PICORNAVIRUSES: RAPID DIFFERENTIATION AND IDENTIFICATION BY IMMUNE ELECTRONMICROSCOPY AND IMMUNODIFFUSION

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PLATES XIII AND XIV

Immune electronmicroscopy (IEM) (Almeida and Waterson, 1969; Doane, 1974) has been used successfully for virus detection, identification and serology. For example, this relatively simple technique has been used to detect viruses that cannot be cultivated in vitro or are difficult to detect because they do not cause a cytopathic effect (Bayer, Blumberg and Werner, 1968; Kapikian et al., 1972, 1973, 1974; Feinstone, Kapikian and Purcell, 1973). In addition, IEM has been used to type viruses and to identify viruses directly from patient specimens (Anderson and Doane, 1973; Vassall and Ray, 1974; Valters et al., 1975). The purpose of this report is to present results of IEM and immunodiffusion (ID) studies of picornaviruses with human and animal sera. We used these techniques to search for group antibody in human sera to human and animal picornaviruses. In addition, we describe the potential use of IEM for the rapid grouping of viruses as human picornaviruses with human serum. The rationale for the use of appropriate human sera for grouping human picornaviruses is based upon the observation that these viruses appear to possess a common C-type antigen (Hughes et al., 1974) and that human sera contain antibody for this antigen. A group or C-type antigen is defined as an antigen that stimulates the production of non-neutralising antibody, and this antibody reacts not only with homologous but also heterologous antigens belonging to the same or related subgroups of viruses.

MATERIALS AND METHODS

Cell culture

HeLa cells were used to propagate viruses for IEM. The materials and methods for the propagation of HeLa cell cultures have been previously reported (Conant and Hamparian, 1968). A porcine-kidney cell line (SK), a feline-kidney cell line (CRFK), a grivet-monkey-kidney cell line (BGM-70) and a rhesus-monkey-kidney cell line (LLCMK2), supplied respectively by Drs Griesemer, Gillespie, Barron and The American Type Culture Collection, were

Received 13 July 1976; accepted 29 Sept. 1976.
TABLE I

Host cells used for virus propagation

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine enterovirus type 1</td>
<td>BGM-70</td>
</tr>
<tr>
<td>Feline calicivirus strain C14</td>
<td>HeLa</td>
</tr>
<tr>
<td>Coxsackievirus type B5</td>
<td>CRFK</td>
</tr>
<tr>
<td>Echovirus type 4</td>
<td>BGM-70</td>
</tr>
<tr>
<td>Echovirus type 9</td>
<td>HeLa</td>
</tr>
<tr>
<td>Encephalomyocarditis</td>
<td>BGM-70</td>
</tr>
<tr>
<td>Equine rhinovirus strain KERV</td>
<td>HeLa</td>
</tr>
<tr>
<td>Rhinovirus types 1A, 2, 14, 22, 31, 46, 54</td>
<td>BGM-70</td>
</tr>
<tr>
<td>Porcine enterovirus strain 036</td>
<td>SK</td>
</tr>
<tr>
<td>Poliovirus type 2</td>
<td>HeLa</td>
</tr>
<tr>
<td>Simian enterovirus type 2</td>
<td>LLCMK2</td>
</tr>
</tbody>
</table>

* See Materials and methods.

TABLE II

Agglutination of unheated picornaviruses as detected by immune electron-microscopy (IEM)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Homologous animal serum neutralisation titre*</th>
<th>Serum dilution used for IEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus type 2</td>
<td>5120/100</td>
<td>4000 (+)</td>
</tr>
<tr>
<td>Coxsackievirus type B5</td>
<td>1920/100</td>
<td>1000 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 2</td>
<td>3840/1000</td>
<td>5000 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 14</td>
<td>3840/3</td>
<td>250 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 54</td>
<td>≥ 5120/300</td>
<td>5000 (+)</td>
</tr>
<tr>
<td>Encephalomyocarditis</td>
<td>15 360/300</td>
<td>5000 (+)</td>
</tr>
<tr>
<td>Bovine enterovirus type 1</td>
<td>480/30</td>
<td>50 (+)</td>
</tr>
</tbody>
</table>

* Serum-dilution endpoint/TCID50 of test viral dose.
(+ ) = Positive agglutination.

propagated with materials and methods similar to those used for HeLa cells. (See table I for the host cells used for propagation of the various viruses.)

Viruses

The viruses used for IEM were poliovirus type 2, coxsackievirus type B5, echovirus type 9, bovine enterovirus type 1, encephalomyocarditis (EMC) virus and rhinovirus types 1A, 2, 14, 22, 31, 46 and 54. The methods of preparing these viruses, their partial purification and titration have been reported (Conant and Hamparian, 1968; Hughes, Thomas and Hamparian, 1973). Purified viral preparations were not used in all experiments because satisfactory results were obtained with crude cell lysates clarified by centrifugation at 400 g. Infectivity titres of viral preparations used for IEM experiments ranged from 5·5 TCID50 to 9·5 TCID50 per ml.
**Table III**

*Identification of agglutinated human picornavirus C-antigens by immune electronmicroscopy (IEM)*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Human serum no.</th>
<th>Serum neutralisation titre*</th>
<th>Serum dilution used for IEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus type 2</td>
<td>1</td>
<td>4/30</td>
<td>100 (+)†</td>
</tr>
<tr>
<td>Poliovirus type 2</td>
<td>3</td>
<td>10/30</td>
<td>500 (+)</td>
</tr>
<tr>
<td>Echovirus type 9</td>
<td>2</td>
<td>192/30</td>
<td>500 (+)</td>
</tr>
<tr>
<td>Echovirus type 9</td>
<td>3</td>
<td>24/100</td>
<td>500 (+)</td>
</tr>
<tr>
<td>Coxsackievirus type B5</td>
<td>2</td>
<td>12/100</td>
<td>500 (+)</td>
</tr>
<tr>
<td>Coxsackievirus type B5</td>
<td>3</td>
<td>20/3</td>
<td>100 (−)</td>
</tr>
<tr>
<td>Rhinovirus type 1A</td>
<td>3</td>
<td>4/10</td>
<td>5000 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 1A</td>
<td>4</td>
<td>24/30</td>
<td>250 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 22</td>
<td>4</td>
<td>8/300</td>
<td>250 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 31</td>
<td>4</td>
<td>4/3</td>
<td>250 (+)</td>
</tr>
<tr>
<td>Rhinovirus type 46</td>
<td>4</td>
<td>24/100</td>
<td>250 (+)</td>
</tr>
<tr>
<td>Encephalomyocarditis</td>
<td>2</td>
<td>4/30</td>
<td>100 (−)</td>
</tr>
<tr>
<td>Encephalomyocarditis</td>
<td>3</td>
<td>20/30</td>
<td>100 (−)</td>
</tr>
</tbody>
</table>

* Serum-dilution endpoint/TCID50 of test viral dose.  
† (+) = Positive IEM response; (−) = negative IEM response.

In addition to the viruses listed above, the following were used in ID tests: porcine enterovirus, strain 036; bovine enterovirus type 1; feline calicivirus, strain C14; equine rhinovirus, strain KERV; echovirus type 4; and simian enterovirus type 2, strain 2382. These viruses were obtained from Drs R. Griesemer, T. Moll, J. Gillespie, J. Todd and The American Type Culture Collection, respectively. All viruses were triple-plaque-purified (Conant, Somerson and Hamparian, 1968). After plaque purification, each virus was tested for the presence of contaminating viruses by the serum-breakthrough-neutralisation method (Hampil and Melnick, 1969).

**Sera**

Immune sera to viral antigens were produced either in guinea-pigs or in rabbits. The procedure for the preparation of hyperimmune sera in guinea-pigs has been reported (Hughes et al., 1974). Rabbit antisera were prepared by weekly intravenous injections of 1 ml of fluorocarbon-treated aqueous viral preparations for a period of 6 weeks. The animals were exsanguinated 7–10 days after the last injection.

Normal human sera (NHS) for IEM were obtained from four persons aged respectively 12, 33, 48 and 74 years. Normal human sera used in immunodiffusion tests were obtained from 21 subjects with ages ranging from 21 to 45 years.

**Serological techniques**

Initially, neutralisation tests were performed with HeLa cell cultures by a macromethod described previously (Conant and Hamparian, 1968). In later experiments, micro-neutralisation tests were performed with HeLa cell cultures in Falcon Microtest II tissue-culture plates (Falcon Plastics, Oxnard, Ca.). Tissue culture wells were inoculated with 20000 HeLa cells per 0·1 ml, and incubated overnight at 37°C in a CO₂ incubator. The next day, cell culture supernates were removed and maintenance medium was added to each well. Antisera were heated at 56°C for 30 min. to inactivate complement. Equal volumes of diluted antiserum and test-virus dose (30–300 TCID50) were mixed in disposable trays, Model 96WS (Joseph E. Frankle Co., Philadelphia, Pa.) and incubated at room temperature for 1 h.
Subsequently, 0.1 ml of each mixture was added to each of two microtest wells. The plates were sealed, placed at 33°C for rhinoviruses and at 37°C for other picornaviruses, and examined daily for cytopathic effects for 5 days. The exact viral concentration used in each neutralisation test was determined by a simultaneous back titration in microplates of the input virus. The serum neutralisation titre was expressed as the highest serum dilution completely neutralising the test-virus dose.

Immunodiffusion tests were done as previously described (Hughes et al., 1974). To minimise cross-reactions due to common host-cell antigens, all ID antigens were prepared in cell cultures grown and maintained in rabbit serum. All antisera were prepared by immunising animals with antigens prepared in cell cultures grown and maintained in media with foetal bovine serum. Where possible, the immunising antigens also were prepared in a completely different host-cell system (see table I). Control antigens were prepared from uninfected cells by identical methods.

Acid inactivation of viruses

Virus inactivation was accomplished by a method similar to that described previously (Hughes et al., 1974). One-ml portions of virus were dialysed against 100 volumes of citrate-phosphate buffer, pH 3.0, for 1 h at room temperature followed by 2 h at 4°C. Controls were dialysed at pH 7.2. Subsequently, both control and experimental preparations were dialysed overnight at 4°C with 500 volumes of 0.01 M Tris buffer, pH 8.1. For rhinovirus type 14, greater than 99.99 % of viral infectivity is lost under these conditions (Hughes et al., 1973).

Immune electronmicroscopy

Sera for IEM were heated at 56°C for 30 min., diluted in phosphate-buffered saline (PBS, 0.005 M phosphate), pH 7.4, and filtered through a 0.45 μm Millipore membrane with a Millipore AP20 prefiltet. Serum controls for non-specific viral agglutination consisted of either pre-immune animal serum or PBS. As an additional control for specificity, reciprocal IEM tests were done with poliovirus type 2, coxsackievirus B5 and their hyperimmune animal antisera. An appropriate serum dilution (0.9 ml) and the virus suspension (0.2 ml) were mixed and incubated for 30 min. to 1 h at 37°C. Some mixtures were held overnight at 4°C. The mixtures were centrifuged in 15-ml Corex centrifuge tubes for 90 min. at 45 000 g by the use of the SS-34 rotor with adapters in a Sorval RC2-B centrifuge.

Pellets obtained by centrifugation were resuspended in one drop of sterile, pyrogen-free water. The pellet area of the tube was vigorously scraped with a Pasteur pipette. One drop of 3% phosphotungstic acid (pH 7.0) was added to the suspension and a drop of the mixture was placed on a 300-mesh Formvar-carbon-coated copper grid. Excess fluid was removed with filter paper within 10–60 s and the grid allowed to air dry. Grids were examined in a Hitachi HU-12 Electron Microscope and pictures were taken at magnifications of 60 000–200 000. Each grid was searched for 20–30 min. If no viral aggregates or free virions were found, a second grid was prepared and examined.

RESULTS

IEM with animal and human sera

Virions agglutinated with antibody were readily recognised upon direct viewing in the electron microscope. Electronmicrographs revealed aggregates of virions surrounded by a diffuse matrix of antibody-like material. Generally three types of aggregates were seen. Two of these types of immune aggregates appeared to be related to the amount of antibody present. At low dilutions of serum, the immune aggregates were loose, contained relatively few virions and were surrounded with a dense halo of antibody-like material (fig. 1). At
Fig. 1.—Negatively stained immune complex of human rhinovirus type 1A converted to C particles by heating at 56°C for 15 min. and mixed with human serum diluted 1 in 100. Notice the empty (stain penetrated) particles. ×210 000.

Fig. 2.—Negatively stained immune complex of unheated poliovirus type 2 mixed with homologous animal antiserum diluted 1 in 4000. The serum-neutralisation-end-point titre was 5120 when tested against a viral dose of 100 TCID50. Notice the intact stain excluded particles. ×275 000.

Fig. 3.—Unheated coxsackievirus B5 mixed with homologous animal serum diluted 1 in 100. The serum-neutralisation-end-point titre was 1920 when tested against a viral dose of 100 TCID50. ×45 000.

Fig. 4.—Unheated poliovirus type 2 mixed with human serum diluted 1 in 100. ×100 000.

Fig. 5.—(A) Coxsackievirus B5 mixed with PBS; (B) encephalomyocarditis virus mixed with normal rabbit serum. Both ×52 000.

Fig. 6.—Immune complex of unheated encephalomyocarditis virus mixed with homologous animal antiserum diluted 1 in 5000. Notice halo surrounding virions. ×375 000.
IMMUNE ELECTRONMICROSCOPY OF PICORNAVIRUSES

Fig. 7.—Precipitation reactions of a normal human serum with echovirus type 4 and rhinovirus type 2.

Fig. 8.—Precipitation reactions of normal bovine sera with and without neutralising antibody diffused against bovine enterovirus type 1.
low antibody concentrations, when serum dilutions close to the neutralisation endpoint were used, the immune aggregates were small and antibody was easily seen (fig. 2). A third type of immune aggregate can be seen in fig. 3. Hundreds of virions in dense matrices are present.

When seven unheated picornaviruses were tested with homologous animal antisera, immune aggregates were seen consistently. The homologous serum neutralisation titres and the serum dilutions used for each IEM reaction are listed in table II. When nine picornaviruses were heated and allowed to react with different human sera diluted past their serum-neutralisation endpoints, positive IEM results were consistently observed only with the human viruses (table III). Although NHS-1 from a 12-year-old child had no detectable neutralising antibody at a dilution of 1 in 4 for the three poliovirus serotypes, it agglutinated heated poliovirus at a dilution of 1 in 100. Fig. 4 shows an immune aggregate consisting of poliovirus type 2 and human serum. The aggregate consists of intact and empty particles. With a single exception, all human sera were used at dilutions 10-fold or greater than their neutralisation-endpoint dilutions and all human sera gave positive IEM results except NHS-3 which did not react with coxsackievirus B5 (table III).

Control preparations consisting of virus and pre-immune animal sera or virus and saline contained only single virions. Figures 5a and 5b are examples of this kind of reaction. Non-specific clumping was not apparent. A control consisting of coxsackievirus B5 and poliovirus with heterologous animal antiserum (diluted 1 in 100) was also negative for immune aggregates. Control mixtures consisting of an unheated animal picornavirus, EMC virus, tested with NHS-2 and NHS-3 diluted 1 in 100 were negative for immune aggregates. No immune complexes were seen when heated EMC virus preparations were used with NHS. When unheated EMC virus was tested with the homologous rabbit serum, immune complexes were readily seen (fig. 6).

**Differentiation of human picornaviruses by IEM**

Preparations of rhinovirus type 14 and poliovirus type 2, after treatment at pH 3.0 and adjustment to pH 7.2, were allowed to react with either homologous animal antisera or with NHS, or both, and examined in the electron microscope. Preparations of both viruses treated at pH 7.2 and mixed with immune serum were included for control purposes. No immune aggregates were observed with the rhinovirus preparations treated at pH 3.0. All other tested preparations were positive for immune aggregates (table IV).

**ID tests with hyperimmune animal sera and normal human sera**

Immunodiffusion tests performed with animal hyperimmune sera formed precipitin lines only with homotypic antigens. No lines were formed when animal hyperimmune sera were diffused against normal-cell concentrates or heterologous viral antigens.

The frequency of antibody to nine picornavirus antigens (three human and six animal) in 21 NHS was determined by ID (table V). Encephalomyocarditis
TABLE IV

Differentiation of human picornaviruses by immune electronmicroscopy (IEM)

<table>
<thead>
<tr>
<th>Viral antigen</th>
<th>Serum</th>
<th>Animal</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinovirus type 14, pH 7.2</td>
<td>+*</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Rhinovirus type 14, pH 3.0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Poliovirus type 2, pH 7.2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Poliovirus type 2, pH 3.0</td>
<td>+</td>
<td>N.T.†</td>
<td></td>
</tr>
</tbody>
</table>

* (+) = Positive IEM response; (−) = negative IEM response.
† Not tested.

TABLE V

Frequency of antibody to picornaviruses in normal human sera

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Number of sera, of 21 tested, that reacted with the indicated virus in neutralisation test†</th>
<th>precipitation test‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human rhinovirus type 2</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Coxsackievirus type B5</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Poliovirus type 2</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Bovine enterovirus type 1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Porcine enterovirus strain 036</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Simian enterovirus type 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feline calicivirus strain C14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Equine rhinovirus strain KERV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Encephalomyocarditis</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The cell cultures used for preparing immunodiffusion antigens are listed in table I.
† All sera were tested at a 1 in 4 dilution against 30-300 TCID50 of each virus.
‡ Immunodiffusion tests were performed with undiluted sera, and all antigens were tested in a homologous system with hyperimmune animal sera to ensure the reactivity of viral antigens.

...
Attempts to strengthen the specific lines by varying the concentration of the reagents and the size of the wells were unsuccessful. With poliovirus type 2, all NHS gave two precipitin lines, and all sera contained neutralising antibody to poliovirus.

A typical immunodiffusion test with human serum and two human picornavirus antigens is displayed in fig. 7. In this test echovirus type 4 and rhinovirus type 2 were diffused against NHS. A specific line for the rhinovirus and a group line for both viruses are present. The group line demonstrates partial relatedness with a weak spur appearing on the rhinovirus group line.

**ID tests with normal bovine sera**

Immunodiffusion tests with bovine enterovirus (BEV) and normal bovine sera (NBS) gave single precipitin ID lines with 13 of 14 sera. Normal bovine sera containing neutralising antibody to BEV also formed only single precipitin lines. The neutralisation titres in all cases were 32 or less. To determine whether the single ID line represented both group and specific reactivities, an immunodiffusion experiment was performed (fig. 8). Two NBS with neutralising antibody {top right (NB9) and right (NB10) wells} and two NBS without neutralising antibody {top left (NB8) and left (NB7) wells} were diffused against bovine enterovirus antigen (centre well). From fig. 8 it can be seen that the precipitin lines with both the neutralising and non-neutralising sera merge without forming a spur, indicating that the line formed with the serum lacking neutralising antibody is by definition a group line. As might be expected, the precipitin line formed with the serum containing neutralising antibody was stronger. These results indicate that, in this system, group and specific antibodies appear to diffuse at the same rate.

**DISCUSSION**

By IEM, Anderson and Doane (1973), working with 11 different enterovirus serotypes, found no cross-reactivity with 15 different animal enterovirus sera at dilutions of 1 in 50 or greater. When we used type-specific animal antiserum at dilutions of 1 in 100, we also did not observe any cross-reactivity among picornaviruses. Serological cross-reactions among picornaviruses have been demonstrated by IEM by Chaudhary, Kennedy and Westwood (1971), but cross-reactions were not observed in all cases. The viruses they used, both for immunisation of animals and for IEM, were propagated in primary monkey-kidney-cell cultures. Moreover, the antigens for IEM were "purified" by differential centrifugation only and the antisera were used at very high concentrations (undiluted or 1 in 2). Under these conditions, the possibility of non-virion host-cell antigens and antibodies being present and affecting the specificity of IEM results cannot be excluded. Furthermore, their virus preparations might have been contaminated with heterologous enteroviruses, since serum-breakthrough-neutralisation tests were not done.

Hummeler, Anderson and Brown (1962) used specific anti-D and anti-C sera and IEM to distinguish between C and D particles of poliovirus type 1.
Kapikian, Almeida and Stott (1972) also showed that complete "full" and incomplete "empty" rhinovirus particles could be seen by IEM, but did not use specific anti-D or anti-C antibody. We used NHS and seven different picornaviruses and were able to detect enterovirus and rhinovirus C-type antigens and antibody as immune complexes. The human sera used for these reactions were diluted in most cases 10- to 50-fold and sometimes more than 1000-fold beyond the homologous serum-neutralisation endpoints (table III).

With IEM, the dilutions at which homologous hyperimmunne animal sera can be used successfully with unheated (D type) picornaviruses appear to be directly related to the neutralising-antibody content of the sera (see table II). The animal antisera for rhinovirus type 14 and bovine enterovirus type 1 were not tested at higher dilutions and no inferences can be made for these antisera. Since no attempt was made to determine the IEM endpoint titres of any of these sera, the comparative sensitivity of the two serological techniques for detection of D antigens in our system remains to be determined. However, Anderson and Doane (1973), using a serum-in-agar IEM-diffusion method, found that their IEM endpoints for three different picornaviruses were approximately equivalent to their serum-neutralisation endpoints.

We did not compare the sensitivity of IEM and the complement-fixation test (CFT) for the detection of C-type antigens of, or antibody to, picornaviruses. However, relatively large amounts of viral antigen are necessary for both CFT and precipitin tests. For these tests, most if not all picornaviruses need to be concentrated. In contrast, the IEM technique was capable of detecting virions in unconcentrated preparations with low infectivity titres.

Previous reports from this laboratory have demonstrated the acid lability of rhinovirus type 14 and the loss of D and C antigenicity after acid treatment (Hughes et al., 1973, 1974). Data presented in this paper (table V) support these observations. By IEM, we were unable to detect either D or C type antigens for rhinovirus type 14 after treatment at pH 3.0. Reeves and Mayor (1973), using acid-treated (pH 3-0) rhinovirus type 14 were not able to detect complete virions by electronmicroscopy. They concluded that the pH at which a marked loss of infectivity occurred correlated with the almost complete degradation of virion capsids. Thus, IEM after acid treatment appears to be a simple and rapid means of differentiating rhinoviruses from enteroviruses.

With one exception, each of the 21 NHS tested by ID had group antibody to the three human picornaviruses. Only a single human serum reacted by ID with one of the six animal picornaviruses (table V). These results corroborate our IEM results with human sera and heated picornaviruses (table III). Possibly, the lack of group antibodies to animal picornaviruses in human sera is due to limited contact with animal picornaviruses; or the host range of these animal picornaviruses may not include man; or a more sensitive test may be necessary to detect group antibodies to animal picornaviruses in human sera. It would be of interest to test sera for group antibodies from persons who have close and long-term contact with animals. The reason for the lack of reactivity by ID of one human serum for coxsackievirus B5, and the lack of reactivity by IEM for this same virus by another human serum is not clear. This might
be due to the presence of an antigen with predominantly subgroup specificity or to the fact that only six serotypes of coxsackievirus group B are known, resulting in limited contact of man with these viruses.

The quality and quantity of group-reactive antibody in a human serum appears to be related to the previous experience of the subject. The presence of group antibody to bovine enterovirus in normal bovine sera, as reported in this paper (fig. 8), suggests that what applies for human sera may also be true for animal sera. By successive infections of monkeys with coxsackieviruses, Schmidt et al. (1965) demonstrated that the spectrum of group-precipitating antibodies broadened to include coxsackievirus serotypes not previously experienced by the monkeys. These workers suggested that the mechanism and pattern of antibody responses to coxsackieviruses in naturally infected human subjects might well be explained by the findings in infected monkeys. Thus, after initial infection with a virus belonging to one of the human picornavirus subgroups, a person would produce group antibody of narrow specificity capable of reacting only with the viruses of that subgroup. After additional experience with viruses containing group antigens, antibody capable of reacting with other group antigens would eventually be produced. This sharing of group antigens between human picornavirus subgroups (fig. 7) suggests that in addition to physicochemical properties, appropriate serological tests can be useful in the classification of human picornaviruses. Previously we suggested the possibility of grouping picornaviruses by acid lability (Hughes et al., 1974).

The current technique of IEM has the decided advantage of being able rapidly to identify viruses directly from patients or after primary isolation in cell culture. Furthermore, the identification of viral isolates that belong to viral groups comprising multiple serotypes should be possible by IEM without specific serotyping of the isolate with numerous monotypic antisera or pools of antisera. Our IEM results with seven picornaviruses and human sera suggest that IEM may provide a simple means for the rapid grouping of human picornaviruses.

**Summary**

Immune electronmicroscopy (IEM) was used to identify human picornaviruses rapidly and to differentiate enteroviruses from rhinoviruses. Human sera, diluted 10- to 50-fold beyond the neutralisation endpoints for homologous virus, readily agglutinated C-type antigens of seven human picornaviruses. Human sera did not react by IEM with a control animal picornavirus. By IEM after acid treatment, differentiation of a human enterovirus from a human rhinovirus was possible. There was an excellent correlation between the results of IEM and immunodiffusion (ID) tests for the presence of antibody to human picornavirus group antigens. By ID, only one of 21 human sera reacted with one of six animal picornaviruses. Immune electronmicroscopy appears to be a sensitive and simple technique for the detection of picornavirus C-type antigens, and may be useful for identifying viruses belonging to groups comprising many serotypes and sharing a common group antigen.
REFERENCES


