Plaques of Rhinoviruses in Human Diploid Cells.* (32477)

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Procedures describing the plaque assay of rhinoviruses have been reported(1-4). However, only a small percentage of rhinoviruses (15 of about 100 strains) have been reported to produce macroplaques. Moreover, it is not known whether the presently described procedures are applicable to the plaque assay of other rhinoviruses. The present report describes a simple procedure which has been consistently reproducible for the plaque assay of an additional 16 rhinoviruses in human diploid cells.

Materials and methods. The rhinoviruses of this study were received from Dr. Maurice R. Hilleman, Merck Institute for Therapeutic Research, West Point, Pa. The numerical designates of these serotypes(5,6) do not correspond numerically with prototype strains recently classified by the Rhinovirus Collaborating Laboratories at Bethesda(7) (see footnote base Table I). Fully grown monolayers of human diploid (WI-38) cells in 1-oz prescription bottles were seeded with various dilutions (0.2 ml/bottle) of the above viruses and incubated at 33°C for one hour. The bottles were then overlaid, each with 6.0 ml of high cystine altered Eagle's medium (HCAEM)(8) containing 10% inactivated newborn calf serum and 0.7% Ionagar No. 2.† After 6 days of incubation at 33°C, the bottles were overlaid, each with 3 ml of the above overlay medium, now containing neutral red (1:10,000 final concentration). All bottles were covered with aluminum foil immediately after addition of the second overlay, incubated at 33°C and examined for plaques on the following day. Incorporation of diethylaminoethyl (DEAE) dextran was made by

TABLE I. Plaque Size, PFU and Log_{10} TCD_{50} of 16 Rhinoviruses in WI-38 Cells.

Virus* (Merck classification)	Strain	Plaque diameter (mm)	PFU per ml	Log_{10} TCD_{50}‡
Coryzavirus 11	68	2†	6×10^4	4.4
" 17	5986	3-4	3×10^4	4.6
" 20	21	2†	1.3×10^5	5.2
" 21	47	3-4	3×10^4	4.0
" 22	127	2	1.1×10^5	5.6
" 25	5146	5-6	8×10^5	6.0
Rhinovirus 34	515	2	1×10^5	5.0
" 35	611	2-3	5×10^5	5.3
" 41	6360	2†	2×10^5	5.5
" 42	6692	2-3	3×10^3	3.8
" 43	1936	2-3	2×10^4	4.7
" 44	6258	3	9×10^4	5.0
" 45	605	3-4	8×10^4	5.0
" 46	1979M	2	8×10^3	3.6
" 48	1983	2†	3×10^4	4.4
" 51	1857	2-3	1.1×10^4	4.6

* Numerical designations are those given in references 5 and 6. Under the new classification CV11 = rhinovirus type 7; CV17 = rhinovirus type 18; CV21 = rhinovirus type 21; CV22 = rhinovirus type 22; and CV25 = rhinovirus type 24. Some of the Merck rhinovirus serotypes probably represent prototypes not yet classified in the current schema.

† Plaque size after addition of 25 μg/ml of DEAE dextran to the first overlay.

‡ These titer values were provided by Miss Margaret Chapin.

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† Obtained from Consolidated Laboratories, Chicago Heights, Ill.

addition of 25 μg/ml to the first overlay medium.

Results. All viruses except coryzaviruses 11 and 20 and rhinoviruses 41 and 48 showed macroplaques of 2 mm or more in diameter without the incorporation of DEAE dextran. While the addition of DEAE dextran increased the diameter of the above 4 viruses from 1 mm to 2-3 mm, it did not affect plaque size of the other viruses. No increase in number of plaques (titer increase) of any of the 16 viruses tested was observed by addition of DEAE dextran. Table I shows plaque diameter, PFU and $\log_{10}$ TCD_{50} (in WI-38 tubes) of these viruses. Photographs of plaques formed by coryzaviruses types 22 and 25 and rhinoviruses types 42 and 45 originally described by the Merck group are illustrated in Fig. 1.

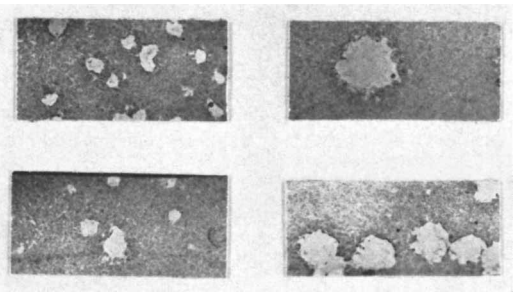

FIG. 1. Plaques of rhinoviruses in WI-38 cells. Magnification: 1×. All plaques were stained by a second overlay containing neutral red (see text). Upper left: Coryzavirus 22; upper right: coryzavirus 25. Lower left: rhinovirus 42; lower right: rhinovirus 45.

Discussion and conclusions. The lack of a uniform and reproducible plaque assay for rhinoviruses (now totaling about one hundred types) has handicapped characterization of these important human pathogenic agents. Different procedures involving variations in the optimal cell culture, composition of the overlay medium and incorporation of anti-inhibitor and/or enhancing substances (*e.g.*, DEAE dextran and Mg^{++} ions) have been described by various workers. In an attempt to develop a simple and reproducible procedure for plaquing a substantial number of rhinoviruses, we found the following conditions to be optimal for plaque formation by all 16 rhinoviruses that have been tested: (1) fully grown WI-38 cell monolayers in prescription bottles; (2) an overlay medium consisting of HCAEM containing 10% newborn calf serum and 0.7% Ionagar No. 2; (3) double agar overlay technique with the second overlay (containing neutral red) added 6 days after the first; and (4) incorporation of 25 μg/ml of DEAE dextran to the first overlay medium as an additional means to improve plaque formation.

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An Androgenic Basis for the Sexual Difference in L-Ascorbic Acid Biosynthesis in Rats.* (32478)

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A sexual difference in hepatic enzymic activities involved in biosynthesis of ascorbic acid, and in concentrations of total ascorbate in a number of tissues from male and female

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