A SEROLOGICAL SURVEY FOR ANTIBODIES AGAINST HERPES SIMPLEX
VIRUS WITH SPECIAL REFERENCE TO COMPARATIVELY
HEAT-LABIILE COMPLEMENT-FIXING ANTIBODIES*

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The demonstration by Andrewes and Carmichael (1930) that a majority of human
population possessed antibodies against herpes virus opened a way to a number of
follow-up investigations, and the total body of data accumulated has supported the
essence of the postulate of Burnet and Williams (1939) that the primary infections,
either apparent or inapparent, might occur mostly in childhood and result in a com-
mensal host-parasite relation lasting for the rest of life with or without occasional
manifestations such as recurrent herpes. The earlier workers, employing neutraliza-
tion test (Weyer, 1932; Burnet and Lush, 1939; Scott et al., 1952; Buddingh et al.,
1953; Tateno et al., 1958), complement fixation test (Hayward, 1950: Holzel et al.,
1953; Yamaguchi, 1959a) or both (Dascomb et al., 1955; Yamaguchi, 1955b; Schmidt
and Lennette, 1961), found antibodies among 60 to 100 per cent of the adult popu-
lations examined. It was also a common observation that newborn babies less than
four months old showed the same positive ratio as adults but after this period a

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markedly low positive ratio was observed up to the age of two years.

The purpose of our investigation was to obtain further informations concerning (1) the distribution of antibodies in various age groups especially among children less than ten years old, and (2) the correlation between the neutralizing and complement-fixing antibodies, on the basis of analysis with a large number of serum samples. Usually, a difficulty facing such an attempt is the cumbersome manipulation required for the neutralization test. However, this was overcome by the application of the simple one-day egg neutralization technique (Yoshino et al., 1959).

The pattern of antibody distribution obtained as a result of this study appeared to differ in some respects from previous surveys performed in this country (Tateno et al., 1958; Yamaguchi, 1959a, 1959b), suggesting perhaps an environmental improvement. In addition, the finding of comparatively heat-labile complement-fixing antibodies seemed noteworthy. The present report summarizes and analyses these results.

**MATERIALS AND METHODS**

**Serums**: Serum samples were obtained by random selection from among children who visited the Pediatric Ward of the Koishikawa Branch Hospital of the University of Tokyo during a period of from May 1959 to October 1960, complaining mostly of upper respiratory illnesses, outpatients who came to the Pediatric Clinic of the First National Hospital of Tokyo in 1960, personnel of the National Institute of Health, Tokyo, and other healthy adult residents of Tokyo City. A total of 359 serum samples were subjected to the following neutralization and complement fixation tests. All the serums were inactivated by heating at 56°C for 30 minutes and stored at -20°C in a deep freezer until being tested.

**Neutralization test**: Details of the one-day egg neutralization test were given in a previous report (Yoshino, Taniguchi & Taniguchi, 1959). The only modification was that the inoculum dose per egg was 0.1 cc, instead of 0.2 cc previously formulated, in order to minimize the amount of serum needed for the test. The HF strain adapted to the one-day egg by serial passage was used. Each serum was diluted 1:5, 1:20, 1:80 and 1:320, and each dilution was mixed with an equal volume of virus, whose strength was so adjusted as to include more or less 25 infective units per 0.05 cc. After an hour's incubation in a waterbath at 37°C, each mixture was inoculated into five one-day eggs. The serum endpoint was determined by candling after a further 7 days' incubation according to Reed and Muench (1938), considering the non-specific death factor as being 20%.

**Complement fixation test (CFT)**: The procedure was a modification of the method developed by the arbor virus research group of the Rockefeller Institute, U. S. A. The diluent was saline containing 0.01 % MgCl₂ adjusted to pH 7.8. For the production of antigen, 12-day eggs were inoculated via the route of the chorioallantoic membrane (CAM) with an appropriate dilution of the egg-adapted HF strain of herpes virus (Yoshino and Taniguchi, 1956) and after 3 days' incubation the CAM's were cut, thoroughly washed and homogenized at 0°C to make a 20% emulsion in buffered saline (Scott, 1956). Usually such an antigen possessed 8 to 16 units as measured by a box titration (Sosa-Martinez and Lennette, 1955) against an immune guinea pig serum prepared by repeated intraperitoneal administrations of HF-infected mouse brain suspensions. Antigen control was prepared in an identical manner without the first inoculation of virus. Both the antigen and antigen control were stored in a deep freezer without clarifying beforehand, and just prior to use thawed but once and spun at 2,000 rpm for 20 minutes. In the test, each serum was diluted duplicately 1:4 through 1:128 in 2-fold steps in the amount of 0.1 cc, and 0.2 cc complement representing 2 exact units was added to each tube. One series received 0.1 cc of an appropriate dilution of antigen which represented 2 units, while the other a similarly diluted antigen control, and fixation was allowed to proceed at 4°C overnight. Then a hemolytic system, comprising a 3% suspension of washed sheep erythrocytes and an equal volume of amboceptor representing 2 units, was added in 0.2 cc amounts, and reading was done after an incubation in a waterbath at 37°C for half an hour. Other controls were set as usual. The highest serum dilution exhibiting more than three plus fixation was taken as the endpoint. When the antigen control series showed non-specific fixation at a titer approximate to that of
the antigen series within a difference of 2 tubes, the serum was heated at 60°C for 20 minutes and the test was repeated.

S and V antigens: The above-described antigen, i.e. standard antigen, was used in all CFT except one, which tested serum pools against two fractions of antigen, S and V, which were prepared as follows. A lot of antigen made as above, immediately following the step of homogenization, was centrifuged in the cold at 17,500 rpm for an hour. The supernatant was called S (soluble) antigen, and the pellet resuspended in buffered saline to the original volume V (virus) antigen. Storage and clarification prior to test were done as in the case of the standard antigen. A preliminary box titration with the above-stated immune guinea pig serum indicated that both the S and V antigens possessed 1 unit.

RESULTS

Distribution of Neutralizing (NT) and Complement-Fixing (CF) Antibodies in Various Age Groups

Out of 359 serum samples tested, thirteen revealed non-specific complement fixation after the first inactivation, i.e. heating at 56°C for 30 minutes, and therefore were subjected to a repeat CFT after a further heating at 60°C for 20 minutes. Of the 13 serums so treated, 7 still fixed complement in the presence of antigen control and were omitted from the present analysis, while the remaining 6 showed disappearance of non-specific CF antibodies, rendering it possible to demonstrate the presence or absence of specific antibodies. Thus, a total of 352 samples were available for a comparative analysis of NT and CF antibody levels. The following description will deal with those serums.

![Fig. 1. Relation between the age and the NT antibody level.](image)

NT antibodies: In Fig. 1 are depicted the NT antibody levels arranged in the sequence of increasing ages. Since the present survey aimed chiefly at an elucidation of the age distribution of antibodies among young children, the serum samples had been collected largely from individuals younger than 10 years. Hence, the grading of
age groups was made with finer intervals in the younger ages.

Among the 17 babies aged less than 4 months, seven possessed measurable antibodies. In this group, therefore, the positive ratio was 41.2%. In the next group aged from 4 months to one year, the positive ratio was 9.3%, all the positive serums exhibiting only low titers. A similar pattern of antibody levels was seen in all the age groups of from one to 4 years; that is to say, the presence of positive serums was only occasional while the greater part of the serums turned out to be negative, and among the NT positives there were few whose titers were higher than 1:20. A change in this pattern was noticed from the age group of 4-5 years, in which about one half possessed antibodies of different levels covering a wide range of from 1:5 to higher than 1:320 with an unbiased distribution. This trend was maintained up to the age of 10 years. In the group of 10-20 years, the positive ratio increased, but the distribution of antibody levels among the group was not very different from what was observed in the adjacent two younger groups. In the next three groups, the positive ratios became apparently higher, and among the positives the antibody levels shifted to the upper hand, being mostly higher than 1:40.

![Fig. 2. Relation between the age and the CF antibody level.](image)

**CF antibodies**: An essentially similar picture of antibody distribution was recognized when CF antibody levels were plotted against ages, as illustrated in Fig. 2. On the average, the CF titers were lower than the NT titers. All these results are retabulated in Table 1, to indicate positive ratios according to the age groups, whereby NT titers of 1:5-20 and CF titers of 1:4 were arbitrarily categorized as weakly positive reactions. It can be seen that the positive ratio among adults as determined by the neutralization test was around 80%, but that determined by the CFT was averagely 60%. 
Table 1. NT and CF positive ratios in various age groups

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of serums</th>
<th>NT*</th>
<th></th>
<th></th>
<th>CF**</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>0-1/3</td>
<td>17</td>
<td>5.9 %</td>
<td>35.3 %</td>
<td>58.8 %</td>
<td>11.7 %</td>
<td>5.9 %</td>
<td>82.4 %</td>
</tr>
<tr>
<td>1/3-1</td>
<td>43</td>
<td>0.0 %</td>
<td>9.3 %</td>
<td>90.7 %</td>
<td>2.3 %</td>
<td>4.6 %</td>
<td>93.1 %</td>
</tr>
<tr>
<td>1-2</td>
<td>51</td>
<td>3.9 %</td>
<td>7.8 %</td>
<td>88.3 %</td>
<td>21.6 %</td>
<td>7.8 %</td>
<td>70.6 %</td>
</tr>
<tr>
<td>2-3</td>
<td>27</td>
<td>0.0 %</td>
<td>14.8 %</td>
<td>85.2 %</td>
<td>14.8 %</td>
<td>3.7 %</td>
<td>81.5 %</td>
</tr>
<tr>
<td>3-4</td>
<td>24</td>
<td>4.2 %</td>
<td>8.3 %</td>
<td>87.5 %</td>
<td>20.8 %</td>
<td>20.8 %</td>
<td>58.4 %</td>
</tr>
<tr>
<td>4-5</td>
<td>28</td>
<td>25.0 %</td>
<td>25.0 %</td>
<td>50.0 %</td>
<td>62.6 %</td>
<td>0.0 %</td>
<td>37.4 %</td>
</tr>
<tr>
<td>5-10</td>
<td>75</td>
<td>23.9 %</td>
<td>21.2 %</td>
<td>54.9 %</td>
<td>46.8 %</td>
<td>9.4 %</td>
<td>43.8 %</td>
</tr>
<tr>
<td>10-20</td>
<td>38</td>
<td>47.2 %</td>
<td>26.4 %</td>
<td>26.4 %</td>
<td>52.5 %</td>
<td>10.5 %</td>
<td>37.0 %</td>
</tr>
<tr>
<td>20-30</td>
<td>22</td>
<td>72.8 %</td>
<td>9.1 %</td>
<td>18.1 %</td>
<td>45.3 %</td>
<td>0.0 %</td>
<td>54.7 %</td>
</tr>
<tr>
<td>30-40</td>
<td>20</td>
<td>80.0 %</td>
<td>5.0 %</td>
<td>15.0 %</td>
<td>60.0 %</td>
<td>5.0 %</td>
<td>35.0 %</td>
</tr>
<tr>
<td>40-50</td>
<td>7</td>
<td>57.1 %</td>
<td>28.5 %</td>
<td>14.4 %</td>
<td>57.1 %</td>
<td>0.0 %</td>
<td>42.9 %</td>
</tr>
</tbody>
</table>

* Positive means titers higher than 1:20, weakly positive 1:5-20, and negative 1:<5.
** Positive means titers higher than 1:8, weakly positive 1:4, and negative 1:<4.

Fig. 3. Correlation between the NT and CF antibody levels.
Correlation between NT and CF Antibodies

Fig. 3 demonstrates the correlation between NT and CF titers of the serums presently examined, in which serums of children younger than 10 years are expressed by crosses and the rest by dots.

Serums with negative CF titers were mostly negative in the neutralization test, too, but some of them exhibited NT antibodies. This may be partially due to different sensitivities of the two tests. However, some serums possessed considerably high NT titers, despite their negative CF titers, and such serum samples were found almost exclusively among persons older than 10 years. In contrast to this fact, some children’s serums were unequivocally positive in the CFT while lacking NT antibodies. Such cases could not be found among persons older than 10 years.

The remainders pointed to a fairly good parallelism between the NT and CF titers. But, there still existed a trend that the older age group had higher NT than CF antibody levels whereas the younger tended to react to the contrary. The oblique broken line drawn in the figure passes through assumptive points having equal endpoints in the two tests. Serums upper to this line, i.e. having higher CF than NT titers, were almost exclusively of the younger age group.

This disparity between the younger and the older persons in the NT-CF antibody balance was first suspected to be due to degradation during storage of NT antibodies in the children’s serums. Actually, the children’s serums had been stored in the freezer before the tests averagely one year longer than the adults’ serums. Although, there has been no indication in literature as to a quicker degradation of NT than CF antibodies under such storage conditions, this possibility was examined by re-testing several adults’ serum samples for NT antibody levels after being kept at -20°C for

Table 2. Comparison of NT titers of some adults’ serums as obtained shortly after bleeding and after 6 months storage at -20°C

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Shortly after bleeding</th>
<th>After 6 months storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus used</td>
<td>Titer</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>1:59</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>1:320</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>1:160</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>1:320</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>1:34</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>1:160</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>1:160</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>1:48</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>1:112</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>1:40</td>
</tr>
<tr>
<td>11</td>
<td>47</td>
<td>1:200</td>
</tr>
<tr>
<td>12</td>
<td>47</td>
<td>1:48</td>
</tr>
<tr>
<td>13</td>
<td>47</td>
<td>1:160</td>
</tr>
<tr>
<td>14</td>
<td>47</td>
<td>1:320</td>
</tr>
<tr>
<td>15</td>
<td>47</td>
<td>1:110</td>
</tr>
<tr>
<td>16</td>
<td>47</td>
<td>1:110</td>
</tr>
<tr>
<td>17</td>
<td>47</td>
<td>1:110</td>
</tr>
<tr>
<td>18</td>
<td>47</td>
<td>1:63</td>
</tr>
</tbody>
</table>

* One-day egg infective units.
The result recorded in Table 2 pointed out that no appreciable degradation of NT antibodies had taken place during the storage.

**The Presence of Comparatively Heat-Labile CF Antibodies in Children's Serums**

An episode in the present investigation was that two children's serums, both of which were negative in the neutralization test and 1:16 positive in the CFT, were doubted as for specificity of the CF titers because both serums showed a titer of 1:4 in the presence of antigen control, and therefore heated at 60°C for 20 minutes for the performance of a repeat CFT. One became negative and its non-specific reaction disappeared; the other turned also negative and the non-specific CF titer was 1:4.

This result insinuated the presence of certain comparatively heat-labile specific antibodies in such serums. To ascertain this point, the remaining children's serums having no NT antibodies but showing CF titers higher than 1:8 were pooled, and aliquots were heated at 60°C for 5 and 20 minutes. As a control, several adult serums which had revealed positive titers in both tests were pooled and treated in an identical manner. The two serum pools were tested for CF titers with undiluted S and V antigens as well as with undiluted antigen control. The result is set forth in Table 3.

<table>
<thead>
<tr>
<th>Serum pool</th>
<th>Heated at</th>
<th>for min.</th>
<th>Antigen</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (CF+NT-)</td>
<td>56°C</td>
<td>30</td>
<td>V 1:8</td>
<td>S 1:&lt;4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5</td>
<td>1:4</td>
<td>1:&lt;4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20</td>
<td>1:&lt;4</td>
<td>1:&lt;4</td>
</tr>
<tr>
<td>Adults (CF+NT+)</td>
<td>56</td>
<td>30</td>
<td>1:64</td>
<td>1:32</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20</td>
<td>1:64</td>
<td>1:16</td>
</tr>
</tbody>
</table>

It was clearly demonstrated that the CF antibodies present in those children's serums were specific since no fixation of complement occurred in the presence of undiluted antigen control, that they were gradually destroyed by heating at 60°C whereas the CF antibodies in the NT-positive adults' serums were little affected by the same heating, and that the reaction occurred only with V and not with S antigen.

**DISCUSSION**

Despite the contributions hitherto made by many investigators to the knowledge of epidemiology of herpes virus infections, most of the earlier serological surveys (Zinsser and Tang, 1929; Andrewes and Carmichael, 1930; Weyer, 1932; Burnet and Williams, 1939; Burnet and Lush, 1939) had recourse to techniques which did not provide a good basis for comparison of data with more recent quantitative studies. Experimental results suitable for precise quantitative analyses have been supplied only after the adoption of CFT antigens causing minimal non-specific reactions (Hayward, 1949; Fowler, 1950; Dudgeon, 1949; Sosa-Martinez and Lennette, 1961; Schmidt et al., 1962).
al., 1960) as well as neutralization tests in sensitive hosts employing the constant virus-varying serum system (Scott et al., 1952; Rose, 1952; Yoshino et al., 1959; Schmidt and Lennette, 1961). In spite of such technical improvements, however, scrutiny into the distribution of NT antibody levels has been limited to a relatively small number of serum samples, mainly because of the laborious efforts needed for the performance of neutralization tests, and as a consequence data have been meager heretofore which dealt with the correlation between NT and CF antibody levels among healthy individuals. In this meaning the development of the one-day egg neutralization technique (Yoshino et al., 1959) had a merit that it paved an easy way to this approach owing to its technical simplicity. That this simple method is as sensitive as other routine neutralization methods has been proved (Umeda et al., 1962).

The present results put forward, first of all, the features of the antibody distribution in various age groups. This was better illustrated by NT antibodies, since the titers roughly paralleled but showed averagely higher levels than the CF titers, and on the basis of NT antibodies the following picture was drawn. The positive ratio among children under the age of 4 years was approximately 10% throughout except among babies younger than 4 months, who showed a positive ratio of about 40% probably due to transmission of maternal antibodies (Buddingh et al., 1953; Scott et al., 1952; Holzel et al., 1953). Generally, NT positive serums of such young children possessed only low titers. From the 5th year on, the positive ratio increased with age. The NT antibody levels of the positives in the age groups of 4 to 20 years covered a wide range from nearly negative to considerably high titers, but most of the positives older than 20 years had high titers within a narrower fluctuation.

When the present results are compared with similar studies conducted by others employing comparable techniques, some differences can be noted. Scott et al. (1952) found an earlier rise of NT antibodies among children; namely, an increase of the positive ratio started from the end of 2 years, and children 3 to 5 years old had the same positive ratio as adults, which was about 60%. This trend was reflected in the data of Buddingh et al. (1953), too, who recorded much higher positive ratios presumably because they tested only undiluted serums without heating. As to the positive ratio among adults, the present survey determined it to be 60% by the CFT and 80% by the neutralization test. With regard to this ratio, figures presented by other investigators have varied.

Obviously, such a pattern of antibody distribution must differ depending upon the socio-economical standard (Scott et al., 1952; Buddingh et al., 1953), crowdiness (Anderson and Hamilton, 1949; Yamaguchi, 1959a), sanitary conditions (Dascomb et al., 1955) and perhaps the race. Hence, a reasonable comparison may be made between our present survey and those of earlier workers of this country (Yamaguchi, 1959a, 1959b; Tateno et al., 1958), in order to know whether any changes in the antibody distribution have taken place during the past years. Unfortunately, however, their surveys coped with relatively small numbers of serum samples taken from young children and consequently gave no exact information about the starting time of antibody increases. Yet, a conspicuous point commonly observed by those authors was a markedly high positive ratio among adults, i.e., more than 90% in CF (Yamaguchi, 1959a), and more than 80% (Tateno et al., 1958) to 95% (Yamaguchi, 1959b) in NT antibodies. Furthermore, although the increase of the positive ratio with age seems to have appeared similarly as in our present results, the actual antibody titers obtained
roughly followed the all-or-none type distribution.

As for the validity of the all-or-none theory concerning the distribution of antibodies against herpes virus (Burnet and Williams, 1939), one is not inclined to date to consider that such a pattern has existed, since Rose (1952) indicated that a thousand-fold difference could be detected by adopting the constant virus-varying serum system whereas no such difference could be discerned by the constant serum-varying virus system. In fact, most of the recent reports have disproved the existence of the all-or-none phenomenon (Kilbourne and Horsfall, 1951; Jawetz et al., 1952; Buddingh et al., 1953; Dascomb et al., 1955), and the average antibody levels were found to be elevated with the increase of age (Schmidt and Lennette, 1961). However, a comparison of the above-cited surveys previously performed here with the present one appears to lend support to a possibility that the all-or-none type antibody distribution did exist in earlier days but has been deformed recently as a result of circumstantial improvements in sanitary conditions. This postulate might be substantiated by the fact that the all-or-none phenomenon was observed in earlier days not only in Australia (Burnet and Williams, 1939) but also in England (Hayward, 1950) as well as over here. Also, there are evidences indicating that sanitary improvements can change the pattern of herpes antibody distribution; namely, sanitary improvements in Australia resulted in a marked lowering of the anti-herpes NT positive ratio from 90% in 1940 to 60% in 1950 (Burnet, cited in Scott, 1954), and in a special community with high sanitary cares a low positive ratio was seen (Dascomb et al., 1953).

A further cogitation on this point will convince one that the persistence of herpes virus in a human body causing the so-called latent infection is established only after repeated infections occurring during adolescence, because otherwise the sanitary improvements diminishing the frequency of contact with virus during the past decade would not have influenced the antibody titers of persons aged 10 to 20 years. A substantial evidence for this may be the rise and fall of NT antibodies observed at primary infections (Buddingh et al., 1953). Should this interpretation be valid, it might be conceivable that an increasing number of persons having no or low levels of antibodies against herpes virus will appear hereafter, being exposed to the risk of having severer disease manifestations upon infection. This seems important in view of the facts that adults have been by no means safe from severe herpetic infections (Zarafonetis et al., 1944; Armstrong, 1943; Kipping and Downie, 1948; Kilbourne and Horsfall, 1951; Dascomb et al., 1955; Matumoto et al., 1958; Tateno et al., 1958; Howard and Kaufman, 1962).

The above-observed change in the antibody distribution pattern as compared with the former status may have some bearing on the irregularity encountered in the correlation between NT and CF antibodies. In the age groups of less than 10 years, the NT titers showed a fairly good parallelism with the CF titers on the average, with some exceptions which will be discussed later. On the other hand, persons older than this age revealed averagely higher NT than CF endpoints, extreme cases being found among those who had considerably high NT titers despite the lack of CF antibodies. Such can be interpreted as being due to a quicker disappearance of CF antibodies than NT antibodies in individuals who have not yet experienced enough reinfections to establish the status of latent virus infection. The quicker disappearance of CF antibodies was actually noted in the case of newborn babies, too (Holzel et al., 1953).
A paradoxical observation relevant to this was the presence of comparatively heat-labile specific CF antibodies in a certain portion of young children. Three alternatives were thought about to explain this finding; (1) the children’s sera had been stored averagely one year longer than the adults’ sera tested, and this caused a more rapid degradation of NT antibodies than CF antibodies during the storage, (2) most of the children herein tested were outpatients suffering from upper respiratory and other diseases while all the adults tested were apparently healthy, and this difference contributed to the above-stated discrepancy, and (3) the primary infection of man by herpes virus stimulates an abundant production of CF antibodies, most part of which is comparatively heat-labile, but such is not the case with reinfections occurring under the presence of pre-existing antibodies.

The first explanation is not tenable, because a high stability of NT antibodies under the storage conditions adopted was demonstrated. The second can not be answered at the moment, but seems unlikely to be the main reason, because the above CF antibodies reacted specifically and did not react with the antigen control. The last one must be most possible after denial of the other two alternatives.

The reason why the above CF antibodies reacted only with V antigen also remains to be solved. This is a problem of importance in view of the general acceptance of S antigen as the main fraction of herpes CF antigen reflecting the immunity (Hayward, 1949; Lippelt and Soltz-Szots, 1959). After a perusal of literature, one can notice that the presence of the comparatively heat-labile CF antibodies in persons lacking NT antibodies was observed by at least two authors, Dascomb et al. (1955) and Yamaguchi.

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**Fig. 4.** A scheme for the course of events taking place in individuals with regard to herpes virus infection.
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1962

1959b). The former group of investigators found one CF positive serum among 36 NT negative persons, and the latter author stated that about a half of CF positive children of 7 to 10 years lacked in NT antibodies while few such cases were seen among adults. Although no emphasis on those findings was placed by themselves, nor was given any solid proof for its specificity, it is interesting that these observations were reported only from laboratories where the serum inactivation was done by heating at 56°C and not from any other places where the heating of serums was done at 60–62°C.

Analysing the present data in light of Budding et al’s results of an extensive examination of antibody rise and fall in primary infections (1953), one can suppose the course of events taking place in individuals throughout the life with regard to herpes virus infection, which is illustrated as a scheme in Fig. 4. Persons represented by Type 1 experience the primary infection apparently or inapparently in a young age, which is followed by repeated reinfections leading to the status of the persistence of latent infection. Persons of Type 2 do not experience enough reinfections to establish the persistence of virus in their bodies, and might appear as positives or negatives depending upon the time of bleeding. In former days Type 1 was predominant in any population, but, as sanitary conditions were improved, an increasingly larger portion of population has been occupied by individuals belonging to Type 2.

SUMMARY

A total of 352 serum samples taken from outpatients suffering from upper respiratory and other diseases and normal healthy adults were subjected to neutralization and complement-fixation tests with herpes virus. The 1-day egg technique was employed for the neutralization test because of its simplicity and satisfactory sensitivity. The antibody distribution was characterized by low positive ratios and low average titers during a 4-year period after birth followed by gradual increases in the positive ratio and in the average titer of positives. From the 5th to 20th years, titers of positive serums covered a wide range from nearly negative to higher than 1 : 320 but thereafter tended to approximate the all-or-none type distribution. Correlation between neutralizing and complement-fixing antibody titers revealed a fairly good parallelism, but some adults’ serums showed considerably high neutralizing antibody levels while lacking complement-fixing antibodies. On the other hand, some children’s serums were found to contain comparatively heat-labile complement-fixing antibodies capable of reacting with V antigen despite the lack of neutralizing antibodies. The meanings of these observations are discussed, especially in comparison with earlier workers’ data. It seems likely that sanitary improvements during the past decade have resulted in changes in the antibody distribution pattern.

REFERENCES

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