EXPERIMENTAL MUTABILITY OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS. PART II. PROPERTIES OF MUTANTS INDUCED BY NITROUS ACID

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EXPERIMENTAL MUTABILITY OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS

Part II: Properties of Mutants Induced by Nitrous Acid

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Introduction

Nitrous acid is one of the mutagens most frequently used in virus experiments. The mutagenicity of this chemical substance was first studied in the tobacco mosaic virus [1] and in the FX-174 and T4 phages [2, 3]. It has now been demonstrated that nitrous acid can cause mutations in bacteriophages and in plant and animal viruses containing RNA and DNA, not only when the native virus is treated, but also in the case of direct action in vitro on viral nucleic acid [4-7].

The object of the present study is to examine how Venezuelan equine encephalitis virus is changed through the action of nitrous acid.

Materials and Methods

The experimental method was similar to that of the previous report. The extracellular virus was treated with nitrous acid (4 M, 5 min.) by the method reported by Mundry and Gierer [1].

Experimental Results

No mutations in plaque size were observed after the virus was treated with nitrous acid (table 1). The form and size of plaques in the experiment did not differ significantly from the control group. Both the initial and mutagen-treated populations consisted of varieties forming large (5-6 mm) and small (0.8-1 mm) plaques, round in form and with distinct even edges. The percentage of small experimental and control plaques equaled 10.0 and 7.15 respectively.

When pathogenicity was determined in 52 clones isolated from the
mutagen-treated virus population, experiments on mice showed that 11 had changed virulence in various methods of infection (table 2); one clone was apathogenic in intracerebral and subcutaneous inoculation; two clones retained their original pathogenicity when inoculated intracerebrally, but lost it completely in subcutaneous inoculation; three clones exhibited decreased peripheral and pronounced cerebral pathogenicity; and five clones showed decreased viral activity when inoculated intracerebrally and subcutaneously.

Table 1

The Effect of Nitrous Acid on Plaque Size in Venezuelan Equine Encephalitis Virus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Plaques</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Small (number)</td>
</tr>
<tr>
<td>Mutagen-treated</td>
<td>260</td>
<td>26</td>
</tr>
<tr>
<td>Control</td>
<td>262</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 2

Change in Pathogenicity for Mice of Venezuelan Equine Encephalitis Virus Treated with Nitrous Acid

<table>
<thead>
<tr>
<th>Pathogenicity</th>
<th>Nitrous Acid</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S+  S-</td>
<td>S+  S-</td>
</tr>
<tr>
<td>ic+ sc+</td>
<td>12  29</td>
<td>12  30</td>
</tr>
<tr>
<td>ic+ sc-</td>
<td>0  3</td>
<td>0  0</td>
</tr>
<tr>
<td>ic+ sc-</td>
<td>0  2</td>
<td>0  0</td>
</tr>
<tr>
<td>ic- sc+</td>
<td>0  5</td>
<td>0  0</td>
</tr>
<tr>
<td>ic- sc-</td>
<td>0  1</td>
<td>0  0</td>
</tr>
<tr>
<td>All clones tested</td>
<td>12  40</td>
<td>12  30</td>
</tr>
<tr>
<td>Clones with changed peripheral activity, %</td>
<td>0  27.5</td>
<td>0  0</td>
</tr>
<tr>
<td>Clones with changed intracerebral activity, %</td>
<td>0  15</td>
<td>0  0</td>
</tr>
</tbody>
</table>

Nevertheless, these clones exhibited marked cytopathogenicity in a CEC culture and had high titers (EID$_{50}$/mm) of 6.0-6.5.

The majority of mutants (9 of 11) proved unstable and after four passages in a CEC culture their pathogenicity was found to revert to the level of the original strain. For further study we took clones A-30 and A-31, which had proven stable and apathogenic for white mice inoculated subcutaneously. When these clones were tested on guinea pigs and rabbits, they were also found to be apathogenic when inoculated subcutaneously.

When guinea pigs were inoculated with clone A-30, the virus was discovered in the blood only on the fourth day (fig. 1) in a titer of
2.5 (EID_{50}/ml), and on the fifth day it once again did not appear. Clone A-31 was also characterized by weak, brief viremia.

Table 3

<table>
<thead>
<tr>
<th>Clone</th>
<th>Virus titer before heating (log EID_{50}/ml)</th>
<th>Virus titer after heating (log EID_{50}/ml)</th>
<th>T_{55}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50°C</td>
<td>60°C</td>
<td>55°C</td>
</tr>
<tr>
<td>A-30</td>
<td>6.0</td>
<td>6.0</td>
<td>0</td>
</tr>
<tr>
<td>A-31</td>
<td>6.5</td>
<td>6.25</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.75</td>
<td>7.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

When white mice were inoculated with these clones, the virus was found to be present only in the brain and spleen, while not appearing in the liver throughout the entire period of observation (fig. 2). After inoculation with clone A-30 the virus was observed in the brain tissue on the fourth day in a titer of 2.25, and in a titer of 2.0 after inoculation with clone A-31; subsequently the virus was not observed at all in the brain. The virus was detected in the spleen on the second and fourth days in titers of 3.5 and 2.25 after inoculation with clone A-30, and in titers of 3.0 and 1.75 after inoculation with clone A-31; on subsequent days it was not found in the spleen.

The clones examined did not change titer after heating for 30 minutes at 50°C, but after 10 minutes' incubation at 60°C their complete inactivation was noted. Heating for 20 minutes at 55°C reduced the titers of clones by 4.5 in comparison with the original titers, permitting us to describe these clones as thermolabile (table 3).

Clones A-30 and A-31 possessed high antigenic properties (table 4). When rabbits were immunized with these clones, the titer of virus-neutralizing antibodies equalled 1:640 21 days after the first injection and 1:3125 after the second injection. Similar results were also obtained in the hemagglutination-inhibition test. Seven days after immunization antihemagglutinins appeared in a dilution of 1:460 (A-30) and 1:460 (A-31); by the 21st day the titer had increased to 1:1066 (A-30) and 1:230 (A-31). Forty-two days after the second injection the titer of antihemagglutins was 1:4120 (A-30) and 1:4265 (A-31).
Table 4

Antigenicity and Immunogenicity of Mutants Induced by Nitrous Acid

<table>
<thead>
<tr>
<th>Clone</th>
<th>Antibody titer 21st day after first immunization</th>
<th>Antibody titer 21st day after second immunization</th>
<th>Resistance index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR HI</td>
<td>NR HI</td>
<td></td>
</tr>
<tr>
<td>A-30</td>
<td>1:640 1:460</td>
<td>1:3125 1:4120</td>
<td>5.5</td>
</tr>
<tr>
<td>A-31</td>
<td>1:640 1:640</td>
<td>1:3125 1:4265</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Note. NR -- neutralization reaction; HI -- hemagglutination-inhibition test.

A single immunization of mice with these clones caused the animals to develop resistance to subsequent infection with pathogenic strains of Venezuelan equine encephalitis virus. The resistance index was high, attaining values of 5.5 and 5.6.

Discussion

Our studies showed that, when Venezuelan equine encephalitis virus was treated with nitrous acid, mutations with regard to pathogenicity could be obtained.

In this instance, as in the experiments described in the earlier report [8], a unilateral correlation was established between plaque size and pathogenicity of the tested mutants, and a close correlation of pathogenicity with the level of viremia induced in animals and with thermoresistance.

The majority of mutants obtained through the action of nitrous acid on Venezuelan encephalitis virus were unstable. When the latter were passed on a CEC culture, 9 of 11 variants had already substantially
changed their properties after the fourth passage. Reversion of mutants produced through the action of nitrous acid to the level of the original strain has been noted by several authors and, in particular, in experiments with Omsk hemorrhagic fever virus [9]. Mutants which retained their characters unchanged after passages (A-30 and A031) were apathogenic in subcutaneous inoculation of mice, guinea pigs and rabbits, possessed marked antigenic and immunogenic properties and are at present being studied as candidates for vaccine strains.

Conclusions

Treatment of Venezuelan equine encephalitis virus with nitrous acid led to the appearance of mutants in pathogenicity. Mutations with regard to plaque size were not noted upon application of the same mutagen.

The majority of mutants thus produced were unstable when passed on a CEC culture.

Pronounced antigenic and immunogenic properties were observed in two induced mutants which were stable upon subcutaneous inoculation in mice.

Four tables, two illustrations, 9 biographical entries.

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7. A. I. Lebedev, "Preparation of Modified Virus Clones through the Action of High Temperature and Nitrous Acid on the RNA of a Virulent Strain of Type SATr-1 Hoof and Mouth Disease Virus", Transactions of the All-Union Institute of Experimental Veterinary Medicine, vol. 33, p. 233, 1967.


EXPERIMENTAL MUTABILITY OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

II. CHARACTERS OF MUTANTS INDUCED BY NITROUS ACID

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Summary

The changes of characteristics of Venezuelan equine encephalomyelitis virus (VEE) induced by nitrous acid were studied. The treatment of VEE virus by this mutagen resulted in the initiation of mutations affecting the character of pathogenicity, but no mutations affecting only the size of negative plaques were observed. Mutants with lacking or reduced pathogenicity for animals were characterized by small negative plaques, by a low rate of virulence and by their mutability. Besides these mutants possessed pronounced antigenic and immunogenic characteristics.