the monkey may be a useful experimental animal in the broad study of influenza, particularly in the present day development of potential antiviral agents.

1. Saslaw, S., Wilson, H. E., Doan, C. A., Woolpert, O. C., Schwab, J. L., J. Exp. Med., 1946, v84, 113.

2. McIntosh, J., Selbie, F. R., Brit. J. Exp. Path., 1937, v18, 334.

3. Vieuchange, M. J., Bull. Acad. Med., Paris, 1939, v121, 100.

4. Burnet, F. M., Australian J. Exp. Biol. and Med. Sci., 1941, v19, 281.

5. Verlinde, J. D., Makstenieeks, O., Arch. ges. Virusforsch., 1954, v5, 345.

6. Henderson, D. W., J. Hyg., (Lond.), 1952, v50, 53.

7. Roessler, W. G., Kautter, D. A., J. Infect. Dis., 1962, v110, 17.

8. Saslaw, S., Carlisle, H. N., Proc. Soc. Exp. Biol. and Med., 1964, v117, 420.

9. Jensen, K. E., in Diagnostic Procedures for Virus and Rickettsial Diseases, 2nd Ed., Am. Public Health Assn., New York, 1956, p241.

10. Saslaw, S., Carlisle, H. N., Slutzker, B., Am. J. Med. Sci., 1963, v245, 387.

11. Guyton, A. C., Am. J. Physiol., 1947, v150, 70.

12. Hatch, T. F., Bact. Rev., 1961, v25, 237.

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Human Aorta Cells for Isolation and Propagation of Rhinoviruses.* (30316)

C. ALAN PHILLIPS, JOSEPH L. MELNICK AND CATHERINE A. GRIM

WHO International Reference Centre for Enteroviruses, Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

A cell line derived from human aorta containing atheromatous plaques (A-39) has recently been shown to support the growth of a large number of viruses(1). During the past 2 years, we have used these cells extensively in the isolation and propagation of rhinoviruses(2) and they have proved a useful addition to WI-38(3) and human embryonic kidney cells(4).

Materials and methods. All materials and methods employed in this study have been described(5).

Results. While studying upper respiratory tract infections in a population of college students, 19 rhinoviruses were isolated from 111 specimens between October 8, 1963 and April 30, 1964 (period 1), 51 rhinoviruses were isolated from 107 specimens between September 29, 1964 and December 31, 1964 (period 2), and 22 rhinoviruses were isolated from 55 specimens between January 1, 1965 and March 6, 1965 (period 3). The isolates were shown to be RNA viruses which were acid-labile at pH 3.0 and ether-stable, thus possessing the properties of a rhinovirus(4). Those that were viewed by electron microscopy measured 18-23 mμ.

During period 1 (Table I) 18 of the 19 rhinoviruses were isolated in A-39, while 11 of 19 were isolated in WI-38, and only 1 was detected in HEK. Seven of these strains were isolated in A-39 alone but later grew well in WI-38, while 1 strain was isolated only in WI-38 but then grew well in A-39.

During period 2, 17 of 51 rhinovirus strains were isolated in A-39, 24 of 51 were isolated in WI-38, and 43 of 51 were detected in HEK. Of these, none were isolated in aorta alone, while 4 were isolated in WI-38 alone, and 23 were isolated only in HEK.

During period 3, 17 of 22 rhinovirus strains were isolated in A-39 and WI-38, while only 9 were isolated in HEK. Three of these strains were isolated in A-39 alone, 4 were isolated in WI-38 alone, and one was detected only in HEK. Isolations in more than one cell line were common during the 3 study periods as can be seen in Table I. It

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TABLE I. Rhinovirus Isolations in 3 Cell Lines.

Period 1*: 19 isolations		Period 2†: 51 isolations		Period 3‡: 22 isolations	
Total No. of isolations in each cell line		Total No. of isolations in each cell line		Total No. of isolations in each cell line	
Aorta	18	Aorta	17	Aorta	17
WI-38	11	WI-38	24	WI-38	17
HEK	1	HEK	43	HEK	9
Isolations in 1 cell line alone		Isolations in 1 cell line alone		Isolations in 1 cell line alone	
Aorta	7	Aorta	0	Aorta	3
WI-38	1	WI-38	4	WI-38	4
HEK	0	HEK	23	HEK	1
Isolations in more than 1 cell line		Isolations in more than 1 cell line		Isolations in more than 1 cell line	
Aorta and WI-38	10	Aorta and WI-38	4	Aorta and WI-38	6
Aorta and HEK	1	Aorta and HEK	4	Aorta and HEK	1
WI-38 and HEK	0	WI-38 and HEK	7	WI-38 and HEK	0
Aorta, WI-38, and HEK	0	Aorta, WI-38, and HEK	9	Aorta, WI-38 and HEK	7

* Oct 8, 1963 to April 30, 1964.
† Sept 29, 1964 to Dec 31, 1964.
‡ Jan 1, 1965 to March 6, 1965.

was repeatedly noted throughout the study that isolations made in any of the 3 cell lines could be readily adapted to grow in both A-39 and WI-38 cells.

To compare further the A-39 and WI-38 cells, 7 rhinovirus strains representing 5 distinct serotypes that had been isolated in both A-39 and WI-38 during the 1963-64 winter in Houston were reisolated in both cell lines from the original nasopharyngeal swabs after 6 months storage at —30°C. Strains isolated in A-39 were (a) passed 4 times in A-39 and then titered in A-39 and WI-38 and (b) passed twice in A-39, twice in WI-38, and then titered in A-39 and WI-38. In each instance, titers in both cell lines were comparable (Table II). Strains isolated in WI-38 were (a) passed 4 times in WI-38 and then titered in A-39 and WI-38 and (b) passed twice in WI-38, twice in A-39, and then titered in A-39 and WI-38. Again titers in each cell line were comparable. Titers as high as $10^{5.3}$ TCD_{50} per ml were noted in A-39. Rhinovirus strains could be passed without adaptation from one cell line to the other without significant loss of infectivity titer.

In order to be certain that sensitivity to rhinoviruses was not peculiar to one aorta cell line (A-39), 5 different cell lines derived from different human aortas were tested with 3 distinct rhinovirus serotypes, Baylor 1 and the Salisbury serotypes HGP and 16/60. All

TABLE II. Comparison of Infectivity Titers of 7 Rhinovirus Strains Isolated During 1963-64 Winter in Houston in Both A-39 and WI-38 Cell Lines.*

		$A\text{-}39_4$ titered in		$A\text{-}39_2$ $WI\text{-}38_2$ titered in		$WI\text{-}38_2$ $A\text{-}39_2$ titered in		$WI\text{-}38_4$ titered in	
Serotype	Strain	A-39	WI-38	A-39	WI-38	A-39	WI-38	A-39	WI-38
Baylor 2	C. Ma.	3.3	3.3	3.5	3.3	3.5	3.5	3.3	3.3
" 2	H. Cr.	4.0	3.3	4.0	3.5	4.3	4.0	3.3	4.0
" 2	T. Mo.	3.8	4.3	2.5	3.8	4.5	4.5	3.8	3.8
Chicago 179E	T. McH.	4.3	4.3	4.5	4.8	4.8	3.8	4.5	4.3
Chicago 147H	D. Mi.	4.5	3.5	5.0	4.5	4.8	4.8	4.8	4.3
NIH 1200	W. Pa.	5.0	3.5	5.0	3.8	4.8	4.8	4.8	3.8
" 1734	M. Co.	4.5	4.5	5.3	4.5	4.8	4.3	4.8	4.3

* Subscripts indicate number of passages in each cell line. Infectivity titer expressed as $\log_{10}$ TCD_{50} per ml.

TABLE III. Comparison of 5 Aorta Cell Lines Using 3 Rhinovirus Serotypes.

Rhinovirus	A-14	A-27	A-32	A-39	A-61
Baylor 1	4.8*	4.5	5.5	4.8	5.0
HGP	4.8	4.8	5.2	4.8	4.8
16/60	2.8	4.8	5.5	3.8	4.5

* Titer expressed as $\log_{10}$ TCD_{50} per ml.

supported the growth of these serotypes, although A-32 appeared to be the most sensitive of the lines tested (Table III).

The cytopathogenic effect (CPE) produced in A-39 by rhinoviruses is easily discernible. The healthy monolayer after 2 to 5 days shows focal areas of involvement with characteristic large swollen cells which often assume the shape of dendrites seen in WI-38 cells. Later, CPE spreads to involve almost the entire cell monolayer. The cells may be kept for long periods (up to 50 days when rolled at 33°C) and retain their normal morphology.

Discussion. In studies carried out during the past 2 years, the A-39 cells have proved very useful for both isolation and propagation of rhinoviruses. Cell sensitivity seems to be similar to that of WI-38, although A-39 may be more sensitive for some strains. Virus titers are comparable in WI-38 and A-39 cells and viruses isolated in one of these cell lines grow readily in the other cell line. Cell lines of human embryonic lung vary greatly in their sensitivity to rhinoviruses, with WI-38 being one of the most sensitive. However, the 5 aorta cell lines studied all appear to be sensitive to rhinoviruses. Since in a given area and even in a single season, different rhinovirus serotypes occur(2,6-9) there seems to be a need for multiple cell lines with broad strain sensitivity in order to obtain maximum isolations. Cell lines of human aorta should be a useful addition to WI-38, and human kidney in rhinovirus investigations. Using these 3 lines, rhinovirus recovery rate in 3 epidemic periods ranged from 17 to 45% of patients with common colds, which are higher rates than those obtained previously.

Summary. Cell lines derived from human aorta have proved useful for the isolation and propagation of rhinoviruses. In the A-39 cell line, which has been used most often, viruses reach infectivity titers of up to $10^{5.5}$ TCD_{50} per ml and produce readily discernible cytopathic effects. The cell line is easily grown and may be maintained for long periods. It proved to be more sensitive to the rhinovirus serotypes prevalent during the 1963-64 winter than the presently employed WI-38 or human embryonic kidney cells. During the fall of 1964, the prevalent rhinoviruses were selectively isolated on human kidney cells, but during the current winter season aorta and WI-38 cell lines were again more sensitive to the new rhinoviruses in the community.

1. Behbehani, A. M., Melnick, J. L., DeBakey, M., Proc. Soc. Exp. Biol. and Med., 1965, v118, 759.

2. Phillips, C. A., Riggs, S., Melnick, J. L., Grim, C. A., J.A.M.A., 1965, v192, 277.

3. Hayflick, L., Moorhead, P. S., Exp. Cell Res., 1961, v25, 585.

4. Tyrrell, D. A. J., Chanock, R. M., Science, 1963, v141, 152.

5. Phillips, C. A., Melnick, J. L., Grim, C. A., Proc. Soc. Exp. Biol. and Med., 1965, v119, 798.

6. Hamparian, V. V., Leagus, M. B., Hilleman, M. R., Stokes, J., Jr., ibid., 1964, v117, 469.

7. Andrewes, C. H., Science, 1964, v146, 1274.

8. Hamre, D., Connelly, A. P., Jr., Procknow, J. J., J. Lab. and Clin. Med., 1964, v64, 450.

9. Mufson, M. A., Kawana, R., James, H. D., Jr., Gauld, L. W., Bloom, H. H., Chanock, R. M., Am. J. Epidem., 1965, v81, 32.

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