ECOLOGICAL BEHAVIOR OF 6 COXSACKIE B AND 29 ECHO SEROTYPES AS REVEALED BY SEROLOGIC SURVEY OF GENERAL POPULATION IN AOMORI, JAPAN

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SUMMARY: Neutralizing antibody titers of 252 random serum samples from different age groups were determined by the microplate method. The results showed marked differences in the grade and pattern of dissemination among the serotypes. Echo-22 (Group I) showed extremely high endemicity with an infection rate higher than 50% among infants within the first year of life. Cox B-1, 3, and 4 and Echo-9, 11, 14, 21, 25, and 31 (Group II) were highly endemic with occasional outbreaks. Cox B-2 and Echo-3, 4, 6, 12, 13, 15, 16, 19, 20, 26, and 29 (Group III) showed relatively low endemicity with extensive dissemination at relatively long intervals. Cox B-5 and Echo-7 (Group IV) gave unique antibody-incidence curves with a sharp peak at a certain age, which was explained by extensive spread of virus in a certain year after many quiescent years and a strong age preference of primary infection for young children. Echo-2, 17, 18, 24, 30, and 33 (Group V) maintained low endemicity with no extensive dissemination. Cox B-6 and Echo-1, 5, 27, and 32 (Group VI) showed the lowest endemicity. Long communicable periods of the infected host seem to be a decisive factor for Groups I and II to maintain the high endemicity, and the possibility of intrauterine or neonatal infection was suggested for Echo-22 in particular. The chronic carrier state was suggested for the very low endemic serotypes to secure continuous transmission.

INTRODUCTION

Vast epidemiologic data for enteroviruses have been accumulated. However, of the numerous viruses in this group only polioviruses and a few other viruses have been studied extensively and our present knowledge of many other viruses is still far from adequate. Serologic surveys by neutralization tests are very useful to reveal the previous infection histories of different age groups within a population. This type of information collected from various populations, rural and urban, in different social and climatic environments, is analyzed to gain an insight into the ecology of the viruses. Comparative analyses of the data of serologic survey of a population with different serotypes constitute a fruitful method for revealing differences in the ecologic behavior...
among the viruses.

In the present study neutralizing antibody titers were determined against 6 Coxsackie B and 29 Echo serotypes with each serum of a collection from residents in Aomori City, Japan. Aomori is a port city with a population of about 240,000, located near the northern end of Honshu, the main island of Japan, and serves as an important point of the traffic connecting between Honshu and Hokkaido, the northern large island of Japan. The work involving antibody titrations of 252 serum samples with 35 different viruses became feasible by adopting the microplate method.

**Materials and Methods**

*Serum specimens:* We collected 252 random serum samples from persons visiting Aomori Prefectural Central Hospital and some other clinics in Aomori City during the period from January to May, 1970. The serum samples included 14, 18, 28, 32, 40, 40, 40 and 40 samples in age groups, 0-6 and 7-11 months, and 1-4, 5-9, 10-19, 20-39, 40-59 and 60 years and over, respectively. All the sera were titrated for neutralizing antibody against each of 35 enterovirus serotypes (see below). However, for some technical reason the antibody titer could not be obtained in some instances, i.e., 3 sera (10-19 years), 1 (40-59), and 1 (60-) for Coxsackie B-3; 1 (40-59) for Echo-13; 1 (1-4) for Echo-16; 1 (40-59) for Echo-19; 1 (20-39) and 1 (40-59) for Echo-20; 2 (1-4) for Echo-25. The sera were stored frozen at -20°C until use.

*Viruses:* The viruses used for the neutralization tests were the following 6 serotypes of Coxsackie B virus and 29 serotypes of Echovirus: Cox B-1 (Conn-5), B-2 (Ohio-1), B-3 (Nancy), B-4 (JVB), B-5 (Faulkner), and B-6 (Schmitt); Echo-1 (Farouk), Echo-2 (Cornelis), Echo-3 (Morrisey), Echo-4 (Du Toit), Echo-5 (Noyce), Echo-6 (D’Amori), Echo-7 (Wallace), Echo-9 (Hill), Echo-11 (Gregory), Echo-12 (Travis 2-85), Echo-13 (Del Carmen 11-4-D), Echo-14 (Tow), Echo-15 (Charleston 96-51), Echo-16 (Harrington), Echo-17 (CHHE-29), Echo-18 (Metcalf), Echo-19 (Burke), Echo-20 (JV-1), Echo-21 (Farina), Echo-22 (Harris), Echo-24 (De Camp), Echo-25 (JV-4), Echo-26 (Coronel 11-3-6), Echo-27 (Bacon 1-36-4), Echo-29 (JV-10), Echo-30 (Bastianni), Echo-31 (Caldwell), Echo-32 (PR 10), and Echo-33 (Tolca-3).

Those strains had been maintained at Department of Bacteriology, Iwate Medical College, Morioka, or supplied by Iwate Prefectural Public Health Institute, Morioka, or Department of Bacteriology, Medical School, Tohoku University, Sendai. Each strain was confirmed by us to be neutralized by the specific type antiserum obtained from Microbiological Associates, U.S.A., or National Institute of Health, Tokyo.

*Cell cultures:* Highly sensitive cells were used for each serotype, i.e., HeLa cells for Cox B-2, 4 and 6 viruses, HEL cells (strain cells of human embryonic lung) (Kawana and Matsumoto, 1969) for Echo-22, and JINET cells (strain cells derived from cynomolgus monkey kidney) (Tauchiya, Takayama and Tagaya, 1969) for the other serotypes. The same type cells was used for preparation of seed virus, the infectivity assay and the neutralization test.

HeLa cells were grown in Eagle’s minimum essential medium (MEM) supplemented with 5-10% calf serum, HEL cells in medium 199 supplemented with 15% calf serum, and JINET cells in a mixture of equal volumes of MEM and YLE media (Earle’s solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate) supplemented with 5-10% calf serum.
Virus stocks: Each strain was passaged at least three times in cultures of the cells mentioned above, and sufficiently adapted virus was used for neutralization tests. The virus stocks used showed a clear-cut endpoint within 7 days after inoculation in infectivity titration and no indication of break-through in neutralization tests.

Infectivity assay: The infectious titer of the virus stocks was determined by the microplate method prior to the neutralization test. Selection of the cells mentioned above was made depending on the serotype. Serial ten-fold dilutions of the virus material were made in tubes with the same medium as used for cell culture. Each dilution was delivered in 0.025-ml amounts into 4 wells on a U-type plate containing 0.025 ml of culture medium. After incubation in a humidified CO₂ incubator at 37°C for one hour, each well received 0.025 ml of a cell suspension in culture medium. The cell concentration was 4×10⁵, 3×10⁵ and 2×10⁵ cells per ml for HeLa, HEL and JINET cells, respectively. The cultures were incubated in a CO₂ incubator at 37°C and observed microscopically for any cytopathic changes. The end point was read in 4 to 7 days of incubation, and the TCID₅₀ titer was calculated.

Neutralization tests: U-type microplates were used. Serial twofold dilutions, starting from 1:4, of the serum inactivated at 56°C for 30 min were made in 0.025-ml amounts with culture medium as diluent, and each dilution received 0.025 ml of culture medium containing 100 TCID₅₀ of virus determined by a preliminary titration. The serum-virus mixtures were shaken, incubated in a humidified CO₂ incubator at 37°C for one hour, and received 0.025 ml of a cell suspension prepared in the same manner as in the infectivity assay. The cultures were incubated in a CO₂ incubator at 37°C for 4 to 7 days depending on the serotype used, and observed for any cytopathic changes. The antibody titer was expressed as the reciprocal of the highest serum dilution showing no cytopathic changes. Each set of tests included an infectivity titration of the virus used. The tests were taken as valid when the titer obtained was within the range of 30 to 300 TCID₅₀ per 0.025 ml. The same lot of calf serum, inactivated at 56°C for 30 min, was used for preparation of culture medium throughout the study.

RESULTS

The pattern of antibody distribution by age varied remarkably depending upon the serotype used in the test. Based on the antibody distribution pattern the serotypes can be divided into several groups as follows.

A few seropositives were found with Cox B-6 and Echo-1, 5, 27 and 32. The typical pattern of antibody distribution is illustrated in Fig. 1 (Cox B-6).

Six serotypes, Echo-2, 17, 18, 24, 30 and 33, showed a gradual increase with age in the percentage of positives at 1:4 serum dilution, reaching a maximum level of about 30% to 50%. Figure 2 shows a typical pattern of this group against Echo-24 as an example.

In many serotypes, steep increase in antibody incidence was shown at a certain age, with subsequent rise to a plateau sooner or later. Of these serotypes, Echo-26 showed a steep rise in antibody incidence at the age of 20-39 years (Fig. 3) ; Echo-3, 12, 13, 16, 19, 20 and 29 at 10-19 years (Fig. 4) ; Cox B-2 and Echo-4, 6, and 15 at 5-9 years (Fig. 5) ; and Cox B-1, 3 and 4, and Echo-9, 11, 14, 21, 22, 25 and 31 at 1-4 years (Fig. 6).
An aberrant pattern of antibody distribution was observed for Cox B-5 and Echo-7. The antibody incidence curve of these serotypes showed a sharp rise at a certain age, but, unlike that of the above-mentioned serotypes, then dipped sharply to a low level (Fig. 7).

An interesting pattern was noticed for some serotypes when the distribution of
Fig. 5. Age-specific distribution of neutralizing antibody titers against Echo-6 (a pattern of steep rise at 5-9 years).

Fig. 6. Age-specific distribution of neutralizing antibody titers against Echo-9 and 221 (a pattern of steep rise at 1-4 years).
antibody titers in the different age groups was inspected. In these cases, while the curves of titers ≥4 rapidly rose to plateaus, the curves of higher titers, ≥8, ≥16, etc., showed sharp declines after rising to maximum levels at or near the age at which the ≥4 curves reached the maximum levels. Such patterns were clearly demonstrated with Cox B-4, and Echo-9, 14, 22 and 25 (Fig. 6). The pattern was reflected on the geometric mean titer of seropositives in the different age groups (Table I). The above-mentioned serotypes showed high mean titers up to the peak of higher titers with a subsequent decline. However, this pattern of change with age in the mean titer of seropositives was obscure or absent for the other serotypes.

DISCUSSION

The most outstanding finding in this study is the remarkable differences in the age-specific antibody distribution depending upon the serotype used in these tests. This indicates that the extent of virus dissemination within the population in the last several decades differed markedly from one type to another.

The present results obviously indicate extremely poor dissemination of Cox B-6 and Echo-1, 5, 27 and 32 in this area in the last several decades (Fig. 1). Serologic data with Cox B-6 in Aomori in 1964 and 1967 (Sato et al., 1968) also support this conclusion.

The antibody incidence curves of Echo-2, 17, 18, 24, 30 and 33 (Fig. 2) indicate that dissemination of these serotypes occurred at a low level but continuously year after year.

Many serotypes demonstrated antibody incidence curves with a steep rise at 5-9 years or older age followed by steady rise to a plateau (Figs. 3-5). This steep rise seems to indicate that a high-level virus dissemination took place in a past year(s) when the persons in that age group were young children and that the virus remained
TABLE I

Geometric mean titers of seropositives in different age groups

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Age groups (years)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1-4</td>
</tr>
<tr>
<td>Echo-22</td>
<td>3.3</td>
</tr>
<tr>
<td>Cox B-4</td>
<td>3.5</td>
</tr>
<tr>
<td>Echo-9</td>
<td>3.4</td>
</tr>
<tr>
<td>Echo-14</td>
<td>2.0</td>
</tr>
<tr>
<td>Echo-25</td>
<td>2.8</td>
</tr>
<tr>
<td>Cox B-1</td>
<td>3.1</td>
</tr>
<tr>
<td>Cox B-3</td>
<td>3.8</td>
</tr>
<tr>
<td>Echo-11</td>
<td>1.5</td>
</tr>
<tr>
<td>Echo-21</td>
<td>1.1</td>
</tr>
<tr>
<td>Echo-31</td>
<td>1.2</td>
</tr>
<tr>
<td>Cox B-2</td>
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</tr>
<tr>
<td>Echo-4</td>
<td>2.3</td>
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<tr>
<td>Echo-6</td>
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<tr>
<td>Echo-15</td>
<td>1.3</td>
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<tr>
<td>Echo-3</td>
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<tr>
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<tr>
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<tr>
<td>Echo-19</td>
<td>1.5</td>
</tr>
<tr>
<td>Echo-20</td>
<td>1.7</td>
</tr>
<tr>
<td>Echo-29</td>
<td>2.0</td>
</tr>
<tr>
<td>Echo-26</td>
<td>1.4</td>
</tr>
</tbody>
</table>

The geometric mean titer is expressed in the logarithm to base 2. The bold-faced figures represent the mean titers in the age group which showed a steep rise in the seropositive rate.

more or less quiet in the following years. This interpretation is supported by the previous reports of outbreaks of Echo-4 and 6.

Nation-wide epidemics of aseptic meningitis due to Echo-4 were reported in Japan in the summer and fall of 1954 and 1965 (Hinuma et al., 1966; Ishii et al., 1965, 1968; Ito et al., 1965; Shibata, Abe and Nakano, 1965; Yoshida and Tanaka, 1965). The steep rise of the Echo-4 antibody incidence at the age of 5–9 years demonstrated in the present study coincides with the fact that Aomori and the adjoining areas were attacked by both the 1964 and 1965 epidemics (Hinuma and Ohi, 1964; Hinuma et al., 1966; Morita, Nakao and Hinuma, 1965; Nakao et al., 1966; Uruno, 1965, 1967). A large epidemic of aseptic meningitis, due to Echo-6 swept over Honshu and Hokkaido during the summer of 1965 (Kawana et al., 1966, 1968; Okuni, 1966; Uruno, 1967). This fact is compatible with a steep rise of antibody incidence curve at the age of 5–9 years (Fig. 6).

Further support for this interpretation of the steep rise of the antibody incidence curve is provided by serologic surveys in Aomori in 1964 and 1967 (Sato et al., 1968),
which revealed steep rises of antibody incidence for Echo-4 and 6 at the age corresponding to the outbreaks due to these serotypes and gave an estimate for the time of extensive dissemination of Echo-16 compatible with that from the present data (Fig. 4).

In agreement with the low percentages of seropositives in children younger than 5 years as demonstrated in this study, no epidemic occurrence of Echo-4 and 6 infections have been reported in this area in the years following these epidemics. The age-specific incidences of Echo-4 seropositives before the 1964 epidemic in Aomori (Hinuma et al., 1966) indicate that during the period 10 to 14 years before the 1964 epidemic there was an Echo-4 outbreak and thereafter the virus was quiet in this area until 1964.

These discussions lead to the conclusion that Echo-4 and 6 epidemics took place at relatively long intervals with intervening periods of low level virus dissemination, and suggest a similar pattern of virus dissemination for the other serotypes, Cox B-2 and Echo-3, 12, 13, 15, 16, 19, 20, 26, and 29 which showed antibody incidence curves with a steep rise at a certain age as did Echo-4 and 6.

The antibody incidence curves for these serotypes (Figs. 3, 4 and 5) steadily rose to a plateau after a steep rise at a certain age. This pattern is quite different from that of Cox B-5 and Echo-7 (Fig. 7) which sharply dipped to a low level of about 30% after a steep rise. These contrasting patterns can be explained by previous serologic data for Echo-4 and Cox B-5.

Hinuma and his associates (Hinuma et al., 1966; Morita, 1965) reported age-specific distribution of the Echo-4-neutralizing antibody before and after the 1964 epidemic. Their data give estimates of 13, 28, 7, 38, 31 and 25% for age groups, 7-11 months and 1-4, 5-9, 10-19, 20-39 and 40 years and over, respectively, for the infection rate among initially seronegative persons in the 1964 epidemic. These figures indicate that infection in seronegative persons occurred widely irrespective of their age.

A quite different picture emerges when the similar serologic data for Cox B-5 (Hinuma et al., 1964) are analyzed. Large epidemics of aseptic meningitis due to Cox B-5 took place in western Japan in the summer of 1960 (Kono et al., 1960) and in northern Japan including Aomori in the next summer (Hinuma et al., 1964; Nakao et al., 1962, 1964b). The age-specific distributions of the Cox B-5-neutralizing antibody in Aomori before and after the 1961 outbreak (Hinuma et al., 1964) reveal that the outbreak induced significant increases in antibody incidence in children less than 10 years of age but little increase in older children, resulting in an antibody incidence curve with a sharp peak at 3-4 years. From their data the infection rates in initially seronegative children are estimated to be 34, 73, 55, 15 and 0% in age groups, 0-2, 3-4, 5-6, 7-9 and 10-15 years, respectively. This explains the sharp decline at 20-39 years from the high level at 10-19 years which was observed in our antibody curve (Fig. 7). The curve further indicates a long quiescent period of Cox B-5 before the 1961 outbreak. Similar interpretation may also be applicable to the results with Echo-7, since the curve of titers ≥4 resembles that of Cox B-5, but with a peak at the age of 20-39 years.

The susceptibility of persons of different ages, not immune as the result of specific previous infection, for the serotypes, Cox B-2 and Echo-3, 6, 12, 13, 15, 16, 19, 20, 26 and 29, with antibody incidence curves of the patterns depicted in Figs. 3-5, is probably of the Echo-4 type rather than the Cox B-5 type, because their antibody curves did not show the pattern of Cox B-5 in spite of extensive dissemination at long intervals.
Figure 6 depicts antibody curves rising steeply at the age of 1-4 years and rapidly reaching a plateau. The curves indicate great extents of yearly dissemination of the serotypes, Cox B-1, 3 and 4, and Echo-9, 11, 14, 21, 22, 25 and 31.

Of these serotypes Echo-22 is extraordinary in that the antibody incidence was very high (56 %) even at the age of 7-11 months. With none of the other serotypes used in the present study such a high incidence was encountered in this age group. Since the titer of passively transferred maternal antibody declines to an undetectable level in most babies during the first half year of life, the above finding indicates more than half of infants acquired infection with Echo-22 by the end of the first year of life. This is further corroborated by the finding that higher antibody titers were detected much more frequently in this age group than the group of 0-6 months. Another support was provided by Sato, Sato and Watanabe (1970), who isolated a number of Echo-22 strains from premature babies in Aomori. The percentages of seropositives for Echo-22 further went up rapidly reaching 100 % at 5-9 years. Similar antibody incidence curves of Echo-22 were reported in Aomori in 1964 and 1966 (Sato et al., 1970). All these findings indicate an extensive dissemination of Echo-22 occurring year after year.

Nation-wide epidemic of aseptic meningitis due to Echo-9 broke out in Japan in 1967 (Sato et al., 1969; Shingu et al., 1968; Tagaya, 1968). When our data were re-arranged, the percentages of seropositives for Echo-9 came to 6 % (1/18), 38 % (7/18), 69 % (9/13) and 75 % (24/32) in age groups, 7-11 months and 1-2, 3-4 and 5-9 years, respectively, agreeing with the fact that Aomori experienced the 1967 outbreak (Sato et al., 1969). These figures further indicate that Echo-9 was not so quiet in the years following the 1967 epidemic. All these findings seem to indicate that Echo-9 was disseminated to relatively great extents from year to year with fluctuation resulting in occasional recognizable outbreaks.

A similar situation is likely to prevail for Echo-14. An outbreak, though not so extensive, of aseptic meningitis due to Echo-14 was reported in Aomori in the summer and fall of 1963 (Nakao et al., 1964a). The present data are compatible with this fact. Previous serologic data in Aomori before and after the epidemic (Morita, 1965; Sato et al., 1968) also agreed with this fact. Our data also suggest a relatively high level of virus dissemination in the years following the 1963 epidemic.

From the antibody incidence curves (Fig. 6), primary infection with Cox B-4 and Echo-9, 14, 22 and 25 mostly occurred early in life and rarely in older children and adults. Although the acquired immunity may not be so solid against intestinal infection with enteroviruses, re-infection, resulting in boosting of antibody titer, may not occur as readily as primary infection in susceptibles. Under these circumstances, the natural decline with time of antibody titer in persons without further antigenic stimulation by re-infection manifests itself as an average titer decline in the older age groups (Table I). Cox B-1 and 3 had a similar tendency but less distinct (Table I). However, Echo-11, 21, and 31 demonstrated low mean titers in all the age groups (Table I). The reason for this observation is obscure.

The four serotypes showing a sharp rise in the antibody incidence at 5-9 years (Fig. 5) had a somewhat low average antibody titer in the group of 5-9 years. This was expected as most seropositives in this age group acquired infection 5 to 9 years ago. The situation naturally tends to obscure the subsequent titer decline (Table I). For the serotypes demonstrating a sharp rise in the curve of seropositive rate at an
older age (Figs. 3 and 4), the mean titer of seropositives was low already at the age of sharp rise in accordance with long intervals after their seroconversion. Therefore, the mean titer was generally low in all the age groups (Table I). However, with Echo-3 and 19 high-titered sera were encountered rather frequently in the age group of 60 years and over. The reason for this finding is obscure, but it is probable that the old persons with high titers had repeated re-infection which tended to keep the titer high for a long time.

The analyses of the present data with reference to the previous studies performed in Aomori as discussed above enabled us to divide the serotypes into several groups on the basis of the grade and pattern of virus dissemination. The grouping is not always clear-cut but some serotypes may represent border-line cases. However, the six groups in Table II seem reasonable.

### TABLE II

**Six groups of serotypes differentiated by the grade and pattern of virus dissemination**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serotypes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Echo-22</td>
<td>Extremely high endemicity.</td>
</tr>
<tr>
<td>II</td>
<td>Cox B-1,3,4, Echo-9,11,14, 21,25,31</td>
<td>High endemicity with some fluctuation occasionally resulting in recognizable outbreaks.</td>
</tr>
<tr>
<td>III</td>
<td>Cox B-2, Echo-3,4,6,12,13, 15,16,19,20,26,29</td>
<td>Relatively low endemicity with unusually extensive dissemination at more or less long intervals.</td>
</tr>
<tr>
<td>IV</td>
<td>Cox B-5, Echo-7</td>
<td>Relatively low endemicity with unusually extensive dissemination mainly in young children at long intervals.</td>
</tr>
<tr>
<td>V</td>
<td>Echo-2,17,18, 24,30,33</td>
<td>Low endemicity with no outburst of dissemination.</td>
</tr>
<tr>
<td>VI</td>
<td>Cox B-6, Echo-1,5,27,32</td>
<td>Extremely low endemicity.</td>
</tr>
</tbody>
</table>

This concept of different patterns of virus dissemination depending upon the serotype generally agree with the results of many other studies in various areas of the world as well as this country. Many of such studies concern the occurrence of clinical illness due to these viruses. Echo-22 in Group I has been reported to be the etiological agent of sporadic cases of aseptic meningitis and diarrheal disease (Kibrick, 1964). This is compatible with the extremely high epidemicity, hence little possibility of epidemic occurrence, which is attributed to the virus in our scheme. Cox B-1, 3 and 4, and Echo-9, 11 and 14 of Group II and Cox B-2 and Echo-4 and 6 of Group III have been regarded as the cause of outbreaks as well as sporadic cases of aseptic meningitis and exanthematic or diarrheal disease (Kibrick, 1964). These results are compatible with the pattern of dissemination of these viruses in our scheme. As to the Group IV viruses, outbreaks of exanthematic disease due to Cox B-5 (Kibrick, 1964) and a subclinical epidemic of infection with Echo-7 (Gelfund, 1959) have been reported. The serotypes in Groups V and VI with very low endemicity are not expected to
cause large epidemics in general population. Outbreaks, rather limited, of aseptic meningitis due to Echo-30 have been reported (Cooney, McLaren and Bauer, 1962; Duncan, 1960, 1961). However, under exceptional circumstances, small outbreaks may occur, for instance in families, hospital wards or institutions in which inmates have intimate contact with each other. Such small outbreaks in nurseries due to Echo-1 (Nakamura et al., 1960) and Echo-2 (Rendtorff et al., 1964) have been reported.

All these studies tend to agree with our scheme, but there are some other studies which contradict our findings. For instance, there are some serologic data indicating low endemicity of Echo-9 in some areas or in some past years in Japan (Kono, 1960; Sato et al., 1968). It seems likely that Echo-9 in Japan had many quiescent years before the 1967 epidemic and has been relatively active in the years following the epidemic. Serologic data with Echo-1 and 5 in Tokyo, Osaka and Okayama in 1960 (Kono, 1960) indicate rather high endemicity. These discrepancies from our data are difficult to explain, but may be fortuitous or suggest strain difference.

The reason for the marked differences in the pattern of dissemination of the different serotypes is not known, but since we examined each serum with all of the 35 serotypes, these differences we observed seem to have been due to differences in the intrinsic properties of the serotypes rather than those in the environment or the host.

The highly endemic serotypes listed in Groups I and II consume susceptible hosts rapidly and keep the density of susceptibles to a low level. This situation imposes in turn difficulty on the viruses for continued transmission. The communicable period of infected hosts varies from one type to another, lasting several weeks to several months (Gelfund, 1961). The serotypes in Groups I and II may have long periods of communicability and this may play a decisive role for the viruses to overcome the difficulty and to maintain high endemicity (Matumoto, 1969). In this connection, it is worthy of note that more than one half of infants acquire Echo-22 antibody within the first year of life. With this virus and probably some other serotypes, too, the possibility of significant role of intrauterine or perinatal infections in maintaining the high endemicity is to be investigated since such infections may lead to the chronic state. Some workers have reported cases of intrauterine or neonatal infections with Echo-22 or 9 or some Cox B viruses (Berkovich and Smithwick, 1968; Cherry, Soriano and Jahn, 1968; Monif, 1969; Rowan, McGraw and Eward, 1968).

The serotypes listed in Group III reduce the density of susceptibles to a low level by extensive dissemination occurring at more or less long intervals, rendering the extent of subsequent dissemination very much reduced. This is so because the inherent communicability of these serotypes may not be so high as that of the serotypes in Groups I and II. Then the viruses are readily affected by the enhanced herd immunity, although they can readily spread in a population with a sufficiently high density of susceptibles. Similar explanations may also be applicable to the serotypes in Group IV.

The serotypes of Groups V and VI with low endemicity consume susceptibles slowly and the density of susceptibles in the population may be maintained at a sufficient level for continued transmission in spite of the low efficiency. However, for such viruses to secure the man-to-man transmission chain, some other means, for instance the chronic carrier state of infection, would also be indicated.

Further field studies in a variety of urban and rural populations in temperate and
tropical areas, coupled with adequate laboratory studies, are needed to confirm and extend the concepts developed in this study and to further our understanding of the ecology of enteroviruses.

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