FURTHER STUDIES ON THE 57-67 VIRUS

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In a previous paper (Fukumi et al., 1957), a cytopathogenic agent was reported to be isolated from an upper respiratory illness resembling an adenovirus infection. This virus was found to be different from each of the viruses such as polioviruses, Coxsackie viruses, adenoviruses, herpes simplex virus etc., but the cytopathic changes induced by it were like those due to the enteroviruses. The comparison of this virus with the ECHO viruses was not conducted at that time because neither the ECHO sera nor the ECHO viruses were not available to us. However thereafter when some of the ECHO viruses which were adapted to HeLa cells were given to us from Dr. M. Kitaoka, Department of Virology & Rickettsiology, National Institute of Health, Tokyo, experiments were carried out to determine whether or not the virus belonged to the group of the ECHO viruses. Further, serological examinations were made on various groups of people in order to know as to how widely the antibodies against the virus are distributed in them.

EXPERIMENTAL MATERIALS AND METHODS

The prototype strains of ECHO group of viruses: The ECHO prototypes 1 (Farouk), 2 (Cornelis), 3 (Morrisey), 5 (Noyce), 6 (D'Amori), 7 (Garnett) and 9 (Quigley) were received from Dr. M. Kitaoka. They had been adapted to HeLa cells and submitted to several passages in these cells when they were given to us. In the present studies, HeLa cells alone were used throughout for the propagation of the viruses employed, the neutralization reactions or any other experiments.

The 57-67 virus: It was described in detail in the previous paper.

The antisera: In order to obtain antisera against the 57-67 virus and some of the ECHO viruses, rabbits were inoculated intravenously with viruses grown in HeLa cell cultures. Thus, the antisera against the 57-67 virus, ECHO 1 and 6 viruses were obtained and used for the present studies.

The technics for HeLa cell cultivation and for the experiments using HeLa cells were given in the previous publication (Fukumi et al., 1957).

EXPERIMENTAL RESULTS

Obtaining the HeLa cells resistant to the virus 57-67 and their susceptibility to the ECHO viruses: As already reported, the virus 57-67 grows well on and makes
cytopathogenic effect upon HeLa cell cultures, but the cytopathic changes never cover the whole sheet of the infected HeLa cell culture, even if this is infected with a massive dose of the virus, and several islands of cells or parts of the cell sheet are usually left undamaged. Such undamaged cells were further propagated and submitted to experiments as follows.

A bottle culture of HeLa cells was inoculated with the 57-67 virus at about $10^7$ TCD$_{50}$ per $10^7$ cells, and left incubated at 37°C for 7 days. On the seventh incubation day, when the cytopathic changes were almost completed, the medium of the culture was replaced with new maintenance medium and further incubated for 3 days. Then, its medium was again replaced this time with propagating medium (20% human serum, 0.5% lactalbumin hydrolysate, and 0.1% yeast extract in Hanks' balanced salt solution), and incubated further. The culture was again infected by the 57-67 virus at the same dose as previously 25 days after the primary infection. A small number of the cells were observed to be damaged by the infection under microscopical checking. Five days after the last infection, the medium was again replaced with propagating medium and submitted to further incubation. The culture was then submitted to further passages in propagating medium by removing cell sheet by means of trypsinization. In both the third and fourth passages, the cells were distributed to culture tubes and tested for their susceptibility to the 57-67 virus, the ECHO prototype viruses, and some other viruses.

The viruses were used at concentrations of about $10^4$ to $10^5$/cc of TCD$_{50}$ for infecting the cultures. It was found in these experiments that the cell culture selected by the 57-67 virus infection was resistant to both the 57-67 virus and the ECHO viruses used in this study. In addition, the culture was found to be of usual sensitivity to the infections of those viruses such as Coxsackie viruses of group B type 1 (Nemoto), and type 3 (Nancy), adenoviruses type 3 (58-83), type 5 (Mayeda), and type 8 (57-140). In short, the cell culture which was obtained among the HeLa cell population by screening by means of the cytopathogenic effect of the 57-67 virus, was resistant to the infection of either the 57-67 virus or the ECHO viruses though sensitive to Coxsackie B viruses and adenoviruses.

Detailed studies were not made on the properties of the resistant cells. Puck and Cieciura (1957) described a persistent viral infection in the variant cells selected by Newcastle disease virus infection. Such resistant cells lost the capacity to produce virus at least temporarily when cultured in the presence of the antibody, but did not lose their resistance. In the case of the cells resistant to the 57-67 virus, the cells passaged 3 times in propagating medium containing human serum were found not to be liberating virus when tested after incubation for several days in maintenance medium at 37°C. Further study remains to be made concerning this point.

Antigenic analyses of the 57-67 virus in comparison with the ECHO viruses: At first, experiments were carried out to know whether or not rabbit antiserum against the 57-67 virus was able to neutralize any of the ECHO viruses that were available to us. The antiserum whose neutralization titer has a value of 1 : 256 was employed at a dilution of 1 : 10, whereas the ECHO viruses at concentrations of about $10^2$ to $10^3$/cc of TCD$_{50}$. The serum and each of the viruses were mixed in an equal amount and then inoculated into HeLa cell tube cultures after 30 minutes'
contact at room temperature. In this experiment the ECHO prototype viruses types 1 and 6 were found to be neutralized at least partly by the antiserum, while the other types of the ECHO viruses had no cross-reaction with it at all.

Next, antiserum against each of these two types, namely types 1 and 6, was obtained by rabbit immunization, and then cross-neutralization reactions were carried out between the three viruses, namely the 57-67 virus, and the ECHO types 1 and 6 viruses. The results are summarized in Table 1. As seen there, the 57-67 virus seemed to have a common antigen with either type 1 or type 6 ECHO virus, but to be more closely related to type 6 virus rather than to type 1 virus.

Table 1. Cross neutralization reactions between ECHO 1 (Farouk) ECHO 6 (D'Amori), and 57-67 virus

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>ECHO 1 (Farouk)</th>
<th>ECHO 6 (D'Amori)</th>
<th>57-67 virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECHO 1 (Farouk)</td>
<td>1:256</td>
<td>1:32</td>
<td>1:16</td>
</tr>
<tr>
<td>ECHO 6 (D'Amori)</td>
<td>1:16</td>
<td>1:128</td>
<td>1:64</td>
</tr>
<tr>
<td>57-67 virus</td>
<td>1:32</td>
<td>1:32</td>
<td>1:256</td>
</tr>
</tbody>
</table>

Karzon (1957) reported that there were variants with modified antigenicity among the type 6 ECHO viruses. From this point of view, it seems to be reasonable to regard the 57-67 virus as one of the antigenic variants of the ECHO 6 virus.

Capacity of the 57-67 virus to agglutinate red blood cells: It has already been reported that some of the ECHO viruses are capable of agglutinating human group 0 or chicken erythrocytes (Goldfield, Srihongse and Fox, 1957; Lahelle, 1958). The 57-67 virus was however found to be unable to agglutinate chick red blood cells. Experiments were carried out in the way that two-fold dilutions of 57-67 virus suspension were mixed with 0.5% chick red cell suspension in an equal amount and the results were read after 1 hour's incubation at 37°C. No agglutination of red blood cells was observed at all.

Distribution of the neutralizing antibodies against the 57-67 virus in populations: As has been mentioned above, the 57-67 virus is considered to belong to the ECHO 6 group of virus. As the viruses of this group have antigens in common with each other, it can not be said that if the antibodies capable of neutralizing the 57-67 virus are found in an individual person, he has ever had an infection due to this virus. It may, however, be of some use to konw the distribution of the neutralizing antibodies against the 57-67 virus because it may give us some informations about the frequency of occurrences of at least some of the ECHO 6 viruses. Five groups of persons were selected for this purpose, namely:

1) Students of the first grade in the Gyoda High School in Saitama Prefecture. Total 202 persons, 18 years of age in average. The school is located in Gyoda City having a population of about 48,000, 50 kilometers north-west of Tokyo City.

2) Students in the Nurse School of the Tokyo First National Hospital. Total 26 students. They were in average 18 to 20 years of age.

3) Children in Ninomiya Branch Nursery of the Tokyo First National Hospital.
Total 32 children, in average 2 to 3 years of age. The nursery is located about 50 kilometers south-west of Tokyo City, surrounded by kitchen-garden and other fields at the seashore.

4) Children in the Chiyoda Nursery in Gyoda City, Saitama Prefecture. Total 67 children, 4 years of age in average. The nursery is located in Gyoda City.

5) Babies in the Kokuryo Nursery in Tokyo Prefecture. Total 22 sucklings. The nursery is located in Chofu City about 15 kilometers west of Tokyo City.

![Graph showing distribution of neutralizing antibodies against 57-67 virus in five institutions.](image)

**Fig. 1. Distribution of neutralizing antibodies against 57-67 virus in five institutions.**

The experimental results for the students in Nurse School in the Tokyo First National Hospital and the children in Ninomiya Nursery Branch were already given in our previous publication, but are included in the present study for comparison. Fig. 1 shows the results that give us the frequency distributions of those possessing the neutralizing antibodies against the 57-67 virus in the above five institutions. More than fifty per cent of the children three years or more than three years of age were found to possess the 57-67 virus antibodies already in their sera, but 87 per cent of the sucklings had no such antibodies yet.

**DISCUSSION**

As already mentioned in the previous publication, the 57-67 virus was recovered by inoculation of HeLa cell culture from the throat swab of an ill child who seemed to be suffering from upper respiratory infection. It seems to be that the ECHO group of viruses are rarely isolated by HeLa cell culture (Archetti, Weston and Wenner, 1957). However, the present study revealed that the 57-67 virus was an antigenic variant of the ECHO 6 viruses. It is well known that some members of the ECHO group, especially ECHO 6 have been found to cause aseptic meningitis (Melnick, 1958), whereas some ECHO viruses were occasionally incriminated to cause undifferentiated febrile illness. Cramblett, Rosen, Parrott, Bell, Huebner and McCullough (1957, 1958) reported an epidemic of upper respiratory tract in a nursery due to a type of the ECHO viruses. Thus, it can be said that the case from whom the 57-67 virus was reported to have been recovered was an example of ECHO 6 virus infection showing upper respiratory tract symptoms.
The ECHO 6 viruses seem to be widely distributed in our communities as suggested by the results of our serological examinations for various groups of people. As there are many antigenic variants in the ECHO 6 group, it is very difficult to determine from sero-epidemiological findings alone what variant or variants are most prevailing in our communities. Therefore, it is not certain in what extent the virus with the same antigenic characteristics as those of the 57-67 virus is prevailing, but anyhow our findings give us some information concerning the distribution of the antibodies capable of neutralizing the 57-67 virus. What symptoms this virus is most frequently developing by the infection is another question that remains to be solved.

**SUMMARY**

The 57-67 virus which was recovered from a case of upper respiratory tract infection by inoculation of HeLa cells is found to be an antigenic variant of the ECHO 6 virus.

**REFERENCES**


