus was always found in Malpighian tubules, while in ovaries the amount of virus was relatively high.

The presence of virus in the nervous system was confirmed by a single test from pharyngeal and abdominal ganglia, when a low amount of virus was detected there on 13th day of the third experiment.

The result on 10th day of the second experiment indicates that multiplication and penetration of virus to organs may be very slow sometimes.

**DISCUSSION**

It is evident from the mentioned results that the Ťahyňa virus remains fixed in the digestive tract of mosquito even after it is released to the lymph and later penetrates to other organs. From the lymph the virus first reaches salivary glands and only later penetrates to ovaries and to Malpighian tubules. If our results are compared with those obtained by LA MOTTE (1960) in his experiments on the penetration of the Japanese B encephalitis virus to organs of *Culex pipiens*, it becomes evident that the Ťahyňa virus penetrates to organs of *Aedes vexans* more quickly. This fact was also established by MCLEAN (1955) in his studies on multiplication of the Murray Valley virus in mosquitoes *Culex annulirostris*.

If we compare the experiments No. 2 and No. 3, we see that on 6th day of No. 2
experiment the amount of virus in organs was lower than that in the organs on 5th
day of No. 3 experiment, when the virus was detected in a single mosquito, although
in No. 2 experiment the mosquitoes fed on food by 2 log LD₅₀ richer in virus.
On 5th day of No. 3 experiment virus was demonstrated in ovaries and Malpighian
tubules, while in No. 2 experiment it was detected in these organs as late as on 8th
day. In harmony with the results obtained by SUDIA (1959) who established the
fact that virus multiplication in mosquitoes was much faster after the ingestion of
a larger dose of virus by the mosquito, the virus multiplication in No. 2 experiment
should have been higher. The neurally passed strain of virus in the mosquito body
seems to be less capable of multiplication than the extraneurally passed strain.
After titration of the infectious tampons and mosquitoes which had fed on them, it
was established that the mosquitoes contain almost 3.5 log LD₅₀ less virus than does
the titre of the tampons. When extraneurally passed strain is used at the same titra-
tion the difference is only 1.5—2.0 log LD₅₀ of virus. Consequently, the mosquitoes
ingest a lower amount of virus from the infectious mixture containing the virus
in brain suspension than from the infectious mixture including virus directly in
blood. However, even in this case the mosquitoes in No. 2 experiment contained
a higher amount of virus after feeding. It is therefore evident, that in order to simu-
late conditions in nature, it is necessary to infect the mosquitoes by using the infec-
tious mixture made from viremic blood, or by using an infected host animal as stated
by CHAMBERLAIN, SIKES, NELSON and SUDIA (1954).

CONCLUSIONS

The purpose of this study was to determine the Tahyña virus penetration to organs
of the Aedes vexans mosquito between 3—15 days after acquisition of virus. Oesoph-
agus with ventral and dorsal diverticles, ventriculus with intestine, lymph, salivary
glands, ovaries and Malpighian tubules were assayed for virus concentration. In
one case ganglia were examined. Virus was detected in all organs examined: first
in both parts of the digestive tract (oesophagus with diverticles and ventriculus with
intestine), then in salivary glands and lymph and finally in ovaries and Malpighian
tubules. In experiments with neurally passed strain “181” the virus penetration to
individual organs was detected in the course of 8 days. In the experiment with
extraneurally passed strain virus was detected in all organs as early as on 5th
day after acquisition of virus. In some cases the lower transmission rate than infec-
tion rate was due to the absence of virus in salivary glands.
Book Reviews


In their opinion, toxoplasmosis seems to be responsible for the majority of foetopathies although an infection of the decidua and thus, the transmission of the parasites to the embryo may be possible. The relation of congenital toxoplasmosis to malformations seems to be only accidental. G. Piekarski, in collaboration with Werner, deals with the morphology of the parasite. His chapter on the interpretation of immunobiological methods in the proof of toxoplasmosis is an analogy of his publication in 1962. In addition, Piekarski is also concerned with epidemiology. J. Boch records a list of problems of toxoplasmosis of domestic animals together with epidemiological and veterinary aspects specific for each species. Kabelitz discusses in detail the clinical symptomatology of acquired toxoplasmosis. On the grounds of brief case reports and typical curves of the titres of serum antibodies he illustrates acute glandular, abdominal, encephalitic, pulmonal and cardiac forms.