Biological Characteristics and Viral Susceptibility of an African Green Monkey Kidney Cell Line (Vero)[1] (34285)

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A line of African green monkey kidney cells (Vero) was found useful for propagation and assay of the Tacaribe group of arboviruses, the etiological agents of South American hemorrhagic fever (1–3). Subsequently, Vero cells were shown to be useful in the assay of rubella virus (4–6), simian virus 5 (7) and certain adenoviruses (8, 9). Studies to determine the susceptibility of this cell line to additional viruses were further carried out. The present report provides an up-to-date list of the viruses so studied. Some of the biological characteristics of the Vero cells are also presented.

Materials and Methods. Viruses. The viruses tested in this study were obtained from various sources. They were passed once or twice in our laboratory under recommended conditions to obtain standard seed stocks and to produce various complement-fixing (CF) antigens for serological surveys (10).

Tissue cultures. Cell cultures other than Vero cells were obtained from Microbiological Associates, Inc., and were maintained at 37° on Eagle's minimum essential medium in Earle's balanced salt solution containing 3% agamma calf serum (obtained from Hyland Laboratories, Los Angeles, Calif.), 4 mmoles of glutamine, 100 U of penicillin, and 100 μg of streptomycin/ml. Vero cells in passage 93 were obtained from Dr. Y. Yasumura, Chiba, Japan, in 1964, and were maintained first at the Laboratory of Tropical Virology, National Institutes of Health, Bethesda, Md. (1–3), and later in this laboratory (4, 8). The Vero cells were subcultured and maintained in medium 199 supplemented with 5% fetal bovine serum (4). They were dispersed with 0.25% trypsin and diluted in the same medium. Cell suspension was adjusted to contain 1.5×10^5 cells/ml of medium. They were seeded into tubes (1 ml), 2-oz bottles (4 ml) or 32-oz bottles (40 ml) and were incubated for 3–4 days at 37°. The medium was then changed, and the cultures were incubated for an additional 2–3 days and were used for inoculation. The passage 107 through 165 levels of Vero cell cultures were used in this study. The passage 113 was frozen as stock, and was also deposited in the American Type Culture Collection repository.

Virus assay, assay for CPE of viruses. Two to four tubes were used for each 10-fold serial dilution tested. Observations for CPE were made for 28 days following inoculation, and fluids were changed every 5–7 days. The 50% end point was calculated by the Reed–Muench formula (11).

Plaque assay. Plaque assays in Vero cells were carried out by the double overlay method previously described (4). For the first agar overlay, the medium consisted of 0.5 % lactalbumin hydrolysate in Earle's salt solution, 2% fetal bovine serum, 0.23% $NaHCO_3$, antibiotics, and 1.5% agar. The second overlay was similar to the first, except that it contained neutral red at 1:45,000.

Neutralization tests. The constant virus-varying serum method, as well as the constant serum-varying virus method, were used for tube and plaque neutralization tests. Hyperimmune rabbit and monkey sera were mixed with the respective viruses, incubated at room temperature for 1 hr, and inoculated into Vero cell cultures.

[1] This work was supported by Contract PH 43-68-705 of the National Cancer Institute, National Institutes of Health.

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TABLE I. Chromosome Number of Vero Cells.

Passage level	Chromosome no.																						Total counted cells
	37	39	40	41	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	62	77	86	
122	3	1	1	1	2	5	2	3	4	2	7	15	7	12	12	4	10	1	4	2	1		1	100
141		1			3	3	2		2	2	6	5	9	12	28	15	8	2	1			1		100

Complement fixation. Preparation of CF antigens from infected Vero cultures was done according to the method described previously for viral and tumor antigens (10). The CF tests were carried out in the microtiter technique described by Sever (12). The sera employed for the CF tests were standard antiviral reagents of the CF laboratory of the Laboratory of Viral Diseases, NIAID, National Institutes of Health. Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement.

Chromosome cytology. For chromosome studies, Vero cultures were subcultured in the usual manner. After 6 hr, they were treated for 16 hr with diacetylmethyl colchicine (Coleceimide, Ciba) at a final concentration of 0.6 μg/ml; and this procedure was followed by treatment for 15 min with 0.9% sodium citrate. The cells were then removed from the surface of the bottle by scraping, and the cell suspension was processed according to the technique for air-dried preparations described for human peripheral blood (13).

Results and Discussion. Growth characteristics of Vero cultures. The Vero line assumed the characteristics associated with established cell lines. The cells grew in a dense, tightly packed sheet. Practically all of the cells were of polygonal morphology, some multinucleate. Fibroblastic cells were rarely observed (Fig. 1). Figure 1 shows that "transformation" as described by Chang (14) was not observed in the Vero line. The simian origin of Vero cells was confirmed by fluorescent antibody species determination (15). The Vero cells grew fast, in a dense, tightly packed sheet, and had higher plating efficiency than BS-C-1 cells, a continuous kidney cell line derived from the African green monkey (16). The BS-C-1 line grows very slowly in our hands.

Studies to determine the rate of multiplication of Vero cells were carried out with passage 129 cultures. Growth experiments initiated with approximately 1×10^5 cells/ml in 2-oz bottles indicated that the cells doubled in 72 hr and tripled in 4 days (Fig. 2).

In preparing large quantities of Vero cells for the experiments described in this report, it was found that the cell sheet from one C-2 bottle (area, approx 20 cm^2) yielded sufficient cells for preparation of 20 tube cultures, or one 32-oz bottle culture. Such cultures were ready for use after 5–7 days of incubation and one change of medium at about the third day. Throughout all the passage levels, the same procedure was employed.

Up to the present time, no pleuropneumonia-like organisms (PPLO) or latent viral agents have been found in Vero cultures maintained in this laboratory. No tumorgenicity of this cell line has been found in hamsters so far. On numerous occasions, cultures of the Vero line, as monolayers in fluid medium, have been shipped by air express to other parts of the world, and usually have survived the vicissitudes of travel with no difficulty.

Storage of the Vero cell line. Propagation of Vero cells from cultures which had been stored in the frozen state for varying periods of time, up to 12 months, was readily accomplished. Attempts were made to test the viability of Vero cells with 7.5% dimethylsulfoxide and medium 199 containing 10% fetal bovine serum in a nitrogen freezer. After thawing, viable-cell counts were performed, using 0.1% trypan blue as diluent. After 12 months of the nitrogen storage, 50–70% of virable cells were recovered, on 10 occasions, with an average of 55%. Cultures derived

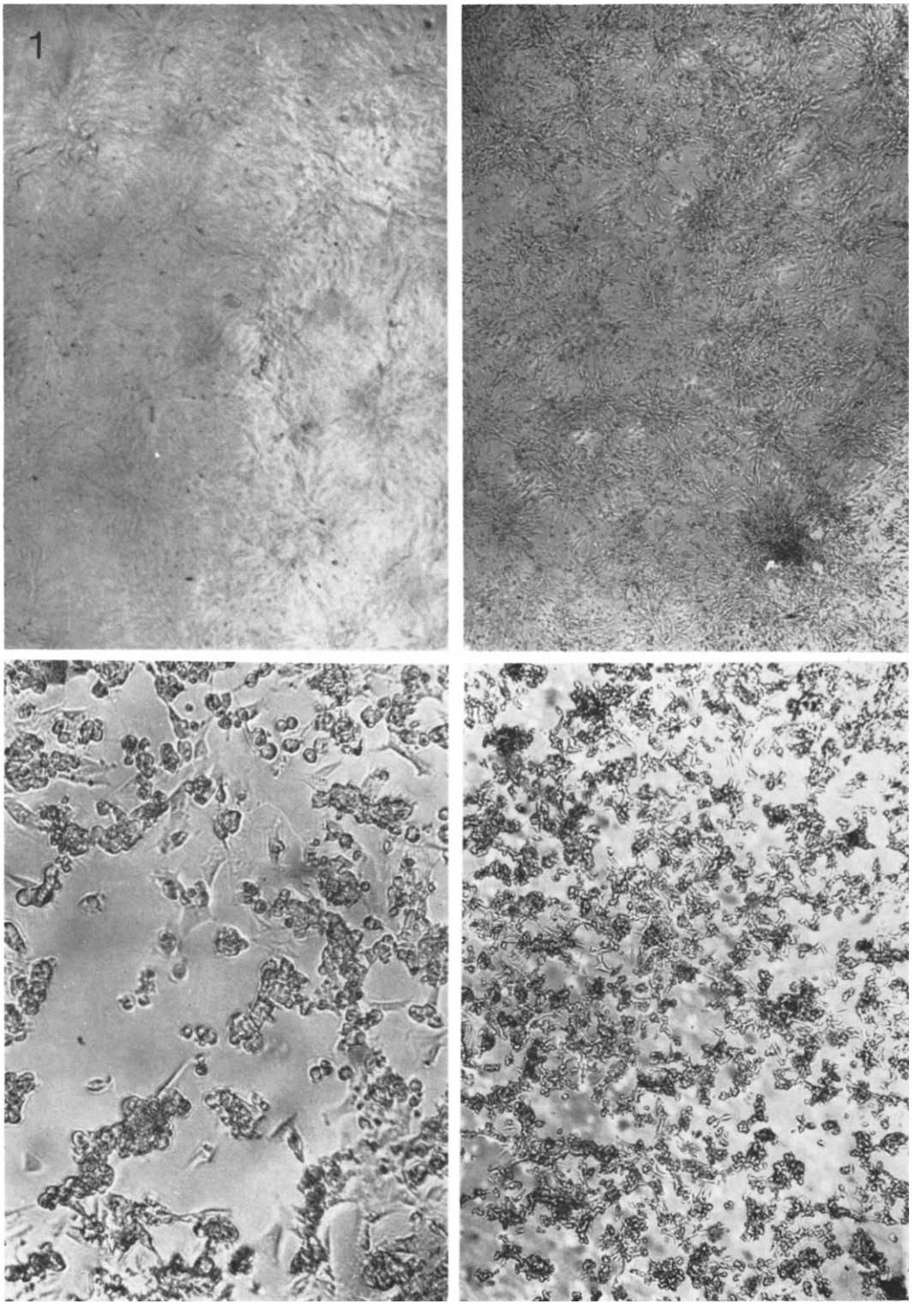
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FIG. 1. Cytopathic effect (CPE) of adenovirus type 11 in Vero cells: (upper left), uninoculated Vero cells (×40); (upper right), CPE of adeno 11 at 37° (×40); (lower right), CPE of adeno 11 at 30° (×40); (lower left), CPE of adeno 11 at 30° (×115).

from the passage 122 and 141 levels, after storage for several months, were examined for SV-40 susceptibility. Both cultures grew well and the revived cells were equally as sensitive to SV-40.

Chromosome studies. Karyology of two different passage levels of the Vero cell line was studied. Continuous *in vitro* cultivation of the line proves to have changed the ploidy of the host species chromosome number. At passages 122 and 141, there was a heteroploid state. No unusual changes were observed in the examination of metaphase spreads: there were no distinctive markers, rings, or consistent breaks. Examination of passage 122 demonstrated that the model number of chromosomes fell in the region of 51–55, with emphasis on 52, 54, and 55. Passage 141 showed a region between 54 and 56 with the emphasis on 55.

A hundred metaphase spreads were counted

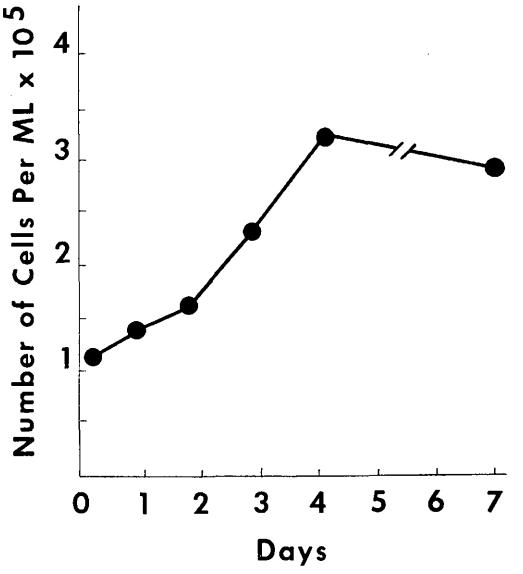

FIG. 2. Multiplication of Vero cells in passage 129 culture.

TABLE II. Susceptibility of Vero Cells to Adenoviruses.

Type	Strain	MOI[d]	CPE in Vero (days post-inoculation)	Infectivity titer[a] in Vero	Infectivity titer[a] in HEK	CF titer[b] in Vero
Human adenovirus						
1		—	++ (4)	6.7	—	>128
2		—	+ (4)	7.2	9.7	>128
3	G.B.	300	+ (7)	6.7(6.0)[c]	9.2	64
4	38558	10	++ (4)	3.5	—	32
5		10	++ (4)	7.7	—	>128
6		10	++ (4)	7.2	—	>128
7	Gomen	300	++ (4)	5.2	8.2	>8
8		—	+++ (5)	6.7	—	—
9		—	+ (9)	3.2	—	—
11	Slobitski	10	++ (4)	5.2	9.2	32
12	Huie	32	++ (2)	6.7	8.2	>128
14	DeWit	100	+ (9)	4.7	7.2	64
16	CH 79	10	+ (5)	6.2	9.2	64
18	DC	3.2	+ (3)	5.2	6.2	64
21	D 56	30	+ (3)	7.7	8.2	>8
31		10	+ (3)	6.2	7.7	>128

[a] Infectivity titer expressed as $\log_{10}TCD_{50}$/ml.

[b] CF titer = reciprocal of dilution giving 3+ to 4+ fixation of 1.8 units of complement.

[c] PFU/ml.

[d] MOI = multiplicity of infection; HEK = human embryonic kidney cells; and — = not done.

TABLE II. (continued) Part II.[a]

	Virus	MOI[d]	CPE[c] in Vero (days post-inoculation)	Infectivity titer[b] in Vero		In Vero	
				Vero	AGMK	PFU	CF[e]
Simian adeno							
I	SV-36	—	++ (4)	6.2	—	—	—
II	SV-15 (m-4)	100	++++ (7)	7.2	8.2	—	—(80)[f]
	SV-17 (m-6)	1	++ (4)	6.2	6.7	—	16
	SV-23 (m-2)	10	++ (3)	6.7	7.7	—	—
	SV-32 (m-3)	300	++ (3)	8.2	8.7	—	—
	SV-37	100	+ (3)	7.2	9.2	—	—
	SV-39	—	++ (3)	7.7	9.2	7.2	16
III	SV-1 (m-1)	1/10	++ (3)	7.2	5.2	—	>128
	SV-20 (m-12)	10	++ (3)	7.7	7.2	7.6	>128
	SV-25 (m-8)	1/10	++ (4)	5.7	5.2	—	>128
	SV-30	1/10	++ (3)	7.2	5.2	—	>128
	SV-33 (m-10)	10	+++ (3)	8.2	5.2	8.3	>128
	SV-34 (A7644)	1/10	++ (5)	5.2	6.2	—	—
	SV-38	100	+ (3)	8.2	7.2	—	>128
	SA-7	10	+++ (3)	8.2	9.2	—	>128

[a] Each virus seed pool was titrated simultaneously in Vero cells and in the indicated control system; 2–4 tubes/dilution were examined daily for 28 days for CPE (cytopathic effects).

[b] Infectivity titer expressed as $\log_{10}TCD_{50}$/ml.

[c] CF titer = reciprocal of dilution giving 3+ to 4+ fixation of 1.8 units of complement.

[d] MOI = multiplicity of infection; and — = not done.

[e] CPE: + = 11–25%; ++ = 26–50%; +++ = 51–75%; ++++ = >75% of cells affected.

[f] Hemagglutination titer.

in each passage. The results indicate that a heteroploid state now exists, with the model number of chromosomes falling in the range of 52–55 (Table I).

Infection of Vero cells with adenoviruses. It has been shown that the Vero cell line can be used for the production of adenovirus tumor (T) antigen relatively free from viral antigen by the thermal separation method (8), as well as for the production of high-titered viral antigen (8, 9). Further studies on the susceptibility of Vero cells to adenoviruses have yielded similar results. High-titered CF antigens for human and simian adenovirus were produced in Vero cells. Infectivity titers of simian adenovirus obtained in Vero cells were comparable to those obtained in primary African green monkey kidney cells. However, infectivity titers of human adenovirus obtained in Vero cells were 1–4 logs lower than those obtained in human embryonic kidney cells (Table II). Thus, the Vero cell line, which is relatively sensitive to human and simian adenoviruses, is more economical to produce than primary human

TABLE III. Cytopathic Effect (CPE) and Infectivity Titers of Adenovirus Type 11 in Vero Cell Cultures at 37 and 30°.

Temp of incubation (°)	Time of harvest (days post-inoculation)	CPE[a]	Infectivity titer[b] in HEK cells
37	4	+	5.4
	11	++	—
	15	++++	6.7
30	4	++	6.0
	11	++++	—
	15	++++C	7.2

[a] CPE: + = 11–25%; ++ = 26–50%; +++ = 51–75%; ++++ = >75% of cells affected; C = complete lysis of cells; and — = not done.

[b] Infectivity titer expressed as $\log_{10}TCD_{50}$/ml; multiplicity of infection was 100 TCD_{50}/cell.

and simian cells, and can be used as a substitute for them in the assay of these viruses.

It has also been found (8) that the highest titers of T antigen for certain adenoviruses were obtained in Vero cells incubated at 30°. Development of CPE and infectivity titers of adenovirus type 11 in Vero cell cultures incubated at 30 and 37° was examined. Vero cultures were inoculated with approximately 100 TCD_{50} per cell of adenovirus type 11 and incubated at 30 and 37° in a stationary position. The cultures were examined for CPE, and fluids were harvested and tested for infectivity at various intervals after inoculation.

There was not only a striking enhancement of CPE in the cultures incubated at 30°, but significantly more virus was obtained. (Fig. 1 and Table III). It is also true, in the case of a Japanese B encephalitis virus study (17) that there was a striking enhancement of CPE in the cultures incubated at 30°, as well as more virus (0.5–1 log) being produced.

Susceptibility to infection with other viruses. Vero cells were further examined for their susceptibility to various other groups of viruses, including herpes, myxovirus, papova, picorna, pox, reo, arbo, and rubella. This work has led to a satisfactory cytopathic and plaque assay for arboviruses, rubella, human and simian adenoviruses (18), SV-40 (19), polio, reoviruses, myxoviruses [measles (20), SV-5], herpes virus, and pox viruses (vaccinia, myxoma). Furthermore, complement-fixing and hemagglutinating antigen yields were also high, although only a limited number of viruses were tested (Table IV).

Since Vero cells were successfully used for

TABLE IV. Susceptibility of Vero Cells to Various Viruses Other than Adenoviruses.[a]

Group and virus (strain)	MOI[d]	CPE in Vero (days post-inoculation)	Infectivity titer[b] in		In Vero	
			Vero	Indicator[e]	PFU	CF[e]
Herpes						
Herpes simplex	1/1000	++++ (3)	6.2	—	—	—
Varicella	—	++++ (7)	6.2	—	5.7	—
Human salivary gland	1/1000	++++ (7)	6.0	—	5.1	—
Canine herpes	1/1000	++++ (9)	6.2	—	—	—
Myxovirus						
SV-5	1/50	++++ (9)	6.2	5.7(RhMK)	5.5	1:32(128)[f]
Mumps	1/100	++++ (7)	5.2	5.2(AGMK)	5.7	—
New Castle	—	+++ (7)	6.7	—	6.3	—
Sendai	—	0 (28)	—	—	—	—
Respiratory syncitial	1/10	++++ (12)	6.2	6.2(AGMK)	6.7	—
Measles	1/50	++++ (5)	6.7	6.7(AGMK)	6.6	—
Bovine paraflu T.3[g]	—	++++ (5)	5.5	5.0(Bk)	—	—
Papova						
Polyoma	1/10	0 (28)	<1.7	6.7(MsK)	—	—
SV-40	10	++ (3)	8.2	8.2(AGMK)	8.2	>128
Picorna						
Polio Type 1	100	++++ (2)	8.7	8.2(AGMK)	8.9	—
2	100	++++ (2)	7.7	8.0(AGMK)	—	—
3	100	++++ (2)	8.2	8.2(AGMK)	—	—
Echo 11 (Gregory)	1/10	++ (3)	5.2	—	—	—
Coxsackie A-9	1/10	+ (5)	6.2	—	—	—
A-21	1/10	+ (10)	2.7	—	—	—
Pox						
Vaccinia	10	++++ (5)	7.2	7.2(AGMK)	7.4	—
Myxoma	—	++++ (6)	6.2	—	6.0	—
Fibroma	—	++++ (5)	5.2	5.2(MA-111)	5.0	—

TABLE IV (continued)

Group and virus (strain)	MOI[d]	CPE in Vero (days post-inoculation)	Infectivity titer[b] in Vero	Infectivity titer[b] in Indicator[e]	In Vero PFU	In Vero CF[c]
Reo						
Reo T. I (Lane)	10	++++ (8)	8.0	8.2(RhMK)	7.8	1:4
(716)	10	++++ (5)	7.7	8.0(RhMK)	7.8	1:32
II (4/48)	1/10	++++ (8)	6.2	—	—	—
III (Dearing)	10	++++ (5)	8.0	8.5(RhMK)	8.2	—
Arbo						
Sindbis	—	+++ (4)	5.7	—	5.8	—
Japanese B. encephalitis (Nakayama)	1/10	++++ (4)	9.0	8.5(HK)	9.4	—
Tacaribe (TR 11573)	1/1000	++++ (5)	8.2	8.1(SM)	8.4	—
Junin (XJ)	1/100	++++ (7)	7.8	7.7(SM)	7.7	—
Machupo (Machupo)	1/1000	0 (28)	—	—	8.8(7.5)[h]	
Amapari (BE An-70563)	1/100	+++ (5)	6.5	7.7(SM)	7.8	—
Vesicular stomatitis	1/10	++++ (3)	6.2	6.2(AGMK)	6.3	—
Miscellaneous						
Rubella (RV)	1/10	++++ (7)	6.4	6.0(AGMK)	5.7	1:8
(M-33)	1/10	++++ (8)	6.2	5.8(AGMK)	—	—
(HP-77)	1/10	++ (8)	3.5	3.7(AGMK)	—	—

[a] Each virus seed pool was titrated simultaneously in Vero cells and in the indicated control system. Two to four tubes per dilution were employed. Cultures were examined daily for 28 days for cytopathic effects (CPE).

[b] Infectivity titer expressed as $\log_{10}TCD_{50}$/ml.

[c] CF titer = reciprocal of dilution giving 3+ to 4+ fixation of 1.8 units of complement.

[d] MOI = multiplicity of infection; — = not done.

[e] RhMK = rhesus monkey kidney; AGMK = African green monkey kidney; MsK = mouse embryonic kidney; BK = bovine kidney; MA-111 = a continuous newborn rabbit kidney cell line; HK = hamster kidney; and SM = suckling mouse.

[f] Hemagglutination titer.

[g] Bovine parainfluenza Type 3.

[h] Plaque forming units in MA-111.

the first time in the U.S.A. for assay of the Tacaribe group of arboviruses (1–3), a number of investigators have reported the susceptibility of Vero cells to infection with various viruses (Table V). Perhaps the most extensive use of Vero cells has been reported for the arbovirus group (1–3, 21–23). Each of 19 arboviruses from Central or South America produced plaques in the Vero cell line and in MA-111, a continuous cell line of newborn rabbit kidney. It has been suggested that Vero cells were superior to the MA-111 cell line (21). This was also true in the case of the Tacaribe group of arboviruses, as shown in comparison studies between the Vero and MA-111 cell lines (1–3).

It has also been shown that Vero cells were useful for primary isolation, propagation, and assay of rubella virus, in lieu of the widely used primary African green monkey kidney cells (4, 5), and this has been confirmed (6). In addition, a recent report showed that Vero cells would furnish a useful alternative for BHK-21 cells for production of rubella hemagglutinating antigen (25).

Summary. A continuous cell line derived from kidney tissue of the African green monkey (*Cercopithecus aethiops*), and designated as Vero, has been maintained and was studied at passage 107–165 levels for susceptibility to various viruses. Vero cells supported the growth of a number of viruses to high

TABLE V. Reported Data on Viral Susceptibility of Vero Cells.

Virus group	Virus	Ref.
Adeno	Adeno	Rhim *et al.* (1968) (8) Schell *et al.* (1968) (9)
Simian Adeno	SV-30	Slifkin *et al.* (1968) (18)
Papova	SV-40	Yasumura and Kawakita (1963) (19)
Myxo	Measles	Sasaki *et al.* (1964) (20)
	SV-5	Rhim and Schell (1967) (7)
Arbovirus		Earley *et al.* (1967) (21) Stim and Henderson (1968) (22)
Group A	EEE, VEE, UNA, Pixuna, Macambo and others	
Group B	Ilheus, yellow fever, SLE, Bussuquara	
	Bunyamwera, Guaroa, Cache Valley, Wycomya	
	California, B-878, B-1113-14	
	Changuinola, BT-104	
	Sandfly fever, Chagres	
	vesicular stomatitis	
Tacaribe		
	Tacaribe	Simizu *et al.* (1967) (1)
	Junin	Rhim *et al.* (1967) (2)
	Machupo	Rhim *et al.* (1969) (3)
	Amapari	Pinheiro *et al.* (1966) (23)
	Rubella	Rhim and Schell (1967) (4) Liebhaber *et al.* (1967) (5) Rafajko and Zur Nedden (1968) (25) Desmyter *et al.* (1968) (6)
	African horse-sickness virus	Ozawa (1967) (24)

titers. Extensive cytopathic effects and plaque formation in Vero cell cultures make possible an efficient and reproducible direct technique for the demonstration of a number of viruses and of specific neutralizing antibodies. In addition, growth of a number of viruses (such as rubella, SV-5, and the Tacaribe group of viruses) to a high titer in a continuous simian cell line provides a convenient source of virus and antigen for other studies. Thus, the Vero cell line provides the virologist with another tool for diagnostic and research work, and perhaps offers advantages for large scale production of viral agents for vaccine.

We thank Mr. H. C. Turner for supervising the complement-fixation tests, and Dr. Robert J. Huebner for helpful criticism and suggestions in the preparation of this manuscript.

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Received June 5, 1969. P.S.E.B.M., 1969, Vol. 132.