Understanding of streptococcal infections in man has been hindered by the existence of a wide variety of clinical manifestations, which stem from the complicated nature of the infectious agent, the difference in the reactivity of man according to age or in the past experience of infections, the characteristic infectious process, etc. Sero-epidemiological study will yield worthwhile informations which have not been disclosed by usual bacteriological or clinical investigations. This way of approach is usually accompanied with characteristics and limitations depending upon the sort of antibody to be chosen.

Among various antibodies produced during human streptococcal infections, anti-streptolysin O (ASL) has been known to be specific and to develop in the majority of cases in a relatively early phase of infection. ASL is, therefore, one of the most suitable indicators for the streptococcal infection, although it is not indicative of the immune status of man (McCarty, 1954; Noguchi et al., 1962; Kusama et al., 1962a). One of the authors (Kusama, 1958) described a method for titrating ASL in human sera which is accurate and wellsuited for serial analyses, and motivated the initiation of the present study, which deals with close examinations of the pattern of the antibody distribution in different age groups. The extent of streptococcal prevalence was thus inferred from the percentage of those who had developed the antibody in a given

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清水利雄 (自衛隊勝田部隊)
population, and the difference in the capacity of antibody production by age was investigated.

MATERIALS AND METHODS

1. Sera examined:
   (a) Sera from infants and young children: Sera were collected from infants and children under 7 years in various parts of the country during a period of July, 1957 to July, 1960. Since it was difficult to secure serum specimens from healthy persons of these ages, sera from patients with various diseases, not related to streptococcal infections, were also utilized. Among 1,674 sera examined, 872 were from healthy infants and children, 314 were from cases with viral diseases (poliomyelitis, encephalitis, mumps, measles, etc.), 309 were from cases with bacillary dysentery, and 106 were acute phase sera of those who had acute respiratory illnesses. The remaining 73 sera were from patients with tuberculosis, diphtheria, congenital diseases, trauma, etc. Since the patient group showed practically the same antibody distribution as the healthy group, both groups were combined together.

   (b) Sera from primary school children: In February, 1958, sera were taken from children in the 1st to 6th grades of a primary school in an up-town area of Tokyo. Sera from 556 children, representing 54% of the total number of children in the school, were available.

   (c) Sera from student nurses: One hundred and seven students in a nursing school in Tokyo and 41 in a school in Chiba had sera taken in October, 1957. About 90% of the students were from 19 to 21 years old.

   (d) Sera from personnel in Katsuta Camp: Sera were obtained from 432 personnel serving in Katsuta Camp of the Japanese Self-Defence Forces in February to April, 1960. The age of examinees ranged from 18 to 40 years.

   Sera were all handled with aseptic precaution, and heated at 56°C for 30 minutes. Most sera were kept frozen at −20°C until being examined.

2. ASL titration: The method previously described (Kusama, 1958) was employed for titrating the ASL content of sera. Some sera other than listed above showed extremely flat neutralization curves and were excluded from the data, because the inhibition of hemolysis of these sera may be non-specific and it was practically difficult to assess reasonable titers to them (Kalbak, 1947; Kusama et al., 1948; Kusama, 1958).

3. Statistical analysis: Comparison of ASL distribution in two or more groups was made by (1) chi-square test for the percentages of the negative and positive groups*, and (2) analysis of variance for the distribution of the positive group*. A test for significance was made at the 5% level.

RESULTS

ALS Distribution in Primary School Children

Percentage distribution of ASL titers in a total of 556 primary school children is shown in Fig. 1. Each column represents the percentage of the respective children who exhibited the titer noted in the subscript. The logarithm of the titer is spaced by 0.1. The bimodal distribution with a bottom somewhere around 25 units/cc evidently implies that the whole population consisted of two heterogeneous groups. One, showing a nearly normal distribution, can be understood as a group which has attained a certain level of antibody content through streptococcal infections in the past, and the other as a group having practically no infection in the past. Since a theoretical curve for the distribution of the latter group can not be estimated with given data, it is difficult to perform elaborated statistical analyses. The demarcation of two groups with a certain level of ASL titer can not exclude the overlapping portion from the other group. However, an almost rectilinear relationship was observed when the

* For definition, see the first chapter of the Results.
cumulative percentages of those who had titers more than 25 units/cc were plotted against the log ASL titer on a normal probability paper. Thus, a separation of the whole distribution into two groups, with titers above 25 units/cc and below 20 units/cc by neglecting the overlapping portion in each group, does not seem to invite any significant obstacle for carrying out further analyses. The former will be referred to as the positive group, and the latter as the negative group hereafter, indicating positive and negative histories of infection, respectively. Among the children examined, 88% belonged to the positive group, the geometric mean titer being 111 units/cc. No significant sex difference was found. Some of the sera from those classified into the negative group showed a titer of 20 units/cc. In many of them, non-specific inhibition of streptolysin O rather than the true ASL may be responsible for exhibiting this titer.

The ASL distribution was further analyzed according to the grade. As shown in Table 1 and Fig. 2, the percentage of the negative group decreased as the grade advanced, i.e., 22% in the 1st grade and down to 7% in the 6th grade. As to the distribution of titers among the positive groups, there was a certain extent of variations in the geometric mean titer of each grade. However, there was no tendency observed that the mean titer shifted to a constant direction (higher or lower) as children age.
Fig. 2. Percentage distribution of ASL titers in primary school children within each grade. The arrow indicates the geometric mean titer of the positive group.

The variations, therefore, can be considered to be due to some factors other than the age.

**ASL Distribution in Infants and Young Children**

It became especially interesting to examine the ASL distribution in infants and young children. Sera collected from various sources as described in Materials and
Methods were measured for the ASL content, and the results are shown in Table 2 and Fig. 3. It was quite the same as in the primary school children that the ASL distribution in each age group consisted of two groups, positive and negative. In infants under 5 months of age, high percentages possessed the antibody, which was of maternal origin. After a rapid loss of the antibody in early months of life, the per cent positive became lowest in 6-11 months of age, almost all infants thus being transferred into the negative group. In later age groups, the per cent negative gradually decreased as the age advanced. For example, 80% observed in the 1-year age group changed to 40% in the 3-year age group, and to 25% in the 5-year age group.

Age trend in the percentage of the negative group is illustrated in Fig. 4. Open circles in the figure represent the data in the primary school children. The number of sera from infants under 5 months of age is rather small, and the circles are connected with a broken line, indicating that the data are not so definite as in older age groups. However, wane of the maternal antibody in early months of life is clearly demonstrated. Since the percentage of the negative group is plotted on a log scale, the slope of the curve reflects the rate of decrease of the negative group according to age, or the conversion rate from the negative to positive group. In age groups older than 1 year, the curve seems to be almost rectilinear.

The observation of the pattern of antibody distribution in the positive groups reveals characteristic features changing with age (Fig. 3 and Table 2). Since a portion of the negative group overlapping into the positive group in the 1-year age group seems to be greater than in other older age groups, the mean and standard deviation of the positive group in the 1-year age group were estimated from the distribution of those who had more than 40 units/cc of ASL by a maximum likelihood method as described by Cohen (1949). The geometric mean titer was lowest (56 units/cc) in the 1-year

<table>
<thead>
<tr>
<th>Age</th>
<th>Number tested</th>
<th>Negative group</th>
<th>Positive group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
</tr>
<tr>
<td>0-2 mos.</td>
<td>27</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td>3-5 mos.</td>
<td>29</td>
<td>25</td>
<td>86</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>117</td>
<td>113</td>
<td>97</td>
</tr>
<tr>
<td>1 yr.</td>
<td>325</td>
<td>259</td>
<td>80</td>
</tr>
<tr>
<td>2 yrs.</td>
<td>292</td>
<td>186</td>
<td>64</td>
</tr>
<tr>
<td>3 yrs.</td>
<td>263</td>
<td>105</td>
<td>40</td>
</tr>
<tr>
<td>4 yrs.</td>
<td>219</td>
<td>64</td>
<td>29</td>
</tr>
<tr>
<td>5 yrs.</td>
<td>255</td>
<td>63</td>
<td>25</td>
</tr>
<tr>
<td>6 yrs.</td>
<td>147</td>
<td>26</td>
<td>18</td>
</tr>
</tbody>
</table>

*: Not calculated.
*=: In the 1-year age group, a greater portion of the negative group seems to overlap in the positive group than in other age groups, as shown in Fig. 3. The figures are, therefore, introduced by a maximum likelihood estimation from a truncated normal distribution (Cohen, 1949).
Fig. 3 (continue to the next page)
Fig. 3. Percentage distribution of ASL titers in 1,674 individuals of infants and children under 7 years of age. The arrow indicates the geometric mean titer of the positive group.
Fig. 4. Age trend in the percentage of the negative group. The slope of the curve reflects the rate at which the negative group is converted to the positive group. ○, from Table 1; ●, from Table 2.

Fig. 5. Percentage distribution of ASL titers in 148 student nurses in two nursing schools. The arrow indicates the geometric mean titer of the positive group.
age group, and increased gradually in later age groups. In the 5-year age group, the mean titer approached 100 units/cc, which was about the same level as observed in the primary school children. The standard deviations of these distributions were variable to some extent from group to group, but were practically in the same range as observed in the primary school children.

**ASL Distribution in Student Nurses**

ASL distributions in student nurses in two nursing schools are shown in Fig. 5 and Table 3. Some differences in the distribution, as evidenced by the percentage of the negative group and the mean titer of the positive group, were observed between two schools. In school A, almost all students can be considered to belong to the positive group, and the mean titer was on the same level as observed in the primary school children. On the other hand, 12% in school B still belonged to the negative group, and the mean titer of the positive group was significantly lower than that in school A. Thus, a certain extent of difference in the distribution pattern is suggested to exist depending upon the group studied, in spite of the same age composition.

**Table 3. Results of ASL titration of 148 sera from student nurses**

<table>
<thead>
<tr>
<th>School</th>
<th>Number tested</th>
<th>Number</th>
<th>Per cent</th>
<th>Number</th>
<th>Per cent</th>
<th>Geometric mean titer</th>
<th>Log standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative group</td>
<td></td>
<td>Positive group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
<td>Log</td>
<td>Units/cc</td>
</tr>
<tr>
<td>A</td>
<td>107</td>
<td>0</td>
<td>0</td>
<td>107</td>
<td>100</td>
<td>2.000</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>41</td>
<td>5</td>
<td>12</td>
<td>36</td>
<td>88</td>
<td>1.878</td>
<td>76</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>5</td>
<td>3.5</td>
<td>143</td>
<td>97</td>
<td>1.972</td>
<td>94</td>
</tr>
</tbody>
</table>

The positive group, and the mean titer was on the same level as observed in the primary school children. On the other hand, 12% in school B still belonged to the negative group, and the mean titer of the positive group was significantly lower than that in school A. Thus, a certain extent of difference in the distribution pattern is suggested to exist depending upon the group studied, in spite of the same age composition.

**ASL Distribution in Personnel Serving in Katsuta Camp**

Fig. 6 shows the percentage distribution of ASL titers in 432 personnel in Katsuta Camp, the Japanese Self-Defence Forces. It was striking that almost all belonged to the positive group and the entire group composed a very well arranged normal distribution, the mean titer being 85 units/cc. The log standard deviation (0.215) was smaller than that in the primary school children (0.314). The age of the personnel studied ranged from 18 to 40 years, but the disintegration of the population into various age groups revealed no significant change in the antibody distribution pattern.

**DISCUSSION**

The most characteristic feature in the present sero-epidemiological studies of streptococcal infections by means of the ASL measurement is that a population has been interpreted as being composed of two groups, positive and negative, according to the pattern of antibody distribution. It seems to be quite logical to discuss (1) the proportion occupied by each group, and (2) the distribution pattern of the antibody exhibited by the positive group. From this standpoint of view, the ASL distributions in various age groups were examined in the present studies. In any analysis of the
antibody distribution, the age factor is of an extreme importance. It is especially true for the streptococcal infection, since the clinical pictures, complications and non-suppurative sequelae are closely related to age, as pointed out by Powers et al. (1948).

1. The proportion of the positive and negative groups in various age groups.

The antibody found in infants under 6 months of age is understood as that passively transferred from their mothers, but almost disappeared by 6 months, the majority now belonging to the negative group. After 1 year of age, children in the negative group were gradually transferred into the positive group as the age advanced, through the acquisition of streptococci. The negative group was 80% in the 1-year age group, 40% in the 3-year age group, and decreased to 20% when children were ready for attending the primary school. The rate of conversion of the negative to positive group seems to be fairly constant until the end of school age. The negative group was hardly recognized in adult groups. Thus, the changes in the percentage of the negative group from the early childhood to adulthood well correspond to serial events conceivable for the increasing frequency of infection. Moreover, the fact that about 50% of a population has the antibody by 3 years of age suggests that the streptococcal infection is one of the most widely prevailing infections in the early childhood.

The examination of antibody distribution in various age groups has been a useful tool for exploring the prevalence of a certain infectious agent which causes many inapparent infections. Poliomyelitis may be a typical example. As to the streptococcal infection, Hammon et al. (1950) compared various populations with each other by the earliest age at which 50% of the population developed the ASL. However, they regarded those who had more than 100 units of ASL/cc as positive for the antibody. Their positive group, therefore, did not include all the members with a positive history of infection, and their results can not be compared with those reported herein.

One may doubt our concept that the negative group as defined herein represents by and large the possession of a negative history of streptococcal infection, and may conceive that a considerable number of those who once developed the antibody but poorly maintained it or those who developed little or no antibody despite of definite infections may be erroneously classified into the negative group. Our studies in scarlet fever patients (Kusama et al., 1962a and b), however, indicate that the cases erroneously classified are quite negligible, being no more than the tailing portion of the
positive group overlapping in the region of the negative group. Almost all of scarlet fever patients who failed to show significant rises in ASL titer belonged to the positive group at the time of hospitalization, and almost all of those who initially belonged to the negative group (primary infection) exhibited significant rises and thus entered the positive group. It was also shown that the scarlet fever patients who initially belonged to the negative group entered the positive group during the course of illness and rarely came back to the negative group again during one year period of follow-up study.

Rantz et al. (1951) reported three antibody response patterns, varying with advancing ages, of which one was feeble response found in infants and a few young children. If their statement is the case, a considerable portion of the negative group defined herein may have a history of infection in spite of the absence of antibody, and far more than a half of the 3-year age group may have been infected. Such a situation is hardly conceivable, unless a population has been heavily exposed to a streptococcal epidemic. Moreover, the existence of an age group with little or nor antibody production is hardly understandable in the light of usual experiences in other infectious diseases or vaccination experiments, although somewhat poor responses were often observed in a certain younger age group.

2. ASL distribution pattern exhibited by the positive group.

The geometric mean titer of the positive group was definitely low in the 1-year age group. It gradually increased thereafter, and approached 100 units/cc by 5 years of age. The difference in the mean titer between the 1-year and 5-year age groups was significant. Hence, the response pattern was almost completed by 5 years of age, and repeated infections occurring in later ages did not seem to exert any boostering effect. The response pattern in the 1-year age group is duly regarded as being developed by the primary infection, whereas the pattern in the 5-year or later age groups has been established mostly as a result of repeated infections. The responses observed in the 2- to 4-year age groups may be considered as mixtures of the above two theoretically distinguishable patterns. Less intense antibody responses in the 1-year age group may be explained by that they have been produced by the primary infection, and repeated streptococcal exposure may be necessary before attaining higher levels of response. Hence, the conditioning of the antibody forming apparatus by previous exposure to streptococci may be one of the factors which determine the extent of ASL response as stated by Rantz et al. (1951). However, our studies (Kurama et al., 1962a and b) in scarlet fever patients to be reported in the subsequent communications support another alternative explanation. In scarlet fever patients over 3 years of age, no difference was found in the pattern of antibody formation and antibody decline between those who had belonged to the positive and negative groups at the beginning of infection. This fact suggests that older children can respond to streptolysin O equally well, whether their infection is primary or secondary. Physiological factor(s) changing with age, therefore, may be responsible for the age difference in the ASL response.

It has been a usual practice to set up a normal value or normal range for the ASL distribution by simply averaging the titers of a whole population, including the negative and positive groups. However, the antibody is acquired as the result of infection and should not be discussed in the same sense as physiological factors such as the blood pressure, blood cell counts, etc. Moreover, the normal value or range has been generally used as a criterion for recognizing pathological materials.
Nevertheless, if one compare the ASL distribution curves in scarlet fever patients at the onset of illness and of the maximum values attained during the course of illness, a considerable portion of each distribution will be found to overlap, suggesting that the interpretation made from the normal range is not only meaningless but also dangerous, except cases in which ASL titers are distant enough from the ASL distribution curve of healthy individuals. Otherwise, the examination of acute and convalescent sera is indispensable for the diagnosis of streptococcal infection. The existence of two heterogeneous groups in a population and their proportion changing with age definitely indicate that a simple average of the values from a whole population is not adequate for serial analyses.

SUMMARY

A total of 2,810 serum specimens from various age groups were examined for the ASL by Kusama’s method, in order to elucidate the ASL distribution in general population, and the following results were obtained:

1. A population can be divided into two groups. One (positive group) is composed of those who have had the antibody by streptococcal infection in the past, showing the ASL titers above 25 units/cc. The other (negative group) consisted mainly of those who have not been infected yet, showing titers under 20 units/cc.

2. The antibody found in the majority of newborn infants was derived from mothers, but mostly disappeared by 6 months of age, the majority now belonging to the negative group. After 1 year of age, children in the negative group were gradually transferred into the positive group as the age advanced, being 40% in the 3-year age group and decreased to 20% in the 6-year age group. The negative group was hardly recognized in adult groups. The conversion rate of the negative to positive group seemed to be fairly constant until the end of school age.

3. The geometric mean titer of the positive group was definitely lower in the 1-year age group than in later ages. The titers of the positive groups in age groups from 5-year to adult composed normal distribution curves, and the geometric mean titers were maintained within a certain range, without shifting to a certain direction with advancing ages. The standard deviation of the ASL distribution of the positive group was smaller in the adult group than in children. The distribution exhibited by the 2-, 3- or 4-year age group was considered to be a mixture of the responses seen in the 1-year age group and in groups of over 5 years.

The authors wish to thank Dr. Shigematsu, National Institute of Public Health, Tokyo, for his valuable advice in statistical analysis. Thanks are also due to many people who were willing to supply us with serum specimens.

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