Virus Multiplication in Organ Cultures of Human Embryo Small Intestine

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In natural virus infections the precise role of the gastrointestinal tract either as the portal of entry or as the initial site of virus multiplication has not been established. It is generally considered, however, that it is an initial site of replication for many enterovirus members of the picornavirus group (Downie, 1963). Twenty years ago Enders and his colleagues demonstrated that the LANSING strain of the poliomyelitis virus multiplied in suspension cultures of human embryo intestine, thus recording the first in vitro multiplication of a virus in human embryo intestinal tissue (Enders, Weller & Robbins, 1949). Cells in organ cultures are similar in structure and function to that of the intact host and thus may provide a convenient experimental model for studying some aspects of the pathogenesis of certain virus infections. In addition, organ cultures have been used to isolate and study the properties, including the structure, of some viruses which cause acute respiratory infection and cannot be grown in monolayer cell cultures (Tyrrell & Bynoe, 1965).

The normal human embryo intestine is sterile and the cells are morphologically and functionally differentiated by about 14 weeks. The purpose of this report is to describe a method whereby human embryo small intestine may be employed as an organ culture system suitable for virus studies and to demonstrate the multiplication of tissue-adapted and wild strains of enteroviruses in the system.

The complete intestines of 14 to 24 week embryos were obtained from Dr H. E. M. Kay, Tissue Culture Bank, The Royal Marsden Hospital, London. Following delivery and sterile dissection the intestines were placed in 199 medium in a universal container and maintained at 4° until processing which occurred within 8 hr of delivery. Initially, the bowel was transferred to sterile disposable plastic Petri dishes where the peritoneal serosal coat was stripped off. Lengths (2 cm.) of the small intestine were prepared. These were placed on previously prepared sterile plastic tissue culture dishes, 60 mm. x 15 mm. Falcon Plastics, which had been scored with a scalpel to allow adherence of the serosal surface of the bowel. The lengths of the intestine were then opened using a pair of fine sterile scissors and the mucosal surface exposed with a minimum of trauma to the intestinal villi. Alternatively, a number of small pieces of bowel, about 4 mm.², were placed on to a single plate and this was convenient if histological examination was required. The sections of small intestine were left undisturbed for about 20 min. to allow for adherence and thus prevent subsequent floating off when the medium was added. Following the addition of 1·25 ml. of organ culture medium the plates were placed immediately in a desiccator where final CO₂ concentration of 2% was achieved using a lighted candle. The desiccator was placed in an incubator at 37°. The maintenance of the organ cultures involved complete medium changes performed on alternate days, 1·25 ml. of organ culture fluid being replaced on each occasion. During early experiments it was observed that sections of intestine exposed to a medium of pH greater than 6·8 did not survive long enough to allow for virus multiplication experiments to be performed. The organ culture medium employed consisted of Eagle’s (MEM) 97 ml., NaHCO₃ (7·5%, w/v) 0·5 ml., calf serum 2 ml., penicillin 100 μg./ml., streptomycin 100 μg./ml.

Initially the organ cultures were observed overnight to exclude contamination with bacteria.
or fungi, but as this proved very infrequent it was considered desirable that the cultures should be utilized as soon as possible. In the growth curve studies with tissue-culture-adapted strains of viruses about 1000 TCID 50 in 0.1 ml. volume of organ culture fluid, was added to the plate. Virus adsorption was allowed to proceed for 15 hr before the fluid was removed, the cultures were washed and fresh organ culture medium was added. When stool specimens were employed, 0.2 ml. of the stool suspension was added to the plate, but otherwise the experiment was as described. In each experiment, between 4 and 6 plates were employed for each virus or specimen and the individual result given in the back titration studies in monkey kidney tissue culture was obtained on the pooled fluids.

Fig. 1. Human embryo small intestine after 8 hr (×256).

Fig. 1 is a section of human embryo small intestine from a 14 week old embryo 8 hr after delivery and presents the typical appearance of the cultures prior to inoculation. It can be seen that most of the epithelial cells are structurally intact and numerous goblet cells are present (g). The appearance of the same culture after 32 hr is presented in Fig. 2 and already some atrophy of the villi has occurred with necrosis of some of the epithelial cells (v). Fig. 3 presents the situation after 4 days and the villi are now atrophied and marked necrosis of the cells has taken place (d).

The growth curve of a tissue-culture-adapted strain of echovirus type 11 in human embryo small intestine organ cultures is presented in Fig. 4. The pattern of behaviour is compared with the fall-off in titre of this virus when samples were titrated for infectivity at selected intervals and therefore is a measure of the thermal inactivation of this strain at 37°. Taking into consideration the multiple dilution factor of complete medium changes and the titres obtained the results indicate that virus multiplication has occurred. The virus obtained from the organ cultures was neutralized, and complete suppression of cytopathic effect was obtained with standard hyperimmune antiserum to echovirus 11 on two separate occasions.
Fig. 2. Human embryo small intestine after 32 hr (×256).

Fig. 3. Human embryo small intestine after 4 days (×256).
Table I presents a quantitative study of the amount of virus present in stool before and after passage in organ cultures of human embryo small intestine. The results indicate that in each case multiplication of the agent has occurred to varying degrees which may represent the tissue tropism of the different viruses. Thus there is evidence that the cultures can act as a multiplier system.

![Growth curve of tissue-culture-adapted strain of echo 11 virus in human embryo small intestine organ culture.](image)

**Fig. 4.** Growth curve of tissue-culture-adapted strain of echo 11 virus in human embryo small intestine organ culture. ○——○, Fall off in titre of echo 11 virus at 37°C; •——•, growth curve of echo 11 virus at 37°C. Titre expressed as log. dilution.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Log. dilution giving TCID 50 Before H.E.I.O.C.</th>
<th>Log. dilution giving TCID 50 After H.E.I.O.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echo 3</td>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Echo 7</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Echo 8</td>
<td>0.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Echo 12</td>
<td>1.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Echo 6</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

(Positive—human amnion)

The results of the histological studies indicate that even after 24 hr some cellular destruction, especially in the centre of the organ cultures, is apparent. Thus the application of the method in its present form is unlikely to provide clearcut histological evidence of the type of cell involved in the initial virus-cell interaction and the subsequent pathogenesis. The histological integrity of the human embryo tracheal organ cultures persists for upwards of 2 weeks under similar conditions, and the present findings may represent the more rapid turnover of intestinal epithelial cells if an analogous situation to that in the adult is present. However, the application of immunofluorescent and electron microscopy before gross cellular damage occurs is likely to give information on the initial infection process.

The growth curve studies indicate that echovirus 11 underwent several cycles of multiplication, and it is not possible to state in which cells the viruses multiplied. The persistence of a high titre at the tenth day after infection with echovirus 11 may mean continued virus multiplication in other than epithelial cells but it may be also a reflection of enhanced virus
release from cells undergoing necrosis. A practical application of the combination of virus multiplication with adequate virus release is that the method may be employed as an initial multiplier system, thus allowing for ease of handling by a secondary indicator system. The results of the experiments quantitating virus in stool specimens before and after passage in organ cultures confirm the ability of the organ cultures to act as such a multiplier.

In summary, the organ culture experiments have two advantages: they allow the initial interaction between an enterovirus and human small intestine to be recorded and they provide a means of enhancing the amount of virus present in a clinical specimen thus providing the baseline for further examination in indicator specimens.

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REFERENCES


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