Virus Susceptibility of Marmoset Kidney Cell Cultures.* (30345)

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During the past three years our laboratory has evaluated the usefulness of the marmoset as a laboratory animal. As a part of this investigation we have made use of marmoset kidney cell cultures. This report will describe the susceptibility of this type of culture to representatives of the major groups of viruses.

Materials and methods. 1. *Viruses.* The virus strains used, and sources, are enumerated in Table I.

2. *Cell cultures.* a) *Primary marmoset kidney cell cultures.* Marmoset kidney cell cultures were prepared by trypsinization using, in essence, the method described by Youngner(1). The cells were grown in a medium consisting of fetal calf serum (FC) 15% and improved basal medium Eagle (BMEI)(2) in Hanks' balanced salt solution (H) 85%. After inoculation, the cultures were maintained in a medium consisting of FC 5% and Medium No. 199(3) with addition of 0.5% peptone.

All media contained 100 units penicillin, 50 μg streptomycin and 5 μg fungizone per ml. The pH of the media was adjusted to a value of 7.2-7.5 by addition of the necessary amount of a 7.5% solution of sodium bicarbonate.

b) *Other cell strains and lines.* The preparation and maintenance of primary human kidney, rabbit kidney, mouse embryo, diploid human embryo fibroblasts, and HEp-2 cell cultures, has been previously described (4). African green monkey kidney cultures were obtained from Microbiological Associates, Bethesda, Md.

3. *Chick embryo techniques.* Myxoviruses were titrated in the allantoic cavity of embryonating hens' eggs using standard techniques(5).

Experimental. Two-tenths of a ml of each agent were inoculated undiluted and at a 1:1000 dilution into each of 4 tubes of primary marmoset kidney cells. The tubes were observed daily, and when 75% destruction of the cell sheet had developed, the tubes were frozen and thawed once and the medium and cells harvested for passage. If no cytopathology appeared, the tubes were harvested at 14 days in the manner described and the undiluted material was passed blindly. This plan was then repeated with the above harvests for a total of 3 passages.

The amount of virus present in the culture at the end of the third passage was determined by inoculation of serial 10-fold dilutions of frozen medium and cells into a susceptible host. In the case of cell cultures 4 tubes and in the egg titrations 4 eggs were used for each dilution. The presence of virus in the egg harvests was evaluated by haemagglutination with 1% chicken erythrocytes.

Virus titers were calculated according to the method of Reed and Muench.

Results. The results are tabulated in Table II. Sixteen lots of marmoset kidney cell cultures were made. Uninoculated tubes from each lot were observed for 21 to 28 days for spontaneous appearance of cytopathology and each lot was checked for haemadsorption with chicken, guinea pig, and human type "O" erythrocytes. In no case was there evidence of indigenous agents in our cell cultures.

There was no evidence for multiplication of influenza A, Newcastle Disease and polyoma viruses. Adenovirus type C-1 and ECHO virus type 9 multiplied without the production of cytopathic effect. Although poliovirus types I and II and the attenuated poliovirus type III strain FOX multiplied well, strain LEON failed to propagate in marmoset kidney cultures.

* This investigation was supported in part by U.S.P.H.S. Research Contract PH43-62-179 from Nat. Cancer Inst., N.I.H.

† Part of this work was done during the tenure of Special Fellowship #1F3 AI-21,183-01 from Nat. Inst. of Allergy & Infect. Dis., N.I.H.

Discussion. It would appear that primary marmoset kidney cell cultures have a fairly wide spectrum of virus susceptibility. Twenty-four of 28 viruses multiplied in the cultures, including members of the entero-, myxo-, and adenovirus groups as well as herpes and pox viruses.

The results with poliovirus type I strain CHAT and adenovirus type Cl strain BERTHA and E9 (low final titers in marmoset kidney cells but high final titers in susceptible hosts) could represent either inapparent infection or lack of complete adaptation of these viruses to the culture system. To our knowledge, this is the first demonstration of the propagation with cytopathic effect of poliovirus strains in cell cultures made from New World monkeys, although Kaplan and Melnick(6) showed multiplication of poliovirus without cytopathology in Capuchin kidney cultures.

The results with SV-40 are inconclusive and are being studied further. They may not represent multiplication but rather carry over from the original inoculum.

Since this source of cell culture is relatively cheap, apparently free of inapparent viral infection and readily available, it is felt that it may represent a useful addition to the virologist's armamentarium.

Summary. Attempts were made to infect primary marmoset kidney cell cultures with 28 different agents representing the major groups of viruses. Although variable evidence for myxovirus multiplication was obtained and poliovirus type II strain LEON multi-

TABLE I. Virus Strains Studied.

Virus	Source	Passage history in our laboratory*	Strain
Poliovirus I	ATCC	2 HeLa	Brunhilde
" I	"	3 HEM	Chat
" II	"	2 HeLa	Lansing
" III	"	2 HeLa	Leon
" III	"	3 HEM	Fox
Coxsackie A-9	Sever, Nat. Inst. Health	2 pHUK	PB (Bozek)
" B-2	ATCC	2 HEp-2	Ohio
" B-6	"	2 HEp-2	Schmitt
ECHO-6	"	3 HEM	D'Amori
" -9	"	3 HEM	Hill
" -10 (REO-3)	Marmoset stool, Presbyterian-St. Luke's Hosp.	4 HEM	
Adenovirus 5	Hillis, Lackland Air Force Base	4 pHUK	HEP
" 9	ATCC	1 HEM, 2 pHUK	Hicks
" Cl	"	1 HEM, 2 pHUK	Bertha
Influenza A	Rafelson, Presbyterian-St. Luke's Hosp.	3 Allantoic	PR8
Newcastle dis. virus	Yamada, Osaka Univ.	28 Allantoic	Victoria
Mumps	Children's Hosp. of Philadelphia	14 Allantoic	Barnes
"	*Idem*	9 Amniotic	Ricky
Parainfluenza 1	ATCC	†	C35
" 3	"	†	C243
Influenza D, Type 1	"	†	Sendai/52
Herpes simplex	"	5 pRK	HF
" tamarinus	Marmoset adrenal gland, Presbyterian-St. Luke's Hosp.	2 HEM, 2 pRK	
Vaccinia	Commercial vaccine, National Drug Co.	3 pRK	
Vesicular stomatitis virus	Lederle Laboratories	8 MCN	Indiana
A-1 agent	O'Malley, Nat. Inst. Health	5 pHUK	
SV-40	Gerber, Nat. Inst. Health	2 pGMK	777
Polyoma	Microbiological Associates	7 pMEM	LID #1

* Numbers represent number of passages. Abbreviations indicate: HEM, human embryo cell cultures, diploid strain; pHUK, human kidney cell cultures, primary; pRK, rabbit kidney cell cultures, primary; pGMK, African Green Monkey kidney cell cultures, primary; pMEM, mouse embryo cell cultures, primary; MCN, subline of Earle's L-cells; ATCC, American Type Culture Collection.

† These agents were not propagated in our laboratory.

TABLE II. Results of Attempted Propagation of Various Agents in Marmoset Kidney Cell Culture.

Virus	Titers* in susceptible host		Marmoset 3rd passage kidney titer*	Passage dilution factor†	Time of appearance of CPE
	Initial	Final			
Polio I Brun	7.2	6.7	2.7	6.7	D7
" I Chat	7.7	7.7	2.0	2.1	D4
" II	6.7	6.2	1.7	5.1	D1
" III Leon	7.7	< .7	< .7	2.1	None
" III Fox	7.2	5.7	5.2	4.4	D5
Coxsackie A-8	6.2	5.7	4.7	2.1	D1
" B-2	6.0	5.2	4.7	11.1	D1
" B-6	6.0	5.2	5.7	6.7	D3
ECHO-6	8.2	7.2	6.2	11.1	D1
" -9	6.0	5.2	< .7	2.1	None
" -10	6.0	6.7	6.7	11.1	D2
Adeno 5	5.2	6.7	4.2	11.1	D3
" 9	5.2	6.2	5.7	2.1	D2
" Cl	4.7	4.7	< .7	2.1	None
Influenza A	4.7	< .7	< .7	2.1	"
NDV	6.5	< .7	< .7	2.1	"
Parainfluenza 1	5.7	ND	4.2‡	8.6	"
" 3	5.7	"	6.2‡	11.6	"
Influenza D, Type 1	8.7	"	7.2‡	11.1	D2
Mumps (Ricky)	5.5	"	6.2‡	9	None
" (Barnes)	5.0	"	3.2‡	4.2	"
Herpes simplex	6.2	7.7	5.2	11.1	D1
" tamarinus	5.2	5.2	4.7	11.1	D1
Vaccinia	5.7	4.2	5.5	2.1	D1
VSV	5.7	3.7	5.2	11.1	D1
A-1 agent	7.2	6.2	7.7	11.1	D5
SV-40	6.2	3.2	< .7	2.1	None
Polyoma	4.7	< .7	< .7	2.1	"

* Log ID_{50}/ml.
† Log total dilution of original inoculum after 3 passages.
‡ These titers determined by hemadsorption.

plied without cytopathology, this type of cell culture proves susceptible to a variety of other agents.

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Received March 5, 1965. P.S.E.B.M., 1965, v119.