

## Comparison of Rhinovirus-Sensitive HeLa Cells and Human Embryo Fibroblasts for Isolation of Rhinoviruses from Patients with Respiratory Disease

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Rhinovirus-sensitive HeLa cells (HeLa "R") and human embryo lung fibroblasts (HEL) were compared for the isolation of rhinoviruses from patients with respiratory disease. In the period May 1970 to December 1974, 526 rhinoviruses were isolated from 517 patients, 32% in both cell types, 59% in HeLa "R" only, and 9% in HEL only. The annual isolations in HeLa "R" were between 1.7 and 4 times greater than in HEL cells and may have been due to changes in the sensitivity of the cells or the prevalence of serotypes favoring the HeLa "R" cells. Acid lability was more easily demonstrated in HeLa "R" than in HEL cells, because the infectivity titers obtained were 10- to 100-fold higher.

For many years rhinoviruses have been isolated in primary human embryo kidney epithelial cells or human embryo lung fibroblasts (HEL). The difficulty in obtaining a regular supply of embryos was alleviated by the introduction of semicontinuous strains of HEL cells, such as the WI-38 strain described by Hayflick and Moorhead (3). Cells of this type can be maintained in flat-sided bottles, trypsinized, and passaged into tubes as required, and reserve stocks may be stored indefinitely at low temperatures in liquid nitrogen.

Most continuous cell lines, such as HeLa and HEp-2 cells, are very resistant to infection with rhinoviruses. However, with the discovery of a strain of HeLa cells sensitive to rhinoviruses (HeLa "R") Hamparian et al. (2) were able to develop tests for serotyping these viruses and for titration of antibodies. Using these cells, Fiala and Kenny (1) developed a plaque assay and Stott and Tyrrell (5) devised a metabolic inhibition test for titration of antibody and identification of rhinovirus types. Stott and Tyrrell (5) also suggested the use of HeLa "R" cells for the isolation of viruses from clinical specimens.

Strizova et al. (6) used HeLa "R" cells for the isolation of rhinoviruses from nasal washings from experimentally infected volunteers. However, there seems to be no documented account of these cells having been used for the routine isolation of rhinoviruses from patients with clinical illness.

This communication compares the isolation of rhinoviruses in HeLa "R" and HEL cells from throat swabs obtained from patients with respi-

ratory disease during the period May 1970 to December 1974.

### MATERIALS AND METHODS

The procedures used for the treatment of throat swabs, preparation of cell cultures, and isolation of viruses were as described previously by Kennett et al. (4). Throat swabs were inoculated into primary cynomolgus monkey kidney epithelial (MK) cells, HEL, and HeLa "R" cells.

MK cells were received weekly from Commonwealth Serum Laboratories (Melbourne) as a suspension, inoculated into phials, and incubated at 37 C until monolayers formed. HEL cells were established from the lungs of local hysterotomy-derived embryos, using the method of Hayflick and Moorhead (3). At passages 2 and 4, the majority of cells were frozen in liquid nitrogen. Tube cultures were prepared from passage 6 of the remaining cells and were inoculated with 10-fold dilutions of low-passage, local isolates of echovirus, herpes simplex virus, cytomegalovirus, and M & H rhinoviruses. The sensitivity of the locally derived HEL strains was found to be equal to or greater than either the WI-38 or MRC V strains, provided that the cells were not used beyond passage 15. In the study reported here, the HEL cells were always used between passages 6 and 15. HeLa "R" cells were obtained from D. A. J. Tyrrell at the Common Cold Research Unit, Salisbury, England, in 1969 and have since been maintained in this laboratory.

Cultures showing a cytopathic effect (CPE) were observed daily, and, when disintegration of the cell monolayer was 50% or greater, the supernatant fluid was harvested and the virus was identified by standard techniques.

Viruses that produced an enterovirus-like CPE in HeLa "R" and/or HEL cells were regarded as candidate rhinoviruses, and confirmation was sought by the following techniques.

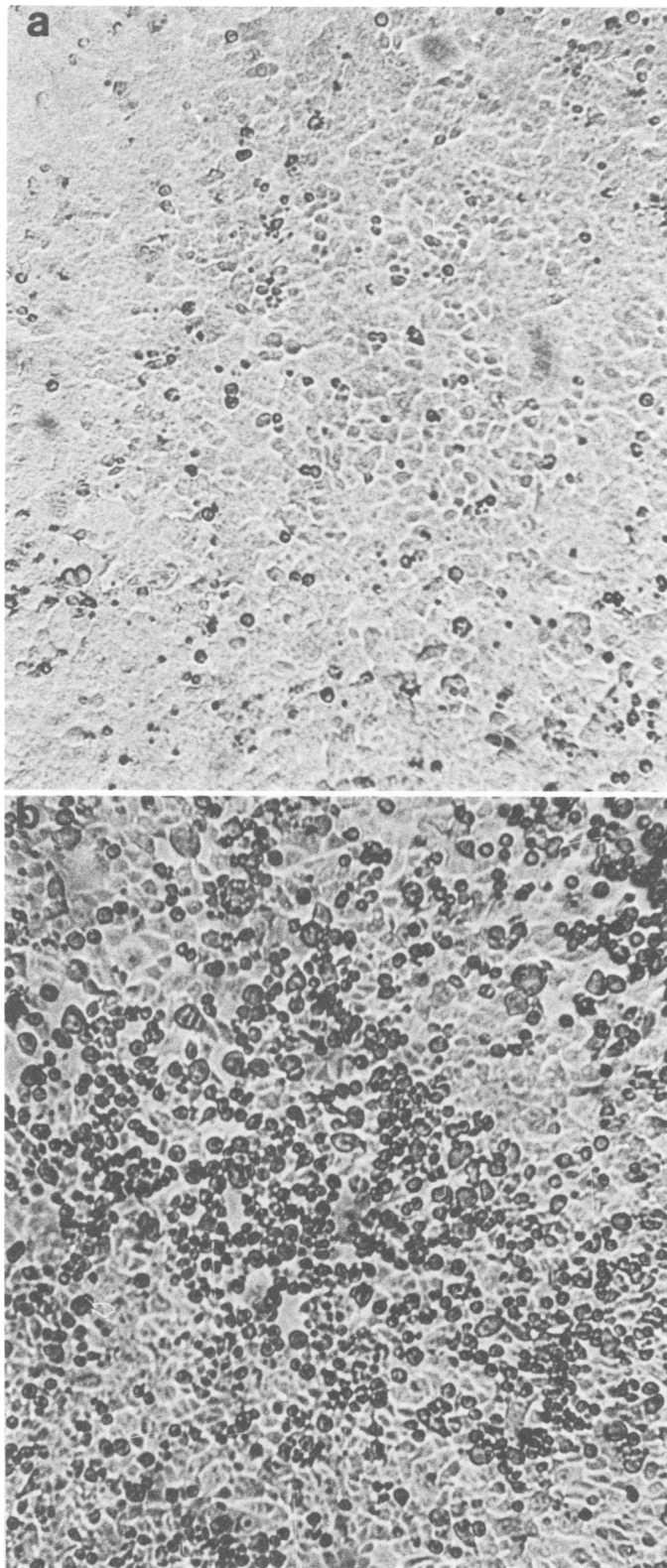


FIG. 1. *Rhinovirus in HeLa "R" cells. (a) Uninoculated cell culture; (b) 10 days after inoculation. ×260.*

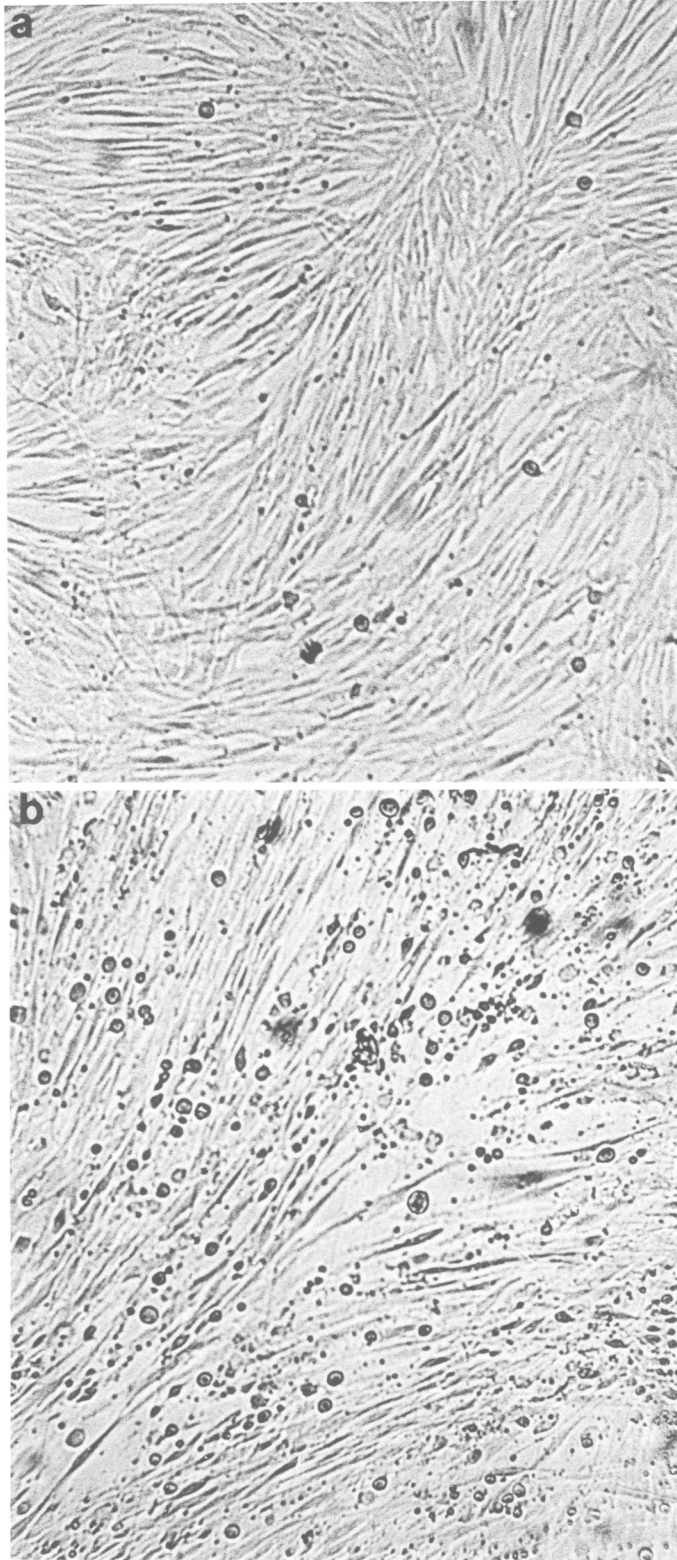


FIG. 2. *Rhinovirus in HEL cells. (a) Uninoculated cell culture; (b) 14 days after inoculation. ×260.*

**Cell specificity and sensitivity.** A 0.1-ml portion of virus suspension was inoculated into duplicate phials of MK, HeLa "R," HEL, and Borrie cells. The phials were incubated on a roller drum at 34 C and examined every 2 to 3 days with an inverted microscope, using a magnification of 40 to 60 $\times$ . Cultures were observed for 7 days if CPE appeared in one or more cell type and for 18 days if no CPE was detected.

Borrie cells were isolated in the laboratory in 1970, when the cerebrospinal fluid from a patient with suspected meningitis was inoculated into HEL cells. The heteroploid epithelial cells that appeared overgrew the fibroblasts and were cloned and established as a cell line. They were used in this study because they are very sensitive to enteroviruses but relatively resistant to rhinoviruses. The Borrie line has not been fully characterized, and it may be possible that it represents a cloned line of HeLa cells.

**Acid lability test.** Acid lability tests were performed in the cells found to be the most sensitive in the above test. One part of virus suspension diluted with 9 parts of Eagle basal medium, adjusted to pH 3.0, was incubated in a water bath at 37 C for 1 h, and 0.1 ml of serial 10-fold dilutions of the mixture was inoculated into duplicate phials of the cells, which were incubated at 34 C on a roller drum. A control consisting of the virus diluted in Eagle basal medium at pH 7.2 was similarly treated. A virus was considered acid labile if the titer at pH 3.0 was reduced at least 100-fold.

All isolates found to be acid labile were stored at -20 C but have not yet been typed.

## RESULTS

### Isolation and confirmation of rhinoviruses.

The rhinoviruses encountered in this series were isolated in HeLa "R" and/or HEL cells. CPE in these cell types occurred in 4 to 21 days. With some strains the CPE in HeLa "R" first appeared as foci of rounded cells similar to those produced by adeno- or herpesviruses. As the change progressed, the cells became shrunken and fragmented and appeared more like that usually encountered with enteroviruses (Fig. 1). Early CPE in HEL cells was more characteristic of rhinovirus infection, with small foci containing round refractile cells of variable sizes (Fig. 2). Sometimes this change went to completion, but at other times it regressed and finally disappeared. Subculture usually permitted recovery and enhancement of the CPE.

In the specificity test at CPE usually appeared more rapidly than in the primary cultures, being evident in 3 to 7 days in HeLa "R" cells and in 7 or more days in HEL cells. On rare occasions a strain of rhinovirus showed CPE more quickly in HEL than in HeLa "R." CPE was generally not seen in MK unless the virus was an M strain. All viruses with a cell

spectrum that differed from this were identified as enteroviruses by neutralization with type-specific antisera.

**Comparison of rhinovirus isolations in HeLa "R" and HEL cells.** In the 55 months of the survey, 526 rhinoviruses were recovered from 517 patients, 59% were isolated in HeLa "R" only, 9% in HEL only, and the remainder in both cell types (see Table 1). Although the annual isolation rates of viruses multiplying in both cells were relatively constant, there was a marked decline in the isolations in HEL only in 1973 and 1974.

The total isolations in HeLa "R" and HEL cells were 479 (91%) and 216 (41%), respectively. Apart from 1970, the annual isolation rates in HeLa "R" were from 1.7 to 4 times those in HEL (Fig. 3).

TABLE 1. *Strains of rhinovirus isolated in HeLa "R" and HEL cells*

Year	No. of isolations	Isolated in:			Total isolations in:	
		HeLa only	HEL only	Both cells	HeLa	HEL
1970 <sup>a</sup>	56	14	11	31	45	42
1971	78	40	14	24	64	38
1972	140	79	16	45	124	61
1973	118	91	2	25	116	27
1974	134	86	4	44	130	48

<sup>a</sup> Seven months only.

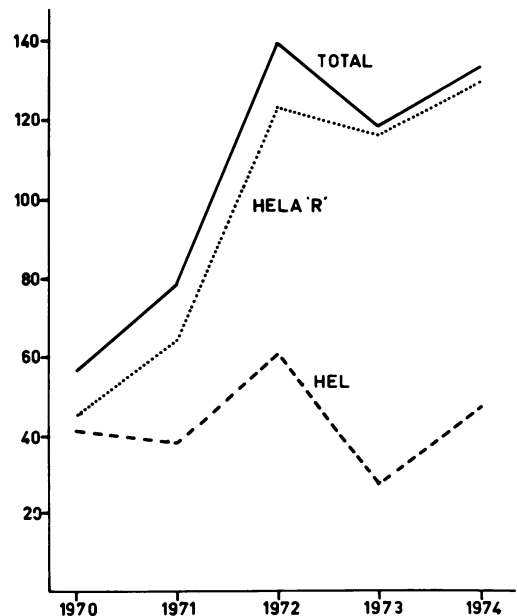


FIG. 3. *Annual isolations of rhinoviruses in HeLa "R" and HEL cells. The figures for 1970 represent 7 months only.*

**Titers of rhinoviruses in HeLa "R" and HEL cells.** The infectivity titers obtained in passage 2 cultures in HeLa "R" cells ( $10^{-4}$  to  $10^{-3}$ /0.1 ml) were 10 to 100 times greater than those in HEL cells.

### DISCUSSION

Although the CPE occurring in HeLa "R" cells was less characteristic of rhinovirus infection than that obtained in HEL cells, once initiated it invariably went to completion, whereas in HEL cells it often disappeared within a few days.

The cell sensitivity test proved most useful for the separation of enteroviruses from rhinoviruses and for the determination of the most sensitive cells, usually HeLa "R," for the acid lability test. In addition, the infectivity titer in HeLa "R" being 10 to 100 times greater than in the HEL cells permitted the demonstration of more pronounced reductions in titer when the virus was subjected to treatment at pH 3.0.

The relative number of rhinoviruses isolated in the two cell types was not constant. In the first 3 years, 41 strains were isolated in HEL only, compared with 133 in HeLa "R" only, whereas in 1973 and 1974 the isolations in HEL and HeLa "R" were 6 and 177, respectively. Whether or not the superior isolation rate in HeLa "R" was due to a predominance of types more easily grown in these cells was not deter-

mined. Alternatively, these results may be due to variations in the sensitivity of the cells, even though the cells were tested periodically for sensitivity to a limited series of viruses.

HeLa "R" have proved satisfactory for the isolation of respiratory syncytial virus and adenoviruses, in addition to rhinoviruses, and together with MK and HEL cells are now routinely used in this laboratory for the recovery of respiratory viruses.

### ACKNOWLEDGMENTS

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