CHIMPANZEE KIDNEY TISSUE CULTURES FOR GROWTH AND ISOLATION OF VIRUSES

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ABSTRACT

DICK, ELLIOT C. (University of Wisconsin, Madison). Chimpanzee kidney tissue cultures for growth and isolation of viruses. J. Bacteriol. **86**:573–576. 1963.—Chimpanzee kidney tissue cultures were employed for propagation of several laboratory strains of viruses that commonly inhabit the respiratory and intestinal tracts, or both, and for isolation of viruses from throat washings of persons with common colds and tonsillitis-pharyngitis. This tissue culture host was found to support the growth of approximately the same viruses as do Rhesus monkey kidney tissue cultures, with two exceptions: (i) chimpanzee kidney tissue culture was much more susceptible to herpes simplex infection, and (ii) cytopathic effects were not produced by either "M" or "H" strains of muriviruses (common cold viruses). The presence of adventitious viruses in some uninoculated chimpanzee kidney tissue cultures is suspected.

Tissue cultures from various hosts differ in their susceptibility to infection by different viruses: for example, it is well known (Schmidt and Lennette, 1961) that tissue cultures from Rhesus monkey kidneys are especially suitable for the growth of many myxoviruses, that human cells in continuous passage (HeLa, HEp-2, etc.) are usually the cells of choice for the isolation of adenoviruses, and that human embryonic kidney tissue cultures or their derivative cell strains (Hayflick and Moorhead, 1961) are most satisfactory or even absolutely necessary (Hamre and Procknow, 1961) for the cultivation of the muriviruses. [The name murivirus for the "common cold viruses" has been suggested by Mogabgab (*see* Weaver, Mogabgab, and Holper, 1963), co-discoverer of the apparent prototype strain of this group. The names rhinovirus, coryzavirus, and ECHO virus 28 have also been associated with these agents.] The use of chimpanzee kidney tissue culture for the propagation of viruses has not been reported; employment of this host system for the growth of several groups of viruses and for the isolation of viruses from throat washings is described herein.

MATERIALS AND METHODS

Preparation of chimpanzee kidney tissue cultures. Freshly obtained kidneys from three adult chimpanzees were immediately minced in growth medium consisting of Eagle's nutrients in Hanks' salt solution supplemented to 10% with calf serum and containing potassium penicillin, streptomycin sulfate, and amphotericin B at concentrations of 200 units, 100 μg, and 1 unit/ml, respectively. The minced tissue was kept at near-freezing temperatures until trypsinization (0.25% Difco trypsin in Hanks' salt solution) approximately 10 hr later. The resultant cell suspensions were washed thrice by centrifugation (International size one, model SB) at 800 rev/min in Hanks' salt solution, diluted in growth medium to a final viable cell concentration of 3×10^5/ml as determined by trypan blue, distributed to culture vessels and tubes, and incubated at 37 C. The growth medium was replaced in 24 hr. When cell monolayers had formed at about 7 days, they were either inoculated within 1 week or trypsinized and frozen by the method of Stuhlberg (1959). For virus inoculation, the cultures were maintained in a mixture of 50% Medium 199 and 50% Eagle's medium with Earle's salt solution; this mixture was supplemented with antibiotics as described above for growth medium and to 3% with Agamma calf serum (Hyland Laboratories, Los Angeles, Cal.). For freezing, the cell monolayer was removed from glass by trypsinization; the suspension was washed once by centrifugation in Hanks' salt solution, and resuspended in Eagle's medium (with Hanks' salt solution) supplemented to 15% with calf serum and to 5% with glycerol. The suspension was slow-frozen at a concentration of 2×10^6 cells

/ml in a Canalco "Slow-Freeze" (Bethesda, Md.) and stored at −70 F. Tissue cultures were prepared from the frozen material by rapid thawing at 37 C and planting 5 × 10^4 cells/ml in growth medium. Medium was replaced in 24 hr; 3 to 5 days were required for a monolayer to form.

Other tissue cultures. Trypsinized suspensions of Rhesus monkey kidney were kindly furnished by Donald Nelson, Chief, Virology Section, Wisconsin State Laboratory of Hygiene, Madison. The WI-26 diploid human embryonic cell strain was obtained from Microbiological Associates, Bethesda, Md. HEp-2 cells were subcultures of a strain furnished by Marc Beem, University of Chicago School of Medicine, Chicago, Ill. All tissue cultures were grown and maintained for virus inoculation as described for chimpanzee kidney.

Viruses. Several strains of muriviruses were furnished by Dorothy Hamre (University of Chicago Medical School), Coxsackie A-21 (Coe) virus by Edwin Lennette (Virus Laboratory, California Department of Public Health, Berkeley), and the parainfluenza viruses and murivirus type 1 (GL/2060/54) by William Mogabgab, Tulane University School of Medicine, New Orleans, La. All agents were tissue culture-grown, and the appropriate tissue culture host is indicated on the tables and figures.

Other procedures. Techniques employed for hemagglutination, hemadsorption, and $TCID_{50}$ titrations have been described previously (Dick and Mogabgab, 1962) and, where necessary, are repeated in the text or in the appropriate table or figure.

TABLE 1. *Days required for cytopathic effect (CPE) to be produced by established viruses in chimpanzee kidney tissue culture (CKTC)*

Virus inoculated[a]	Tissue culture origin of inoculum	Degree of CPE[b] in CKTC		
		2+	3+	4+
Adenovirus				
Type 3	Tonsil	2	4	8
Type 4	Tonsil	2	6	8
Type 5	Tonsil	3	6	8
Coxsackie virus				
A9	MK[c]	4	—[d]	—
B2	MK	10	—	—
B4	MK	10	—	—
B6	MK	3	5	—
ECHO virus				
Type 9	MK	4	—	—
Type 21	MK	10	—	—
Herpes simplex	HEp-2		2	4
Poliovirus				
Type 1	MK		2	3
Type 2	MK			2
Type 3	MK			2
Reovirus				
Type 1	MK	4	6	7
Vaccinia	Tonsil		2	—

[a] Coxsackie A-21 (Coe) virus and five strains of muriviruses failed to cause any consistent CPE.

[b] Results expressed in terms of days after inoculation when CPE appeared. Symbols: 2+, 3+, and 4+ equal 30, 60, and 90%, respectively, of cell monolayer exhibiting CPE.

[c] Rhesus monkey kidney tissue culture.

[d] Indicates that CPE did not progress to this point during the slightly more than 2 weeks in which most tissue cultures were examined.

RESULTS AND DISCUSSION

Growth of established viruses. Twenty-one viruses known to produce cytopathogenic effects in other cell cultures were tested in chimpanzee kidney tissue cultures. Herpes simplex, vaccinia, and several representatives from the adenovirus, Coxsackie virus, ECHO virus, poliovirus, and reovirus groups caused typical pictures of cell degeneration, whereas Coxsackie A-21 (Coe) virus and five strains of muriviruses (3 "M" and 2 "H" strains) did not (Table 1). [It has been suggested (Taylor-Robinson and Tyrrell, 1962) that those common cold virus strains which grow only in human-embryo kidney cells be termed "H" strains, and those which also grow in monkey kidney culture be termed "M" strains.] This spectrum of cytopathogenicity was similar to that shown by these strains when grown in monkey kidney tissue culture, with two major exceptions: (i) M strains of muriviruses caused no significant changes in chimpanzee kidney tissue cultures, and (ii) herpes simplex infections progressed much more rapidly in this tissue culture host than in monkey kidney tissue cultures or, in fact, in any other tissue culture host tested (Table 2).

Four viruses recognized primarily by the production of hemagglutinin in tissue culture were then tested in monkey kidney and chimpanzee kidney tissue cultures. Hemagglutinin production by parainfluenza types 1 and 2 was equally luxuriant in both systems, but parainfluenza type

TABLE 2. *Progression of cytopathic effect produced by herpes simplex virus[a] in several tissue culture hosts*

Tissue culture	Cytopathic effect[b]			
	1+	2+	3+	4+
WI-26		3	4	6
HEp-2		2	3	4
CK[c]			2	3
MK	15	30	—[d]	—

[a] This virus was HEp-2-grown; 0.1 ml was inoculated undiluted.

[b] Results expressed as days after inoculation when degree of CPE indicated appeared. Symbols: 1+ = five to ten distinct foci of infection; 2+, 3+, and 4+ equal 30, 60, and 90%, respectively, of cell monolayer exhibiting CPE.

[c] CK and MK = chimpanzee kidney and monkey kidney.

[d] Indicates CPE did not progress to this point.

TABLE 3. *Hemagglutinin production by several myxoviruses in chimpanzee kidney (CK) and in monkey kidney (MK) tissue cultures*

Virus inoculum*	Tissue culture inoculated	Reciprocal of hemagglutination titer†	
		Human type O erythrocytes	Guinea pig erythrocytes
Parainfluenza 1 HA-2 strain	MK	256	512
	CK	256	—‡
Parainfluenza 2	MK	32	64
	CK	32	256
Parainfluenza 3	MK	64	256
	CK	16	128
Influenza B Great Lakes strain	MK	128	—
	CK	16	16

* Monkey kidney tissue culture virus was used in all cases.

† For testing, 0.25% erythrocytes were sedimented at room temperature (parainfluenza 1 and 2) or 4 C (parainfluenza 3 and influenza B).

‡ Not done.

3 and influenza B (Great Lakes strain) produced less hemagglutinin in chimpanzee kidney cultures (Table 3). Additionally, parainfluenza type 1 was found to produce hemagglutinin in chimpanzee kidney tissue culture as rapidly or nearly so as was reported previously (Dick and Mogabgab, 1962) in monkey kidney culture (Fig. 1).

The quantitative susceptibility of chimpanzee kidney and monkey kidney tissue cultures to infection with three myxoviruses, and of chimpanzee kidney tissue culture and HEp-2 cells to infection with herpes simplex, was measured by $TCID_{50}$ titrations (Table 4). Judging from this criterion, initiation of infection by these agents in chimpanzee kidney tissue culture required no

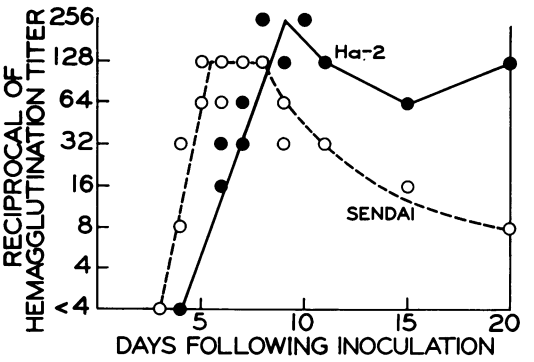

FIG. 1. *Production of hemagglutinin to type O human erythrocytes by two parainfluenza 1 strains. Hemagglutination titers were determined with 0.25% erythrocytes sedimenting at room temperature.*

TABLE 4. *Titration of some myxoviruses and herpes simplex in monkey kidney (MK) or HEp-2 and chimpanzee kidney (CK) tissue culture*

Virus inoculum*	Tissue culture for titration	$TCID_{50}$/0.1 ml of inoculum†
Parainfluenza 2 (MK)	MK	10^6
	CK	$10^{6.5}$
Parainfluenza 3 (MK)	MK	10^6
	CK	$10^{6.5}$
Influenza B Great Lakes strain (MK)	MK	10^3
	CK	$10^{2.5}$
Herpes simplex (HEp-2)	HEp-2	10^5
	CK	10^6

* Tissue culture host for inoculum is indicated parenthetically.

† After 1 week's proliferation of the appropriately diluted virus, $TCID_{50}$ titers of myxoviruses were determined by hemadsorption and hemagglutination of a 0.5% suspension of guinea pig erythrocytes.

TABLE 5. *Isolation of viruses from throat washings from persons with tonsillitis-pharyngitis or common colds**

Clinical classification	No. of specimens	No. of isolates	Isolation in				Tentative identity
			WI-26	CK	MK	HEp-2	
Tonsillitis-pharyngitis	13	2	+	0	0	0	Unknown
		1	+	+	0	+	Herpes simplex
		2	0	+	+	+	Enterovirus
		2	+	+	+	+	1 Myxovirus, 1 enterovirus
Common cold	13	6	+	0	0	0	Murivirus

* Each specimen was inoculated into WI-26, chimpanzee kidney (CK), monkey kidney (MK), and HEp-2 tissue cultures. Tubes were examined for cytopathic effect daily; hemagglutination (human type O erythrocytes sedimenting at room temperature and 4 C) and hemadsorption (guinea pig erythrocytes adsorbed at room temperature and 4 C) determinations were carried out on representative tubes at least weekly.

greater a number of virus particles, and may have required fewer, than were needed to infect the two other tissue culture hosts.

Isolation of viruses from garglings. Throat washings from persons ill with common colds or acute tonsillitis-pharyngitis were inoculated in parallel into WI-26 and HEp-2 cells and monkey kidney and chimpanzee kidney tissue cultures. All cultures were incubated at 33 C in a roller drum, examined daily for cytopathic effect, and tested at least weekly for adsorption of guinea pig erythrocytes and the presence of agglutinins to human type O red blood cells. The results are shown in Table 5. None of the persons with common colds yielded agents which grew in any tissue culture host other than the WI-26 diploid strain, and all isolates elicited cell changes cytopathically compatible with those caused by muriviruses. Throat washings from tonsillitis-pharyngitis patients yielded several agents causing cytopathic effects in chimpanzee kidney tissue cultures as well as other tissue cultures; no viruses were isolated in chimpanzee kidney tissue culture exclusively.

Presence of adventitious agents. During prolonged incubation (2 weeks or more) of uninoculated chimpanzee kidney tissue cultures, the cells frequently exhibited changes similar to changes caused by the growth of viruses; the maintenance medium from several of these cultures was stored at −70 F for further study. No hemagglutinins were detected in uninoculated cultures.

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