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Isolation of Echoviruses with Human Embryonic Lung Fibroblast Cells

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More echovirus isolants were obtained from clinical specimens with a strain of human embryonic lung fibroblast cells (RU-1) than with primary rhesus monkey kidney cells.

Several recent reports have indicated the sensitivity of diploid human embryonic lung cells (WI-38 strain) for isolation of echovirus type 30 from clinical specimens (1-3). We have had similar experiences with echovirus 30 isolations with a strain of human embryonic lung fibroblast cells established at the Center for Disease Control (CDC) by the Respiratory Virology Unit (designated RU-1 cells). In addition, we observed that these cells isolated certain other echovirus types from clinical specimens in some instances where primary rhesus monkey kidney (PMK) cells failed to yield a virus. Our results in isolating echoviruses with RU-1 and PMK cells are summarized here.

RU-1 and PMK cell cultures were supplied by the Tissue Culture Unit, CDC. RU-1 cells were grown on Eagle's minimal essential medium with 10% fetal calf serum and maintained on modified Eagle's medium (4) with 2% agamma calf serum. No studies were made of the chromosomes of these cells. Division of the cells in vitro essentially ceased at about the 36th passage. Growth medium for the PMK cells was Melnick's medium A with 5% fetal calf serum; a lactalbumin hydrolysate, yeast extract, Earle's salt solution medium without serum was used for maintenance. Standard methods were used for preparing stools, rectal and throat swabs, and cerebrospinal fluids for inoculation of cell cultures (5). Inoculated cultures were incubated in stationary racks at 36 C for 8 days and observed every other day for the presence of cytopathic effect. Blind passages were made where indicated by freezing and thawing cultures three times and inoculating samples into fresh cells. Viruses were typed by neutralization tests with pools of enteroviral antisera followed by neutralization with appropriate single antisera. Specimens were received from 19 states and the District of Columbia. Material from 41 cases of aseptic meningitis in Memphis, Tenn., in the summer of 1968 was intensively studied. The predominant virus isolated from this outbreak was echovirus type 30.

The comparative number of isolations of echoviruses in RU-1 and PMK cells is shown in Table 1. Thirty specimens yielded an echovirus only in RU-1 cells (21 echovirus type 30, 9 other echoviruses). One or more blind passages were made of 25 of these 30 specimens in PMK cells without recovery of any virus. An echovirus was isolated from seven specimens only in PMK cells. Blind passage of these seven specimens at least once in RU-1 cells failed to reveal any virus. Thirty-eight specimens were found positive for an echovirus with both cell types. The data as a whole show that 91% (68 of 75) of the echoviruses represented here would have been isolated if only RU-1 cells had been used, whereas 60% (45 of 75) would

TABLE 1. *Comparison of isolation of echoviruses in human embryonic lung fibroblast and primary rhesus monkey kidney cells*

Echovirus type	No. isolated in fibroblasts only	No. isolated in rhesus kidney only	No. isolated in both cell types	Total
1	0	0	1	1
3	1	0	6	7
4	2	1	9	12
6	5	1	5	11
9	0	3	3	6
11	0	0	1	1
13	1	0	0	1
20	0	0	1	1
30 (various states)	4	1	7	12
30 (Memphis, Tenn.)	17	1	5	23
Total	30	7	38	75

have been isolated with only PMK cells. Overall, the RU-1 cells were more sensitive for isolating echoviruses than the PMK cells. It should be emphasized, however, that 7 of the 75 echoviruses (9%) were isolated in PMK but not in RU-1 cells.

Our experience has indicated the value of a strain of human embryonic lung fibroblast cells (RU-1) other than WI-38 for isolating several echovirus types from clinical specimens. The wide availability of WI-38 or similar cells and their apparent sensitivity for isolation of echoviruses emphasize the desirability of using such cells along with primary monkey kidney cells in studies of specimens from suspected enteroviral infections.

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