titis had predominantly α chylomicra (Group 2). Consideration of the diet of each subject and of serial observations of a subject from each group during dietary manipulation leads to these conclusions: β chylomicronemia occurs primarily because of increased synthesis. Clearing may be grossly normal or moderately decreased at moderately elevated TG levels, and may become severely impaired at high TG levels, due to these levels *per se*. α-Chylomicronemia occurs primarily because of impaired clearing but is regularly accompanied by retention of small quantities of β chylomicra.

The author is indebted to Doctor Christine Waterhouse for critical review of the manuscript.

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Received February 24, 1964. P.S.E.B.M., 1964, v116.

Applicability of the HeLa (Gey) Strain of Human Malignant Epithelial Cells to the Propagation of Arboviruses.* (29246)

SONJA M. BUCKLEY (Introduced by J. Casals)
The Rockefeller Foundation Virus Laboratories, New York City

Scherer and Syverton(1), while investigating the viral range of the HeLa strain of human malignant epithelial cells(2), obtained encouraging results with some arthropod-borne (arbo) viruses of groups A and B. Since it seemed desirable to study one *in vitro* host system extensively, efforts have been continued to determine the practical value of HeLa cells for propagation of arboviruses. A total of 94 distinct arbovirus strains have now been tested in these laboratories for their ability to produce a cytopathic effect (CPE) in HeLa cells. The purpose of this report is to provide an up-to-date list of these viruses and to discuss their cytopathology in HeLa cells.

Materials and methods. Viruses. The viruses tested are listed in Table I, grouped according to the classification in use at the Rockefeller Foundation Virus Laboratories at the end of 1962. For the initial *in vitro* passage, infected newborn mouse brain suspensions, liver suspensions and sera were sources of virus. Inocula consisted of liquid or lyophilized virus stocks stored in glass-sealed ampules at —70° and —20°C, respectively. Occasionally, fresh virus preparations were used.

Cellular cultivation and inoculation and incubation of cultures. The procedures for growing and maintaining HeLa cells have varied somewhat through the years(3,4); however, standard methods have been developed and described in detail(5). It has been the experience in these laboratories that a pH of 7.2 $\pm$ 0.2 in the inoculated cultures is optimal for growth of arboviruses at an incubation temperature of 37°C.

Assays for CPE of viruses. Increasing 10-fold dilutions of each strain were inoculated

* Stocks of viruses as well as immune sera were obtained from Drs. Jordi Casals, D. H. Clarke, Max Theiler and Loring Whitman. Appreciation for their generous cooperation is expressed. The valuable technical assistance of C. Mullen, E. Rodriguez and B. Dvorak is also gratefully acknowledged.

TABLE I. List of Arboviruses Tested.

Virus	Strain designation	Source of inoculum and passage level	CPE	TCD_{50}/ml ($-\log_{10}$)
Group A				
AMM 2021	AMM 2021	Mouse, 2	3+	7.5
AMM 2354*	AMM 2354	" 3	3+	8.0
Aura	BeAr 10315	" 6	0	0
Chikungunya*†	Ross, original	" 16	3+	8.7
	Vereeniging	" 9	2+ to 3+	5.7
Eastern equine encephalitis*	TenBroeck	" >100	3+	10.7
	BeAn 5122	" 4	3+	10.7
	Tr 25714	" 3	3+	9.0
Mayaro†	Tr 4675	" 6	3+	8.0
	BeH 256	" 7	4+	>8.5
Middelburg	SA AR 749	" 12	0	0
O'nyong-nyong*	Williams & Woodall	" 8	4+	8.5
Semliki Forest*†	Smithburn *et al.*	" 11	4+	10.3
Sindbis	EgAr 339	" 7	3+	6.0
Una*	BeAr 13136	" 3	3+	7.5
Venezuelan equine encephalitis*	Beck & Wyckoff	" 5	4+	8.0
Western equine encephalitis*	McMillan	" 19	3+	7.7
Group B				
Bussuquara	BeAn 4073	Mouse, 4	3+	7.5
Dengue, type 1*†	Hawaii	" 78	2+ to 3+	3.2
	S 601	" 27	2+ to 3+	5.0
" , type 2*†	New Guinea C	" 52	3+	9.5
	S 843	" 41	3+	>6.5
" , type 3*†	H 87	" 28	3+	8.7
" , type 4*†	H 241	" 12	2+ to 3+	4.5
	A 5902	" 9	2+ to 3+	3.7
H 336	H 336	" 6	4+	7.5
Ilhéus†	Original	" 10	3+	9.5
Japanese B*†	Nakayama	" 50	3+	7.2
	G 8924	" <10	3+	6.5
Kokobera	MRM 32	" 7	2+ to 3+	6.5
Kunjin	MRM 16	" 5	2+ to 3+	7.0
Kyasanur Forest disease*†	P 9605	" 5	3+	10.7
	G 1138	" 6	3+	10.5
	H 377	" 5	3+	11.5
Louping ill*†	Moredun	" 17	3+	7.5
Murray Valley enceph.	MRM 66	" 6	2+ to 3+	>7.5
Negishi	Original	" 9	3+	10.5
Omsk hemorrhagic fever†	Kubrin	" 25	2+ to 3+	9.5
Powassan	Byers	" 5	2+ to 3+	7.5
St. Louis encephalitis	Hubbard	" 120	3+	8.5
Tick-borne encephalitis, type Russian spring-summer encephalitis*†	Sophy	" >100	2+ to 3+	8.7
Tick-borne encephalitis, type Central European*†	Diphasic milk fever	" 3	2+ to 3+	9.2
	Yugo TBE	" 19	2+ to 3+	8.0
	Kumlinge A 59	" 2	2+ to 3+	9.7
	Swedish H	" 8	2+ to 3+	11.2
	Hypr	HeLa, 36	3+	7.7
	Graz	Mouse, 10	2+ to 3+	9.2
West Nile	Eg 101	" 1	3+	7.5
Yellow fever†	Asibi	" 1	2+ to 3+	6.5
	17 D	" 1	3+	6.0
Zika	MR 766	" 146	4+	5.5
Group C				
Apeu*	BeAn 848	HeLa, 19	4+	5.2
Caraparu*	BeAn 3994	" 20	4+	6.3
Caraparu-like*	Tr 34053-1	" 9	4+	6.5
Itaqui*	BeAn 12797	" 7	4+	5.7
Marituba*	BeAn 15	" 22	4+	6.5
Murutucu*	BeAn 974	" 22	4+	5.2
Oriboca*	BeAn 17	" 18	4+	7.0

into each of 3 HeLa cell cultures, which were thereafter examined for development of CPE at 1- to 3-day intervals for 21 days. A Zeiss Standard Universal microscope was used, at magnifications of 78.75 ×, 100.8 × and 126 ×.

TABLE I (continued)

Virus	Strain designation	Source of inoculum and passage level	CPE	TCD_{50}/ml ($-\log_{10}$)
Bunyamwera group				
Bunyamwera	Original	Mouse, 42	4+	>8.5
Cache Valley*†	6V-633	" 20	4+	7.2
	BeAr 7272	" 7	4+	5.7
Germiston	SA Ar 1050	" 9	4+	7.2
Kairi	Tr 8900	" 10	4+	6.7
Sororoca	BeAr 32149	" <10	2+ to 3+	4.5
Wyeomyia	Roca-Garcia	" 206	2+ to 3+	7.7
A 9 171 b†	A 9 171 b	" 19	4+	7.2
California complex				
California enceph.*†	BFS-283	Mouse, 12	4+	8.2
Lumbo*†	SA Ar 1881	" 3	4+	7.0
Melao*†	Tr 9375	" 4	2+ to 3+	8.0
Tahyna*†	Original	" 19	4+	8.5
Trivittatus*†	933	" 6	4+	7.5
Guamá group				
Guamá†	BeAn 177	Mouse, 16	2+ to 3+	>8.5
Catú*†	BeH 151	" 18	2+ to 3+	>6.5
Tacaribe group				
Junín*†	XJ	Mouse, 30	2+ to 3+	6.7
	F	" 1	2+ to 3+	2.5
	R	" 2	2+ to 3+	5.7
Phlebotomus fever group				
Naples phlebotomus fever†	Naples type (Sabin)	Mouse, 51	2+ to 3+	5.5
Sicilian phlebotomus fever†	Sicilian type (Sabin)	" 35	3+	8.0
Vesicular stomatitis group				
VSV Indiana*†	Original	Mouse, 5	4+	>7.5
VSV New Jersey*†	Original	" 5	4+	>7.5
Cocal*†	Tr 42033	" 5	4+	>7.5
Irituia group				
Irituia†	BeAr 41067	Mouse, 2	3+	3.7
BT 436†	BT 436	" 1	4+	6.0
Koongol group				
Wongal	MRM 168	Mouse, 8	0	0
Ungrouped				
Chaco	BeAn 42217	Mouse, 4	0	0
Chenuda	EgAr 1152	" 24	3+	6.3
	SA Ar 3441	" 4	2+ to 3+	3.5
EgAr 492	EgAr 492	" 17	0	0
"Dry Tortugas"*†	"Dry Tortugas"	" 13	2+ to 3+	3.3
Marco†	BeAn 40290	" <10	M + F	4.5
Mirim†	BeAn 7722	" 4	2+ to 3+	5.5
Quaranfil*†	EgAr 1095	" 15	4+	5.3
	SA Ar 2939	" 4	4+	6.5
	SA An 4671	" 7	4+	4.3

* Neutralization test performed.

† Serially propagated in HeLa cells for from 3 to 82 passages.

Note: Viruses listed above but not yet reported in the literature were supplied by the following laboratories: AMM 2021 and AMM 2354 by Walter Reed Army Inst. of Research, Washington, D. C.; Tr 34053-1 by Trinidad Regional Virus Laboratory, Port of Spain, Trinidad; A 9 171 b by the Communicable Disease Center, Atlanta, Ga.; Sororoca, Irituia, Chaco, Marco and Mirim by the Belém Virus Laboratory, Belém, Brazil; BT 436 by Middle America Virus Laboratories, Panama; Chenuda, EgAr 492 and Quaranfil by NAMRU-3, Cairo, United Arab Republic.

In vitro *passages of viruses*. Strains were propagated serially by employing 0.1 ml of either diluted fluid phase or combined cell and fluid phase. Viral transfers from infected to new HeLa cell cultures were carried out as soon as cytopathic activity was detected, i.e., 1-7 days following inoculation, the time interval depending on the prepatent period of the virus.

Infected HeLa cell stocks. These were prepared from combined cell and fluid phases, lyophilized in 1-ml amounts and stored at −70°C.

Infectivity titers were based on the regular occurrence of destructive changes over the 21-day period. They were expressed as TCD_{50} per ml and were calculated by the method of Reed and Muench(6).

In vitro *neutralization tests*. The constant virus-varying serum method as well as the constant serum-varying virus method were used. Hyperimmune mouse and rabbit and immune guinea pig sera were mixed with the respective viruses, incubated at 37°C for one hour and inoculated into HeLa cell cultures.

Experimental results. It was essential that cultures contain uniform cells with a negligible degree of necrobiosis, the normal process by which individual cells die and are replaced by new cells. Cell counts made with a No. 100 Hausser HY-Lite Chamber showed that the uninoculated cultures, under so-called "maintenance conditions," increased in number about 3-fold during a 7-day period. Thus, while cultures exhibited a thin, clear monolayer of cells at time of inoculation, they showed multiple layers of cells, some of which were granular, at time of the final examination, 21 days after inoculation.

For detection of early changes, observation with a magnification of 126 × proved advantageous. With low multiplicities of viruses, the initial lesion consisted invariably in death of a group of cells surrounded by normal appearing cells. The affected cells were rounded and granulated. Later, the necrotic cells tended to detach from the glass with resulting formation of a hole in the cell sheet. This manifestation of one focal lesion or one microplaque was graded as "+F" (Fig. 1). When such lesions became multiple, the picture was graded as "M+F," to denote pressence of many focal areas of necrosis throughout the cell sheet. With prolonged incubation, more and more cells throughout the monolayer underwent necrosis and detached from the glass. Detachment of 25-50% of cells was graded as "2+ to 3+;" subtotal necrosis of the cell sheet, with 50-90% of cells detached, was graded as "3+" (Fig. 2); and total necrosis, 90-100% of cells detached, was graded as "4+" (Fig. 3). As shown in Table I, one-third of the arboviruses studied caused 2+ to 3+ CPE, another third caused 3+ CPE and the remainder caused 4+ CPE.

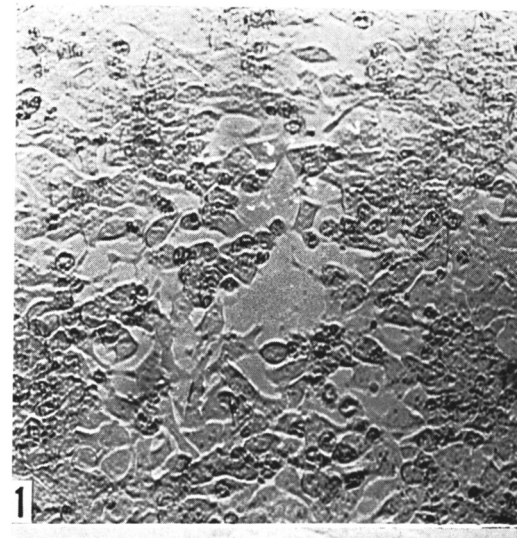
With high multiplicities of viruses, the early stages of +F and M+F were not observed. Instead, disseminated individual cell necrosis or partial necrosis of the cell sheet occurred rapidly, progressing from 2+ to 3+, to 3+ or 4+ as described.

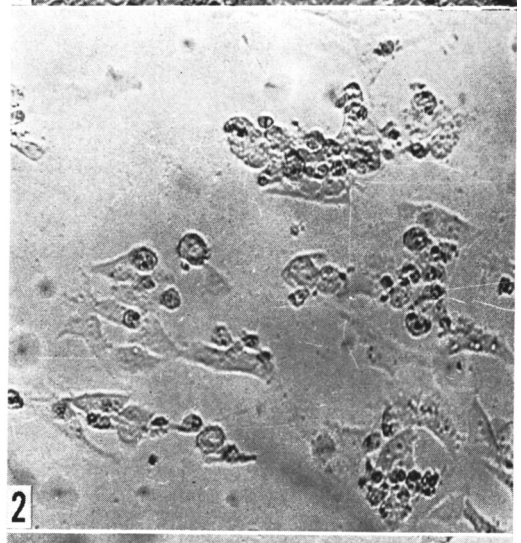
Fifty-four of the strains were propagated serially without difficulty. Inocula of viruses obtained from infected HeLa cell cultures gave results similar to those obtained from mouse passage material. In one instance, with Marco virus (BeAn 40290), a lizard isolate, CPE was arrested at the M+F stage in the initial as well as in the 4 serial passages.

Two group A viruses, Aura and Middelburg, failed to cause CPE in HeLa cells; however, both strains multiplied with concomitant CPE in primary chick embryo tissue cultures. Wongal virus, an Australian mosquito isolate, EgAr 492, an ungrouped Egyptian tick isolate, and Chaco virus (BeAn 42217), a lizard isolate, failed to produce lesions.

The infectivity titers did not depend on the severity of CPE induced; indeed, some of the highest values were observed with group B tick-borne viruses which, on the whole, produced only partial destruction of HeLa cells (Table I). TCD_{50} titers were rather conditioned by the quality of the virus source, lyophilized stocks giving lower titers than frozen or fresh preparations.

The prepatent periods observed with high multiplicities of HeLa cell passage viruses varied from 18 hours to 7 days. It must be emphasized that all HeLa cell adapted virus strains induced lesions within 7 days. Frequent fluid changes during the period of viral synthesis caused a decrease in the prepatent





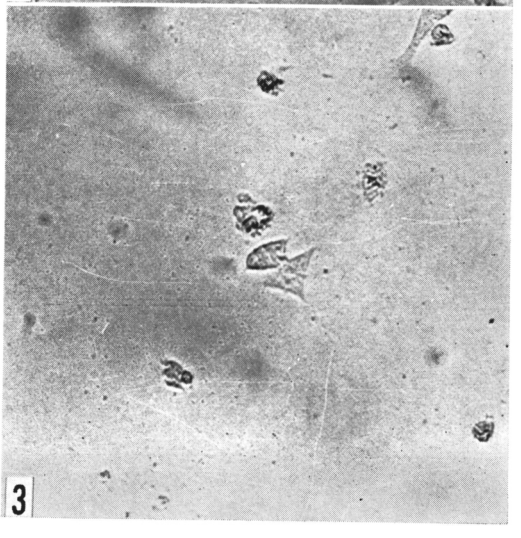


FIG. 1. HeLa cell culture, unstained, 5 days after inoculation with Quaranfil virus (SA Ar 2939), 1st passage in HeLa cells, 10^{-5} dilution. Note area of focal cellular destruction. CPE = +F. 114 ×.

FIG. 2. HeLa cell culture, unstained, 8 days after inoculation with "Dry Tortugas" virus, 5th passage in HeLa cells, 10^{-1} dilution. Most HeLa cells detached from glass. A few islands of cells still attached to glass, made up of rounded and granulated as well as normal appearing cells. CPE = 3+. 114 ×.

FIG. 3. HeLa cell culture, unstained, 8 days after inoculation with "Dry Tortugas" virus, 5th passage in HeLa cells, 10^{-1} dilution. Over 90% of cells detached from glass. CPE = 4+. 114 ×.

period. The beneficial effect of daily fluid changes has been demonstrated for Tahyna virus(7).

As far as tested, the development of CPE was inhibited by homologous hyperimmune mouse or rabbit serum or immune guinea pig serum (Table I).

Summary. These studies have shown that the HeLa cell strain in fluid media is a satisfactory host cell system for arbovirus titrations as well as for neutralization tests. Of 94 arbovirus strains tested, 89 grouped as well as ungrouped viruses caused constant and regularly occurring destructive changes in HeLa cells. Partial destruction of the cell sheet was observed with one-third of the viruses tested, subtotal necrosis with another third and total necrosis with the remainder. In one instance, the cytopathic effect progressed only to a stage characterized by presence of multiple focal areas of necrosis. In 5 instances, no CPE was obtained. As far as tested, specific antiserum prevented development of CPE. Fifty-four strains were propagated serially without difficulty.

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Received January 31, 1964. P.S.E.B.M., 1964, v116.