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Adaptation of Coxsackie Virus Strain A-10 to Human Amnion Cells in Tissue Culture.* (26173)

T. H. DUNNEBACKE AND C. F. T. MATTERN† (Introduced by W. M. Stanley)
Virus Laboratory, University of California, Berkeley

Following the purification of poliovirus(1) and Coxsackie virus A-10(2), an effort has been made to compare a number of properties of these 2 enteroviruses. Much is known about the biology of the polioviruses, but since Coxsackie virus A-10 could be grown only in suckling mice it was impossible to make a comparative study of the biological behavior of these physically similar viruses. Therefore, attempts were made to adapt Coxsackie virus A-10 to tissue culture. Coxsackie virus A-10 was inoculated onto cultures of cells of human origin (primary amnion(3), HeLa, FL cells(4), and amnion strains A_1-A_7(5)). It was found that the virus multiplied in and caused a cytopathic change in the cells in the primary human amnion cultures but not in the cells of the other cultures tested.

A sample of highly purified, suckling mouse grown Coxsackie virus A-10 was diluted in medium 199 containing 20% lamb serum and sterilized by filtration through a Seitz pad. The sterile filtrate was further diluted to have a titer of about 8.4×10^7 suckling mouse LD_{50} per plate of cells, or about 50-100 LD_{50} per cell.

The virus inoculum was placed on 8 petri plates, 4 from each of 2 different preparations of primary human amnion cells (A-847, A-852). Two additional plates from each amnion preparation served as controls and contained only medium 199 with 20% lamb serum.

The control and the inoculated plates were observed daily, and on days 1, 3, 6 and 10, one inoculated plate from each cell preparation was removed from the incubator. The supernatant medium was removed and frozen; the cells on the plates were washed with phosphate buffer, fixed in Bouin's fluid, and stained with haematoxylin. Additional medium (4 ml 199-20% lamb serum) was added to the remaining plates on day 5. Control plates were sampled on days 6 and 10.

On days 1 through 6, no noticeable difference between control and inoculated cells was observed either before fixation or after fixation and staining. On the 8th day, the inoculated plates appeared to differ somewhat from controls, and by the 10th day they were grossly cytopathic. The cells on the control plates appeared to be healthy and well-maintained at 10 days.

The supernatant fluid from each of the 10-day inoculated cultures was diluted 1:5, and each was placed on 2 plates of amnion preparation A-856. The cells of A-856 were grossly cytopathic 3 days after inoculation with the supernatants of both A-847 and A-

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† Present address: Lab. of Biology of Viruses, Nat. Inst. of Allergy and Infect. Dis., N. I. H., Bethesda, Md.

852. An aliquot of the A-847 supernatant was inoculated into suckling mice at log dilutions −2, −3, and −4. None of the mice showed any of the symptoms typical of Coxsackie A-10 infection.

A neutralization test was carried out on the supernatant medium from A-856. Dilutions of 10^{-1} to 10^{-6} were placed on plates of amnion preparation A-857 containing 4 ml 199 with 2% normal monkey serum and 0.1 ml normal rabbit serum, and on plates containing 4 ml 199 with 2% normal monkey serum and 0.1 ml rabbit antiserum to Coxsackie A-10‡. On the third day after inoculation the plates with the Coxsackie A-10 antiserum contained cells of normal, healthy appearance. The plates with no A-10 antiserum were grossly cytopathic at dilutions 10^{-1} through 10^{-4}. The plates with dilutions of 10^{-5} and 10^{-6} became grossly cytopathic after 5 days of incubation. The cytopathic agent was completely neutralized by Coxsackie A-10 antiserum and presumed to be a tissue culture adapted strain of Coxsackie virus A-10 (A-10TC). The virus has been subsequently passaged in many amnion preparations. It shows a cytopathic change within 24 hours and after 11 passages in amnion cells, it was again completely neutralized by Coxsackie A-10 antiserum.

Assays of the tissue culture adapted virus A-10TC, were repeatedly attempted in suckling mice. Titers of $<2.5 \times 10^{2}$ were occasionally found; however, it appeared that A-10TC had lost most of its mouse virulence.

Samples of A-10TC were inoculated onto a monolayer culture of primary human amnion cells in the routine manner for plaque assays of poliovirus(6,7) and plaques 1-3 mm in diameter developed 3-5 days after inoculation. Titers of 1×10^{6} to 6×10^{7} plaque-forming units have been obtained for various stock preparations. Individual preparations of amnion cultures appeared variable in their ability to support plaque formation of A-10TC. Occasionally an amnion yielded no plaques ($<10^{-4}$ titer), whereas simultaneous assays of the same virus sample on other preparations yielded, for example, titers of 1.5×10^{6}, 1.8×10^{6}, and 3.0×10^{6}.

Cytological observations of human amnion cells inoculated with A-10TC showed that the cells undergo the same cytopathic changes observed in human amnion cells after inoculation with poliovirus(8).

This evidence indicates that a strain of Coxsackie virus A-10 has been adapted, or selected, to grow in tissue cultured cells of the human amnion. The virus A-10TC has lost considerable mouse virulence. It is neutralized by Coxsackie A-10 antiserum, and elicits a cytopathic response in the human amnion cells which appears indistinguishable under the light microscope from that caused by infection with poliovirus.

Summary. A tissue culture adapted strain of Coxsackie virus A-10 has been obtained from primary human amnion cells inoculated with virus from a highly purified lot of suckling mouse grown material. The adapted virus (A-10TC) was neutralized by Coxsackie virus A-10 antiserum. It was no longer virulent in mice and caused gross cytopathic changes in primary human amnion cells indistinguishable from those changes caused by the polioviruses. Plaque assays of the virus were obtained on monolayer cultures of primary human amnion cells.

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