Recovery and Identification of Adenovirus in Infections of Infants and Children


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INTRODUCTION

During the past 6 years, the major concern of our laboratory has been to define the etiological role, ecology, and importance of new or newly recovered viruses and Mycoplasma in childhood respiratory-tract illness. Between October, 1957, and May, 1961, we studied 7,509 children, and were able to recover adenoviruses from 504 individuals. This experience made it possible to define certain facets of behavior of adenoviruses in the laboratory as well as in natural infection. In this paper, we will describe the frequency of recovery of different adenoviruses from throat and anal swab specimens, the sensitivity of primary simian and continuous-passage human heteroploid tissue culture for recovery of adenoviruses, and the complement-fixing (CF) antibody response of individuals from whom adenoviruses were recovered.

MATERIALS AND METHODS

Population Studied

Specimens for virus isolation were obtained from 7,509 infants and children from the Washington, D.C., metropolitan area during the period from October, 1957, through May, 1961. Of these, 1,879 individuals were admitted to the hospital for croup, bronchopneumonia, severe bronchitis, pharyngitis, or bronchiolitis, and 2,726 patients were seen in the Outpatient Department of the hospital for mild bronchitis or pharyngitis. In addition, control patients free from respiratory-tract disease were selected to match the first two groups as closely as possible in age, week of study, and other attributes. The control group consisted of 1,974 ambulatory patients and 930 individuals admitted to the hospital. Almost all individuals in this study population were from a low socioeconomic group and were under 6 years of age.

Specimen Collection, Tissue Inoculation, and Virus Recovery

Serum specimens were collected during the acute phase of illness and during convalescence from the infants and children who were hospitalized. Throat and anal swab specimens were collected by use of dry cotton swabs which were immersed in Hank's saline containing 0.5% gelatin, 500 units of penicillin, 500 µg of streptomycin, 50 µg of tetracycline, and 125 µg of either nystatin or amphotericin B per ml. Specimens obtained in the clinics or at bedside were kept refrigerated from time of collection until they were inoculated into tissue culture approximately 2 to 3 hr later. During periods when tissue cultures were not available, the specimens were immediately frozen at -65 C and stored until tested. Over 90% of the specimens were
inoculated into tissue culture immediately, whereas those specimens stored frozen were usually inoculated into tissue culture within 14 days after collection.

Adenovirus isolation was attempted in monolayer tissue cultures of continuously passaged (CP) human heteroploid epithelial cells (KB or HEp-2 cells) and primary monkey kidney (MK) epithelial cells. Two CP and two MK tubes were each inoculated with 0.2 ml of throat or anal swab specimen. Usually one tube of rhesus MK (Macaca mulatta) and one tube of vervet MK (Cercopithecus aethiops) were inoculated concurrently during the latter half of the study. There were periods, however, when only one kind of MK tissue was available. Rhesus MK was used exclusively during the first 2 years. From October, 1957, to March, 1958, KB cells were utilized, whereas from March, 1958, through May, 1961, HEp-2 cells were used. CP cultures were either purchased or prepared in the laboratory from KB or HEp-2 cells subcultivated twice weekly in bottles. Throat swabs were tested in MK and CP cultures during the entire study period. Anal swabs were also routinely tested in CP cultures only after August, 1959.

Tissue culture maintenance media were varied only slightly during the study. During the first 2 years, MK cell cultures were maintained with Eagles' basal medium and 0.2% SV-5 antiserum. During the last 2 years of the study, equal parts of Eagles' basal medium and medium 199 with 0.2% SV-5 antiserum were used. Maintenance medium of the primary cell cultures was exchanged every 5 to 7 days. Eagles' basal medium with 3% inactivated chick serum was used to maintain CP cell cultures during the study period; this medium was exchanged every 3rd or 4th day. Antibiotics were present in all maintenance medium in the following concentration per milliliter: penicillin, 100 units; streptomycin, 100 μg; tetracycline, 10 μg; and nystatin or amphotericin B, 25 μg.

Both MK and CF cells (KB and HEp-2) were incubated for the first 3 years of the study in stationary racks at 35°C for 2 to 3 weeks. During the last year, inoculated HEp-2 tissue cultures were placed on a drum, rotating 10 rev/hr, for 7 days and were then transferred to stationary racks for the remaining 2 weeks. Tissue cultures inoculated with throat and anal swab specimens were examined every 2nd or 3rd day for cytopathic effect (CPE). During the first half of the study, when KB tissue culture was used, each specimen was subpassaged routinely. The subpassaged KB cultures were observed for 10 to 14 days. During the last half of the study when HEp-2 tissue was used, cultures were observed for 21 days but were not routinely subpassaged.

Identification

Isolates which produced a CPE consistent with adenovirus and isolates which showed other CPE but which could not be otherwise identified were tested by complement fixation (Fig. 1). Preliminary identification was accomplished with a human serum pool containing adenovirus group-reactive CF antibody. Confirmatory identification was accomplished by use of paired sera from adenovirus-infected patients who developed a rise in group-reactive CF antibody during convalescence.

Adenovirus isolates thus identified were then placed in one of the four categories originally described by Rosen; these categories are based upon the hemagglutination pattern displayed with rat or rhesus erythrocytes (5). Viruses of group 1 agglutinate only rhesus erythrocytes and serotypes in this group include types 3, 7, 11, 14, 16, 20, 21, 25, and 28. Agents in group 2 produce complete agglutination of rat erythrocytes as well as rhesus erythrocytes, but titers are lower with the latter cells. This group includes adenovirus types 8, 9, 10, 13, 15, 17, 22, 23, 24, 26, 27, "29," and "30" (6). Viruses in group 3 produce incomplete agglutination of rat erythrocytes, but fail to agglutinate rhesus erythrocytes. Included in this group are types 1, 2, 4, 5, and 6. Viruses of group 4 do not agglutinate either rat or rhesus red cells. Thus far, this group includes only types 12 and 18. Agents falling into this category were tested by tissue culture neutralization with rabbit antisera for type 12 and type 18 adenoviruses. All the other isolates were typed by hemagglutination inhibition (HI) with the use of type-specific rabbit sera for those adenoviruses which produce the type of hemagglutination pattern observed. All dilutions for the HI tests were performed with isotonic saline,
except for viruses in group 3. The hemagglutination reaction of agents in this group is enhanced when a heterotypic antiserum from group 3 is added to the reaction mixture (4, 5). Since type 6 adenovirus was one of the least frequently encountered group 3 viruses in our studies, a 1:100 dilution of type 6 rabbit antiserum was used as a diluent for isolates of group 3. If an isolate in this group could not be identified as type 1, 2, 4, or 5, the test was repeated with another heterotype group 3 antiserum for enhancement.

Selection and preparation of erythrocytes. The concentration of erythrocytes used in hemadsorption (HA) and HI tests was determined visually. This procedure differed from the original method of Rosen, who used spectrophotometry for this purpose (5). Erythrocytes were diluted serially in saline to give successive concentrations ranging from 0.1 to 1.0%. A 0.4-ml amount of saline was placed in each tube, followed by 0.2 ml of erythrocyte suspension. The mixture was allowed to settle at 37°C for 1 to 2 hr. The highest dilution of erythrocytes that produced a compact button in the bottom of a tube was taken as the dilution to be used in the HA test. This usually corresponded to a 0.9% suspension for rat erythrocytes and a 0.6% suspension for rhesus erythrocytes.

Monkeys which yielded red cells suitable for adenovirus HA continued to yield suitable erythrocytes when such animals were tested over a 2-year interval. Blood was collected in Alsevers' solution (4 parts Alsevers' to 1 part blood) and stored at 4°C for a few days before it was washed three times in dextrose-gelatin-Veronal solution (DGV).

Those erythrocytes which had a satisfactory HA titer with type 3 adenovirus were also suitable for all other serotypes belonging to group 1. Antigen was prepared by growing the prototype strain of type 3 virus in CP cells; the inoculum was diluted so that CPE was maximal not earlier than 3 days. A single pool of type 3 virus was used throughout the study period to assess the agglutinability of different lots of rhesus erythrocytes. Rhesus erythrocytes were considered satisfactory if they could be agglutinated by type 3 virus diluted at least 1:32. However, antigen titration end points of 1:64 to 1:128 were obtained in most tests. Of the monkeys tested, 30 to 40% yielded satisfactory erythrocytes. Those erythrocytes which did not agglutinate type 3 adenovirus were used for adsorption of typing sera before such sera were used in the HI test.

Blood obtained by cardiac puncture from rats (Sprague-Dawley strain) was collected in Alsevers' solution (4 parts Alsevers' and 1 part blood) and kept at 4°C overnight. This material was then washed three times in DGV, and stored as a 10% suspension in DGV no longer than 7 days. Because of the unusual sensitivity of rat red cells to washing compounds, best results were achieved when disposable tubes were used. Although rhesus red cells were not as prone to spontaneous agglutination as rat red cells, disposable tubes were nevertheless used with rhesus erythrocytes as well.

Rat erythrocytes were satisfactory if a standard type 4 virus preparation produced agglutination at a dilution of 1:32 or greater. Rat erythrocytes which agglutinated satisfactorily with type 4 virus were also suitable for use with all other rat cell reactive adenoviruses. Approximately 75 to 80% of the rats tested were found acceptable. Rats yielding nonhemagglutinating erythrocytes were exsanguinated, and the red cells were used for adsorption of rat cell agglutinins from typing sera.

Treatment of hyperimmune sera. Hyperimmune rabbit sera were prepared by use of prototype strains of adenovirus grown in KB cells. These sera were then absorbed with kaolin and rat or rhesus monkey red blood cells (5). Equal amounts of 25% acid-washed kaolin suspended in saline and heat-inactivated (56°C for 30 min) rabbit serum diluted 1:5 were mixed and incubated for 20 min at room temperature with occasional agitation. The kaolin was sedimented by centrifugation at 325 g, after which the serum was decanted. To each 1 ml of diluted serum, 0.1 ml of a 50% suspension of either rat or rhesus red cells was added, and the mixture was incubated for 1 hr at 4°C. The type of red cell used for adsorption was determined by the subgroup in which the serum belonged. Erythrocytes were removed by centrifugation and the treated serum was tested for red-cell agglutination. This adsorption procedure was repeated if agglutinins were not removed by the first treatment.

Each serum was titered against the homologous antigen as well as against antigens within its own erythrocyte-reactive group. The virus (0.2 ml)-serum (0.2 ml) mixtures were shaken and were then incubated for 1 hr at room temperature before the addition of 0.2 ml of erythrocyte suspension. The erythrocytes were allowed to sediment for 2 to 3 hr at room temperature. The lowest dilution of each serum was tested for its ability to agglutinate the appropriate erythrocytes in the absence of antigen. The highest dilution of serum which completely inhibited rhesus or rat erythrocyte agglutination was considered to contain 1 unit of antibody. These
sera from different rabbits having similar homologous HI titers when tested against prototype antigen were pooled. After the titer of the pooled sera was rechecked, the serum was diluted to contain 8 antibody units per 0.2 ml and then was stored at -20 C.

HI test. Disposable flint-glass tubes with hemispherical bottoms were used in all tests.

A preliminary virus titration was performed in which each isolate was diluted 1:8 to 1:512 in twofold steps. To 0.4 ml of each dilution, 0.2 ml of the appropriate erythrocyte suspension was added. Erythrocytes were allowed to sediment at room temperature. The end point of an antigen titration was read as the last tube showing complete agglutination of rhesus or rat erythrocytes, or, in the case of partially agglutinating types, the last tube showing approximately 50\% agglutination of rat red cells.

Duplicate tests were performed with each serum and 8 HA units of virus. The virus-serum mixtures were incubated for 1 hr at room temperature. Then 0.2 ml of erythrocytes was added to each tube. The test was read after it had been incubated for 2 to 3 hr at room temperature. The test was not considered satisfactory unless replicate HI readings with a given serum agreed. A simultaneous antigen titration of each isolate was included in the test. If the isolate could not be identified and if more than 8 units of antigen were used, the test was repeated.

Adenoviruses that did not hemagglutinate initially were passaged in CP cells until all or almost all culture cells were destroyed after 3 to 5 days. Then the isolate was restested for hemagglutination with rat and rhesus erythrocytes.

Neutralization test. In the neutralization test for type 12 and 18 adenoviruses, an equal volume of undiluted infected tissue culture fluid and a 1:10 dilution of inactivated hyperimmune rabbit serum was incubated for 1 hr at room temperature and was then inoculated into rhesus monkey kidney, human embryonic kidney, or HEp-2 tissue culture. The test was read when the virus control cultures exhibited 50 to 75\% tissue destruction while CPE was not detectable in cultures containing either adenovirus 12 or 18 serum.

**Complement-Fixation Test**

In the complement-fixation test, 4 to 8 units of adenovirus type 2 antigen were used, and the technique was a modification of that described by Rowe (7). Type 2 was the adenovirus serotype most often recovered during the study period.

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of children</th>
<th>Per cent of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>166</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>14</td>
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<td>4</td>
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<td>0.4</td>
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<td>56</td>
<td>11</td>
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<td>12</td>
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<td>16</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>0.4</td>
</tr>
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<td>27</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>29</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Untyped</td>
<td>6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* A total of 506 virus strains were recovered from 504 infants and children. Type 3 and type 22 were recovered from one patient and type 5 and an untyped strain from another.

**Table 2. Comparison of recovery of adenoviruses from throat and anal swab specimens of 3,830 infants and children (August, 1959-May, 1961)**

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of individuals from whom adenovirus recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Respiratory disease patients</td>
</tr>
<tr>
<td></td>
<td>Control individuals</td>
</tr>
<tr>
<td></td>
<td>Throat only</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6, or 7</td>
<td>32</td>
</tr>
<tr>
<td>9, 10, 12, 16, 18, 22, 23, 25, 26, 27, 29, 30, or untyped</td>
<td>0</td>
</tr>
</tbody>
</table>

* A total of 506 virus strains were recovered from 504 infants and children. Type 3 and type 22 were recovered from one patient and type 5 and an untyped strain from another.
TABLE 3. Sensitivity of primary monkey kidney and continuously passaged heteroploid human cell cultures for recovery of 411 adenoviruses (August 1959-May, 1961)

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of isolates</th>
<th>Per cent of isolates recovered in indicated culture system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monkey kidney only</td>
<td>Heteroploid human cell only (HEP-2 or KB)</td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 6, or 7</td>
<td>361</td>
<td>0.8</td>
</tr>
<tr>
<td>9, 10, 12, 16, 18, 22, 23, 25, 26, 27, 29, 30, or untyped</td>
<td>50</td>
<td>2</td>
</tr>
</tbody>
</table>

![Fig. 2. Time required for detection of adenovirus in monkey kidney tissue culture (October 1955-May, 1961).]

RESULTS

Virus Recovery

Of the isolates, 89% were adenoviruses of types 1 to 7, and most of these were type 1, 2, 3, or 5 (Table 1). The remainder of the isolates were adenoviruses of the higher numbered designations, including three of proposed type 29 and two of proposed type 30 (6). In addition, six strains were recovered which have not yet been identified and may represent new serotypes. Thirteen of the adenoviruses recovered were type 12, and four were type 18.

Adenoviruses were recovered approximately two to three times more frequently from anal swab specimens than from throat swab specimens, as indicated in Table 2. This was true not only for patients with respiratory-tract illness but also for individuals in the control group. Adenoviruses of type 9 and above were recovered exclusively from anal swab specimens, although some patients with respiratory-tract illness yielded these viruses, and the throat swab specimens from these patients were obtained in the early phase of the illness.

The sensitivity of primary MK and CP heteroploid human cell cultures for recovery of adenoviruses is compared in Table 3 for that period during which comparable testing was performed. The CP HEP-2 or KB cells were far more sensitive than were MK tissue cultures. Lower numbered adenoviruses, i.e., type 1, 2, 3, 4, 5, 6, or 7, were frequently recovered on both tissues, but those of type 9 or above were much less frequently recovered in MK tissue culture. In fact, most adenoviruses above type 18 were recovered only in the heteroploid human cell cultures. Had we not used monkey kidney tissue cultures, we would have missed less than 1% of the adenoviruses of types 1 through 7 and only 2% of the adenoviruses above type 7.

Extensive experience with certain adenovirus serotypes permits comparisons to be made concerning (i) the time required for detection of viruses by use of different types of tissue culture, (ii) the time required for detection of virus from throat swab and anal swab specimens, and (iii) the time required for detection of CPE produced by different serotypes. These data are depicted in Fig. 2 to 5.

Several conclusions can be drawn from a review of these data.

(i) With most of the adenoviruses of lower numerical designation, 50% of the virus-positive throat swab specimens produced a CPE in monkey kidney tissue culture by the 6th or 7th day after inoculation, and 90% did so by the 11th or 12th day. Although continuous human cell cultures were more sensitive for detection of these adenoviruses, the time required for detection of CPE was longer than in MK. Thus, 50% of the virus-positive throat swab specimens containing a lower numbered adenovirus produced a CPE by the 9th day in heteroploid cell cultures, and 90% did so by the 15th day.

![Fig. 3. Time required for detection of adenovirus in monkey kidney tissue culture (October 1957-May, 1961).]
(ii) Throat swab specimens contained slightly greater amounts of virus than did anal swab specimens, or possibly some other factor operated to result in an earlier detection of pharyngeal adenovirus in both MK and continuous human cell culture.

(iii) Adenoviruses of types 1, 2, 3, and 5 seemed to produce CPE at similar rates. Adenovirus type 7 throat swab isolates produced CPE more rapidly in continuous cell cultures than the other types.

(iv) Those adenoviruses of a lower numerical designation were detected much sooner than those of types 9 and above. In general, the higher types not only exhibited slower growth in tissue culture but were often confirmed only on subpassage, a presumptive CPE having appeared at the time of last reading.

**CF Antibody Response**

Table 4 shows the CF antibody response by age of infants and children from whom adenoviruses were recovered during the period from 1957 through 1963. Of 225 infants and children, 19% developed a group-reactive antibody response to the type 2 CF antigen. The highest percentage of responses occurred in infants between the ages of 7 and 12 months.

In Table 5 the CF antibody response of patients from whom an adenovirus was recovered is analyzed to compare patients with or without illness, to show the response according to various adenovirus types, and to indicate the response according to whether adenovirus was recovered from a throat swab specimen or anal swab specimen, or both. CF antibody rises occurred almost
of infants and children from whom adenovirus 1, 2, 3, 5, or 7 was recovered and who were ill. CF antibody responses were infrequent in infants and children in whom virus was recovered only from the anal specimen.

In Table 6 the CF antibody response of patients from whom an adenovirus was recovered is analyzed according to the presence or absence of group-reactive CF antibody in the acute-phase sera. A CF antibody response occurred in 36% of ill patients from whom adenovirus 1, 2, 3, 5, or 7 was recovered and who did not have antibody in their acute-phase serum. Only 8% of patients from whom these agents were recovered but who had prior group-reactive antibody showed a response. This difference was not seen in the few patients from whom other types of adenoviruses were recovered. Antibody responses occurred frequently in control patients; however, the number of subjects was too few to draw any conclusions.

An inhibition of human CF antibody was observed when an excess of antigen was employed in the CF test. This phenomenon, however, was not responsible for the limited CF antibody response of adenovirus-positive infants and children described in this report. Chess-board titrations involving both antigen and serum dilutions indicated that the antigen dilution used in our routine tests was in the zone of optimal reactivity.

Thus, it appears that several factors are correlated with the group-reactive CF antibody response of patients from whom adenovirus is recovered. Factors which favor a CF antibody response include an age of 7 to 12 months, the occurrence of illness, adenovirus serotype (types
1, 2, 3, 5, or 7), the absence of prior group-reactive antibody, and recovery of adenovirus from the oropharyngeal area rather than the lower gastrointestinal tract.

**Discussion and Conclusions**

Although adenoviruses were among the earliest reported of the “newly uncovered” viral agents producing illness in man, the multiplicity of types and the expense and tedium of neutralization tests for identification of those viruses has limited the extent of epidemiological studies. Thus, there has been a need for sensitive, simple, accurate, and rapid isolation and identification procedures. The procedures reported in this paper, modified slightly from those originally described by Rosen, were found to meet these requirements.

The key to these procedures is that all but types 12 and 18 of the 28 previously recognized serotypes of human adenoviruses and apparently certain other newly defined serotypes hemagglutinate either rat or rhesus erythrocytes (5). As described in Materials and Methods, these adenoviruses can be divided into three hemagglutinating subgroups and one nonhemagglutinating group. The accuracy of this “differential hemagglutination” screening procedure is indicated by the fact that each of the adenovirus serotypes identified by this procedure exhibited the hemagglutination pattern characteristic of the prototype strain of its serotype. Selection of rat or rhesus erythrocytes which are sensitive in this procedure is important, and failure to screen erythrocytes might account for the difficulty in using the hemagglutination technique. Rhesus erythrocytes were used throughout our study, but grivet erythrocytes are also suitable (8).

In this study, a majority of the serotypes recovered from children were those previously thought to be prevalent in children, namely, types 1, 2, 3, and 5. Of all the serotypes, only 11% were of the higher numerical designation and all but 1% (six isolates) could be identified. Probably the presently untyped adenoviruses are indeed new adenovirus serotypes.

Although many of the adenovirus isolates in this study were recovered in both MK and heteroploid human tissue culture cells, 68% of the adenovirus types 1, 2, 3, 4, 5, 6, and 7, and 85% of the so-called “higher” types of adenovirus, were recovered only in heteroploid human tissue culture cells. Only 0.8 to 2% of the isolates would have been missed had heteroploid tissue culture alone been used. These findings confirm those reported by Grayston et al. (1) and Pal et al. (3). Direct comparison of the HEp-2 and KB types of heteroploid cells was not made, but the recovery rate of adenoviruses seemed to be about the same in both of these tissue lines.

In most instances in which an adequate comparison could be made, it appeared that CPE was evident sooner in tissue cultures inoculated with throat swab specimens than with anal swab specimens. This suggests that the amount of virus present in throat swab specimens may have been slightly greater, although it also is possible that a slight delay in production of cytopathogenicity could have been occasioned by the presence of some viral inhibitor in anal swab material. Nonetheless, in all types of subjects and with all serotypes, more adenoviruses were recovered from anal swab specimens than from throat swab specimens. During the period of this study, no adenoviruses of the higher numerical designation were recovered from throat swab specimens. (Three were recovered during a subsequent period.) More than likely, these findings result from the fact that these adenoviruses are present in the nasopharynx or oropharynx for a brief period early during human infection and that they then persist for a longer interval either in gastrointestinal mucosa or lymphoid tissue. A longitudinal study in which specimens are taken from the same subjects over a period of time would help to clarify some of these questions.

Only 19% of 225 individuals from whom an adenovirus was recovered showed a rise in CF antibody when type 2 adenovirus was used as a cross-reactive antigen. This fact should be considered in interpreting clinical or epidemiological studies based on complement-fixation tests. The fact that infants in the age group of 7 to 12 months showed the highest percentage of cross-reactive CF antibody rises probably reflects the fact that this is the period during which there is the highest incidence of infection with adenoviruses (2). This is the time of life when primary infection is most likely to occur, and, therefore, virus isolation is most likely to be associated with acute infection. Virus recovery from older infants and children is more likely to be associated with long-term or intermittent shedding of virus as a result of chronic or subacute infection. Group reactive CF antibody responses would be detected less frequently during such infection than during acute infection. The apparent influence of other factors, such as the greater likelihood of a CF antibody rise if the adenovirus was recovered from the throat rather than the feces and if the adenovirus was of a lower numerical designation, is also probably related to the likelihood that the subjects concerned were undergoing acute infection. The apparent limitation of antibody responses in subjects whose acute serum contained some cross-reactive antibody might result from...
several factors, the most likely of which is that CF antibody present as a result of infection with a different serotype in the recent past would mask a rise in antibody due to the presently infecting serotype.

**Summary**

In summary, the development of differential erythrocyte hemagglutination and the HI test as a standard laboratory tool for the typing of adenoviruses has been most effective in furthering the ability of a virus laboratory to identify adenoviruses simply and rapidly. Once an isolate was placed into a rhesus or rat erythrocyte hemagglutinating group, typing was readily accomplished. Adenovirus types 1 to 7 were recovered from both anal swab and throat swab specimens. They were detected more often from an anal swab than from a throat swab specimen but they were detected sooner from throat swab specimens. Adenoviruses of the higher numerical designations were recovered only from anal swab specimens and in general were detected with greater difficulty and later than the lower adenovirus types. CP human cell tissue cultures were more sensitive for adenovirus recovery than primary MK tissue culture. A limited number of infants and children from whom adenovirus was recovered displayed a group-reactive CF antibody response, and several factors were noted to increase the likelihood of such a response. The highest percentage of responses occurred in infants in the second half year of life, in patients from whom types 1, 2, 3, 4, or 7 adenoviruses had been recovered, and in those from whom the adenoviruses were recovered from throat swab specimens.

**Acknowledgments**

This is to acknowledge gratefully the assistance of Leon Rosen in rechecking the typing of a majority of the adenoviruses of higher numerical designation reported in this paper.

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**Literature Cited**