The Cytopathic Effects of HGP Virus and Coxsackievirus B Type 5 on HEp2 Cells

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It is generally accepted that the rhinoviruses are closely related to the enteroviruses (1). Part of the evidence for this is the close similarity of the cytopathic effects observed in cell cultures infected with members of either of these groups of viruses (2, 3, 4). There is, however, no detailed description of the cytopathic effects produced by a rhinovirus. This report describes in detail and compares the development of cellular changes observed in HEp2 cells infected with HGP virus, a member of the rhinovirus group, and with Coxsackie virus B type 5.

The propagation of the HEp 2 cells employed in these experiments has been described elsewhere (5). Coverslip cultures were prepared by inoculating approximately 120,000 cells into 16 x 125 mm screw cap roller tubes containing 6 x 22 mm coverslips. Within 24 hr most of the coverslips were covered with a monolayer of cells. There were also many cells growing on the adjacent walls of the roller tube.

The HGP virus (6) was received from Dr D. A. J. Tyrrell. It had been propagated 4 times in human embryonic kidney cells and once in rhesus monkey kidney cells. In this laboratory it was cultured many times in rhesus monkey kidney cells and subsequently in HEp2 cells before it was used in these experiments. The preparations of HGP virus employed in these experiments contained 10^{4.5} tissue culture infective doses (TCID 50) per 0.1 ml., assayed in HEp2 cells and calculated by the method of Reed & Muench (7). Two strains of coxsackie virus B5 were studied in these experiments with identical results. One strain had been isolated and repeatedly propagated in HEp2 cells; the other had been isolated and propagated many times in rhesus monkey kidney cells and 3 times in HEp2 cells before it was used. The seed pools of coxsackie virus B5 studied contained approximately 10^{7.0} TCID 50/0.1 ml., assayed in the same way as HGP virus.

Some coverslip cultures were treated with Bouin's fixative before staining with Harris's haematoxylin and alcoholic eosin Y. Other cultures were stained with acridine orange (8); the specificity of RNA and DNA staining was confirmed by inhibition of staining after treatment with the appropriate nuclease. Half of the coverslip cultures were inoculated with 0.1 ml. undiluted virus while the remainder served as uninoculated controls. The cultures were then incubated at 33° in a roller apparatus revolving at 12 rev./hr. At intervals three cultures from each group were removed for examination by phase contrast microscopy and for staining with haematoxylin and eosin and acridine orange.

Observations at a magnification of 40 diameters were made by light transmitted through the wall of the culture tube. When equal concentrations of coxsackie virus B5 and HGP virus were employed, cytopathic change was first seen about 6 hr after inoculation of the coxsackie virus and 20 to 36 hr after inoculation of HGP virus; it consisted of groups of 5 to 10 adjacent cells which had pulled away from their neighbours and had refractile edges. Infected cells progressively rounded and became more...
shrunken and refractile. Most of them ultimately detached from the glass; it was not possible to discern nuclear changes in infected cells. Thus, apart from the earlier appearance of cytopathic effects with coxsackie of virus B 5, there were no differences detectable by this means in the evolution of HEp 2 cell changes after infection with either virus.

In cultures infected with HGP virus, earliest cytopathic effects were noted 20 to 22 hr after inoculation and consisted of cells which had begun to contract from their neighbours and round up. The nuclei were slightly shrunken. There was some perinuclear cytoplasmic condensation and retraction and occasionally cytoplasmic projections (Pl. 1, fig. 2). During the ensuing 4 to 6 hr cells became completely rounded containing only nuclear remnants, and eventually became detached from the glass (Pl. 1, fig. 4). In cultures infected by coxsackie virus B 5, cytopathic effects were first recognized 4 to 6½ hr after inoculation. The cytoplasm was divided into a dense endoplasmic zone sharply demarcated from a lacy ectoplasmic zone. Cytoplasmic projections were abundant (Pl. 1, fig. 3). During the next 1 to 3 hr the nuclei became pyknotic and the cells rounded up and were indistinguishable from similar cells infected with HGP virus (Pl. 1, fig. 4). Phase contrast observations were made at magnifications of 100 ×, 200 × and 400 × on preparations mounted in phosphate buffered saline. The cytopathic effects observed with both viruses were the same as those seen in similar cultures stained with haematoxylin and eosin.

Acridine orange stain revealed cytoplasmic and nuclear alterations that were not otherwise apparent and occurred in cells infected with both viruses. Brightly stained RNA inclusions were frequently seen in the cytoplasm of cells in the advanced stages of rounding. There was a general loss of RNA staining of the cytoplasm of these inclusion-bearing cells. As the nucleus shrank the chromatin became coarser and clumped and DNA staining increased in intensity (Pl. 1, figs. 5, 6). RNA staining of the nucleolus was not consistently altered. When the nucleus was grossly shrunken and the nucleolus had disappeared, RNA was diffusely distributed in the nucleus. This nuclear RNA was not seen in the final stage of cell damage.

Pereira (9) described the cytopathic effects of the enterovirus group as consisting of cellular retraction, nuclear pyknosis, and disintegration in stained preparations. Our observations were consistent with this description. We did not observe the large refractile cells described by others (2, 3, 4, 10) in cell cultures infected with HGP virus. This may have been due to a difference in the HEp 2 cells we used since such cells were seen in monkey and human kidney cell cultures infected with our strain of HGP virus. The cytoplasmic alterations observed in HEp 2 cells infected with coxsackie virus B 5 were previously seen in human amnion cells infected with coxsackie virus B type 3(10).

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Short communications

REFERENCES


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EXPLANATION OF PLATE

Fig. 1. Uninfected HEp2 cells. Haematoxylin and eosin stain.

Fig. 2. HEp2 cells infected with HPV virus, stained with haematoxylin and eosin. Arrows identify some of the affected cells. The cytoplasm has retracted and some cytoplasmic projections are evident. The nuclei are slightly shrunken and the chromatin is more coarse than in normal cells.

Fig. 3. HEp2 cells infected with coxsackie virus B5, stained with haematoxylin and eosin. Almost all the cells are affected. The cytoplasm is separated into a dense inner zone which is sharply demarcated from a less dense, lacy outer zone. The nucleus is shrunken and many cytoplasmic projections are evident.

Fig. 4. HEp2 cells infected with HPV virus, stained with haematoxylin and eosin. Almost all the cells are affected. The cells are completely rounded up and the nuclei are pyknotic. Identical cells are also seen in HEp2 cell cultures infected with coxsackie virus.

Fig. 5. HEp2 cells infected with HPV virus, stained with acridine orange. Brightly stained, irregular inclusions are evident in the cytoplasm of the cells. The black and white prints seen here and in fig. 6 were prepared from colour transparencies.

Fig. 6. HEp2 cells infected with coxsackie virus B5, stained with acridine orange, showing cytoplasmic inclusions similar to those in fig. 5.
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