Early attempts to grow rhinoviruses in HeLa and similar transformed cell lines were unsuccessful (Tyrrell, Bynoe, Hitchcock, Pereira, and Andrewes, 1960; Hamparian, Keller, and Hilleman, 1961) but later certain rhinoviruses were adapted to these cells (Mogabgab, 1962; Keller, Hamparian, and Hilleman, 1962; Johnson and Rosen, 1963). It was clear that some cell lines were much more susceptible to infection than others (Taylor-Robinson, Hucker, and Tyrrell, 1962) and viruses usually needed several passages to achieve adaptation. More recently Hamparian, Leagus, and Hilleman (1964) have used HeLa cells for the titration of antiviral antibodies in the serotyping of viruses.

Porterfield (1962) reported the production of plaques under agar with several rhinoviruses in human diploid cell strains (HDCS) of embryonic lung fibroblasts. More recently Fiala and Kenny (1966) have shown that the M-HeLa cells used by Hamparian et al. (1964) are particularly sensitive to rhinoviruses and that, if 30 mM of magnesium chloride is added to the medium and purified agar is used, plaques may readily be produced by a number of rhinoviruses.

A sensitive system for the study of rhinoviruses is needed which is not based on cultures of either primary cells or even of human diploid cell strains; we have therefore repeated and extended the work of Fiala and Kenny (1966) and have shown that M-HeLa cells may be used in several ways to improve techniques for the handling and study of rhinoviruses.

Materials

Viruses. Rhinovirus type 2 (HGP) was originally isolated in rhesus monkey kidney cells and has since been passed in HDCS and HeLa cells.

Rhinovirus type 43 (GT) was originally isolated in organ cultures but later adapted to HDCS and M-HeLa cells.
Other rhinoviruses used included prototype strains representing 12 different serotypes and 50 strains isolated in Salisbury and Glasgow between 1962 and 1966.

**Cells.** M-HeLa cells were supplied by Dr. V. V. Hamparian. They were grown in Eagle's basal medium (Gibco G 13) containing 10% ox serum filtered through a collodion membrane, 0.10% NaHCO₃, penicillin (100 units/ml), streptomycin (100 μg/ml), achromycin (22.5 units/ml), and maintained in a similar medium with ox serum reduced to 2% and supplemented with 30 mM MgCl₂ and 5% tryptose phosphate broth (Difco). Cultures were trypsinized each week and bottles or tubes reseeded at 10⁴ cells/ml. M-HeLa cells were contaminated with *Mycoplasma orale* and numerous attempts to eliminate this with antibiotics were unsuccessful.

HDCS were cultured as previously described (Brown and Tyrrell, 1964). The two strains used were both derived locally and were shown to be rhinovirus-sensitive.

**Media.** Magnesium chloride was prepared as a 3 M sterile stock solution which was added to media at 1% as required.

The media used in the metabolic inhibition test were modified from those described by Shand (1961) and were made up as follows:

A. Diluent for viruses and sera

- Parker's medium 199 85 ml
- Ox serum 5 ml
- 4.4% NaHCO₃ solution 4 ml
- 3 M MgCl₂ solution 1 ml
- 10% glucose solution 3 ml
- additional 0.1% phenol red solution 2 ml

B. Diluent for cells

- Eagle's basal medium 88.5 ml
- Ox serum 5 ml
- 4.4% NaHCO₃ solution 2.5 ml
- 3 M MgCl₂ solution 1 ml
- 10% glucose solution 3 ml

**Results**

The work of Fiala and Kenny (1966) was confirmed using rhinovirus type 2 (strain HGP). Thus, this virus produced irregular plaques 1—5 mm in diameter under an overlay of Eagle's basal medium containing 0.4% ionagar and additional MgCl₂. The plaque count was directly proportional to the amount of virus added. Plaque production was inhibited by specific antiserum, Bacto-agar or Noble agar. The optimal MgCl₂ concentration in a range from 0 to 60 mM was 30 mM. In addition, it was found that better cell monolayers and higher plaque counts were obtained if 30 mM MgCl₂ was added to the growth medium as well as to the overlay medium. The concentration of sodium bicarbonate in the overlay could be varied between 0.04% and 0.26% without significantly altering the plaque count.