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A comparative study of susceptibility of primary monkey kidney cells, Hep 2 cells and HeLa cells to a variety of faecal viruses

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INTRODUCTION

During the routine virological and research investigations of this laboratory, many enteroviruses and adenoviruses were isolated on different tissue culture cell lines: the cell lines in use being primary monkey kidney cells and continuous HeLa and Hep 2 cells. Hsiung (1962) made a comparative study of the susceptibility of monkey kidney and human cells to enteroviruses using tissue culture passaged prototype strains while other workers (Archetti, Weston & Wenner, 1957; Stulberg, Page & Berman, 1958) had found that ECHO viruses grew less readily in permanent lines of human cells. It seemed useful to compare the primary isolation rates of the different viruses, including the vaccine strains of poliovirus, ECHO 6, ECHO 11 and other enteroviruses, and a variety of adenoviruses on the different cell lines used in this laboratory with particular reference to human malignant cells and primary monkey kidney cells.

MATERIALS AND METHODS

The HeLa cell line was grown in Hanks basic salts medium fortified with 0·5% lactalbumen hydrolysate, 10% human serum and 10% calf serum and antibiotics (penicillin, streptomycin and nystatin). The Hep 2 cell line was originally obtained a number of years ago from the M.R.C. Virus Research Unit at Sheffield who in turn had obtained it from Dr R. Chanock; it was grown in the same medium as HeLa cells, except that there was no human serum present. Both these cell lines were maintained in medium 199 with 2% calf serum added.

The primary cynomologous monkey kidney cells were grown in Hanks basic salt solution containing lactalbumen hydrolysate, 5% horse serum and antibiotics and were maintained in serum-free medium 199. The specimens used in this study were the faeces of patients sent to the laboratory. Volumes of 0·2 and 0·1 ml. of a supernatant of a 10% suspension of faeces were inoculated into two tubes. When the first sign of virus growth was observed, the number of days from the inoculation of the material was recorded. Cultures showing the toxic effect of faeces, or those cultures showing changes due to long maintenance, were passaged blindly. This was achieved by freezing and thawing them before they were inoculated into fresh tissue culture tubes. When the presence of a virus agent was suspected, confirma-

tion and identification was obtained by a neutralization test and in the case of adenovirus a complement fixation test as well. The techniques of virus isolation and neutralization were based on those originally described by Munro-Ashman, Gardner, Taylor & McDonald (1958) and Gardner, Knox, Court & Green (1962). All HeLa and Hep 2 cultures were kept a minimum of 28 days, while monkey kidney cells were kept about 14 days (see Table 1). When a child had recently been fed with oral poliovirus vaccine, it was assumed that the virus being excreted was a vaccine strain and no tests for virulence were performed.

RESULTS

During this study, vaccine strains of poliovirus, ECHO 6, ECHO 11, other enteroviruses and a variety of adenoviruses were isolated in the three different cell lines used. The results of isolation of vaccine strains of poliovirus in the cell lines are shown in Table 1.

Table 1. *Comparison of the three tissue culture lines for excretion of vaccine strains of poliovirus*

	Total no.	Positive monkey kidney	Positive Hep 2	Positive HeLa	Negative Hep 2 positive monkey kidney	Negative monkey kidney positive Hep 2
No. of poliovirus isolations	66	64 (97%)	64 (97%)	9 (14%)	2 (3%)	2 (3%)
Average time for isolating poliovirus	—	5 days	7 days	18 days	11 days	10 days
Average time of keeping negative cell lines	—	14 days	28 days	28 days	—	—

Of the specimens suspected of containing poliovirus, 97 % were positive both on monkey kidney and Hep 2 cells. The average time taken for isolating poliovirus on monkey kidney cells was 5 days and slightly longer on Hep 2 cells. In two specimens poliovirus was not isolated on Hep 2 cells but was recovered on monkey kidney cells and in a further two specimens virus was isolated only on Hep 2 cells. In nine specimens (14 %) only, were polioviruses isolated on HeLa cells and this was after prolonged incubation.

The results for the isolation of ECHO 6 virus on these cell lines are shown in Table 2.

On Hep 2 cells, ECHO 6 virus was isolated from 89 specimens, whereas only 42 % of these specimens showed positive results on monkey kidney cells. The average time taken for isolating ECHO 6 virus on Hep 2 cells was 9 days and slightly longer on monkey kidney cells. No isolations of ECHO 6 virus were ever made on HeLa cells even after 28 days incubation.

The results for the isolation of ECHO 11 virus on these cell lines are shown in Table 3.

ECHO 11 virus was isolated on Hep 2 from twenty-three specimens but only 52% of these specimens showed positive results when examined on monkey kidney cells. The average time for isolation of this virus on Hep 2 cells was 10 days and on monkey kidney cells slightly longer.

The results for the isolation of the various adenoviruses on these cell lines are given in Table 4.

Table 2. *Comparison of ECHO 6 virus isolations on the three cell lines*

	Total no.	Positive monkey kidney	Positive Hep 2	Positive HeLa
No. of ECHO 6 virus isolations	89	37 (42%)	89 (100%)	0
Average time for isolating ECHO 6 virus	—	11 days	9 days	—
Average time for keeping cell lines	—	14 days	28 days	28 days

Table 3. *Comparison of ECHO 11 isolations on the three cell lines*

	Total no.	Positive monkey kidney	Positive Hep 2	Positive HeLa
No. of isolations of ECHO 11 virus	23	12 (52%)	23 (100%)	0
Average time for isolating ECHO 11 virus	—	13 days	10 days	—
Average time for keeping cell lines	—	14 days	28 days	28 days

Table 4. *Comparison of adenovirus isolations on the three cell lines*

	Total no.	Positive monkey kidney	Positive Hep 2	Positive HeLa	Positive Hep 2 negative HeLa	Negative Hep 2 positive HeLa
No. of adenovirus isolations	25	9 (36%)	24 (96%)	22 (88%)	3 (14%)	1 (4%)
Average time for isolating adenoviruses	—	12 days	11 days	12 days	—	—
Average time for keeping cell lines	—	14 days	28 days	28 days	—	—

Table 5. *Comparison of other enterovirus isolations on the three cell lines*

Virus	Total isolated	Monkey kidney	Hep 2	HeLa
Coxsackie A9	2	2	—	—
Coxsackie B2	3	1	3	—
Coxsackie B3	3	2	3	—
Coxsackie B4	1	1	1	—
Coxsackie B6	2	1	2	—
ECHO 5	1	—	1	—
ECHO 9	1	1	—	—

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Adenoviruses were isolated on Hep 2 cell lines from 96 % of suspected positive material and on the HeLa cells from 88 % of the same material. On three occasions HeLa cells showed complete negative results whereas an adenovirus was recovered on the Hep 2 cell line and in one specimen adenovirus was isolated on HeLa cells but not on Hep 2 cells. The average time for isolation of adenoviruses was 11 days on Hep 2 and 12 days on HeLa cells. Only 36 % of the specimens showed positive results on monkey kidney cells and the average time for isolation was again 12 days.

Table 5 shows a number of other enteroviruses which were isolated in the laboratory and which had been examined on all three tissues.

DISCUSSION

The results of this investigation have shed some light on to the susceptibility of a few of the common tissue cultures used in the laboratory for the isolation of faecal viruses.

The vaccine poliovirus strains grow equally well on monkey kidney cells and the Hep 2 cell line which were in use in this laboratory. It appears that where monkey kidney cells are not available, Hep 2 cell lines can be profitably used for the isolation of vaccine poliovirus strains in epidemiological problems without loss of sensitivity. On HeLa cell lines the vaccine strains did not grow well, only 14 % of these specimens showed positive results even after prolonged incubation. Virulent strains of polioviruses are known to grow as well on HeLa cells as on monkey kidney cells (Plotkin, Carp & Graham, 1962). Continuous cell lines have been used as markers for determining virulence of strains of polioviruses, viz. the M.S. marker (Kanda & Melnick, 1959), but in this case, Hep 2 cells are no use as a marker for avirulence.

Remarkably good results were obtained with Hep 2 cells for the isolation of both ECHO 6 and ECHO 11 virus when a comparison was made with monkey kidney cells, the Hep 2 cell line being far more sensitive for both these strains. The growth of ECHO 6 prototype strains on monkey kidney and Hep 2 cells is variable. Hsiung (1962) used a different cell line of Hep 2 (Sabin line) which was completely insusceptible to any of the ECHO prototype strains. He had, however, previously used a cell line, now lost due to bacterial contamination, which had been susceptible to a number of ECHO viruses. Hsiung also obtained a consistent positive result with rhesus monkey kidney cells, using ECHO 6 prototype strains. Other workers (Fukumi, Nishikawa & Mitzutani, 1958) found that certain virus strains related to ECHO 6 grew on HeLa cells but failed to grow on monkey kidney cells. The examination of prototype strains of ECHO viruses was purposely avoided in this study as they were all monkey kidney cultures and would have borne no resemblance to primary isolations.

Bell, Turner, Macdonald & Hamilton (1960) compared the susceptibility of different human cell lines (both HeLa cells and embryonic human tissues) to adenovirus type 3. They showed that human embryonic tissues were more susceptible than HeLa cells and compared the mean isolation time for their

respective isolations; it was found that embryonic tissues gave better results. Grayson, Loosli, Smith, McCarthy & Johnston (1958) demonstrated that different laboratory lines of HeLa cells varied in their sensitivity to adenovirus. The Hep 2 cell line used in this laboratory appeared to be slightly better than HeLa cells in their susceptibility to adenoviruses, though the line of HeLa cell in use was satisfactory (22 positive out of 25); the results also confirmed the observations of many that monkey kidney cells are not a good method for adenovirus isolation. In the small number of comparisons made with other enteroviruses there appear to be no significant differences in the susceptibility of Hep 2 cells and monkey kidney cells, with the possible exception of Coxsackie A 9.

The Committee on ECHO Viruses (1955) adopted the preferential susceptibility of monkey kidney cells as one of the criteria for the inclusion of a virus in the ECHO group. In view of our findings and those of others (Fukumi *et al.* 1958) either the various strains of viruses that have been isolated are not ECHO virus or the original definition of the ECHO group should be modified.

No hard and fast rules can be made as to which cell lines one should use in the laboratory. It has been shown that continuous cell lines, in this case Hep 2 cells, have a part to play in the isolation of enteroviruses. Laboratories where primary monkey kidney cells are difficult to obtain might do well to reconsider the host range of their continuous cell lines, in view of the experience of this laboratory.

SUMMARY

A comparative study of the susceptibility of monkey kidney, Hep 2 and HeLa cells to enteroviruses and adenoviruses is made. It seems that this Hep 2 cell line is as effective as the monkey kidney cells in their susceptibility to vaccine strains of polioviruses and far better than monkey kidney cells in their susceptibility to strains of ECHO 6 and ECHO 11 from clinical material. It is as effective as, or slightly better than, HeLa cells for the isolation of adenoviruses. It also compares favourably with monkey kidney cells for the isolation of other enteroviruses.

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