tute for folic acid. As with the co-factors, the present experiments do not exclude differential cell permeability as the basis of these differences.

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Viral Susceptibility of a Human Carcinoma Cell (Strain KB). (22263)

HARRY EAGLE, KARL HABEL, WALLACE P. ROWE, AND ROBERT J. HUEBNER.
Laboratory of Infectious Diseases, National Microbiological Institute, National Institutes of Health, Bethesda, Md.*

The cultivation of a human epidermoid carcinoma of the floor of the mouth (strain KB) by direct implantation in a fluid medium has been described(1). As will be here shown, this cell line has proved susceptible to a number of human and animal viruses.

Methods. The basal medium used in these experiments was the same as that used in the original isolation, and consisted of the amino acids, vitamins and salts essential for the growth of the HeLa cell, at the concentrations optimal for that cell(2-5). In the initial cultivation this was supplemented with 10% whole human serum. In the present experiments, the same basal medium was supplemented with 4% whole horse serum, in order to avoid the complication introduced by the presence in pooled human serum of antibodies to some of the viruses. Four-day cultures of the cell line in T-15 flasks(2), then containing approximately 4.5 x 10^6 cells, were washed twice with the horse serum medium, and refed with 2.5 ml of fresh medium. One-tenth ml of the viral suspensions of Table I, diluted as there indicated, were then added. The medium was then replaced at 2-day intervals. This first passage test was terminated 7 or 8 days after the original inoculation. The times required for the earliest cytopathogenic effect, and for the destruction of more than 75% of the cells, are indicated in Table I. Fluids harvested at the time of maximal cytopathogenic effect (or after 7 to 8 days in the case of those viruses with no apparent effect) were then similarly inoculated into somewhat larger (T-30) culture flasks containing approximately 4.2 x 10^6 cells at time of inoculation. The virus inoculum in this second passage was 0.5 ml in a total of 5 ml. As in the first passage, the fluids were changed every other day, beginning the day after inoculation. The time required for the earliest visible cytopathogenic effect, and for the almost total destruction of the cells is shown for each virus in the last 2 columns of Table I. Material harvested from this second passage at the time of maximal cytopathogenic effect (or after 6 days in the case of those viruses with no apparent effect) was assayed for viral content by either complement-fixation, pathogenicity in tissue culture, or animal inoculation. The results of those tests are summarized in Table II.

Results. Cytopathogenic effects were produced in the KB cell in both first and second passages by type 1 poliomyelitis (Mahoney), herpes simplex, vaccinia, types 1, 3, and 4 adenoidal-pharyngeal-conjunctival (APC) viruses, lymphocytic choriomeningitis (LCM), and encephalomyocarditis (EMC) viruses (*cf.* Tables I and II). In all these, the titers of the second passage fluids indicated that the viruses had multiplied. The titer of complement-fixing antigen produced by the type 1, 3 and 4 APC viruses was significantly higher than had previously been obtained in

* Public Health Service, U. S. Department of Health, Education and Welfare.

TABLE I. The Susceptibility of the KB Cell to a Number of Human and Animal Viruses.

Virus	Source of virus	Primary inoculation*			Second passage†		
		Final dilution of viral suspension in culture medium	Days for beginning of cytopathogenic effect	Days for more than 75% of cells to degenerate	Harvested from first passage on day No.	Days for beginning of cytopathogenic effect	Days for more than 75% of cells to degenerate
Poliomyelitis (Mahoney)	Monkey kidney culture	1: 100	1	2	2	1	1
Herpes simplex	Adult mouse brain	"	2	4	4	1	2
Vaccinia	HeLa culture	1: 250	1	2, 3	2, 4, 5 (pool)	1	2
APC, type 1	*Idem*	"	4	6	6	1	2
3	"	"	4–6	>8‡	8	1	1
4	"	"	3–4	5–6	6	1	1
Mumps	Suckling mouse brain	1:1000	4	7	7	2	3
"	Allantoic fluid	1: 100	2	3	7	2	3
LCM	Adult mouse brain	1:1000	3	7	7	3	5
EMC	*Idem*	"	1	3	3	6	6
Rabies	Adult mouse brain	1:1000	4	5	7	>6‡	—
GD VII	*Idem*	"	6	>7‡	7	>6	—
Yellow fever	"	"	5±	>7	7	>6	—
APC (SV_1) (6)	Monkey kidney culture	1: 25	1	1–2	4	>6	—
(DC) (7)	HeLa culture	1: 10	6(?)	>8	8	>6	—
(Type 8) (8)	*Idem*	"	6(?)	>8	8	>6	—
Coxsackie A9	Monkey kidney culture	1: 250	>8‡	>8	8	>6	—‡
Influenza (type B)	Allantoic fluid	1: 100	>8	>8	7	>6	—
Idem	Monkey kidney culture	"	6(?)	>7	7	>6	—

* In T-15 flasks containing 450 × 10^4 cells, and 2.5 ml fluid. Culture fluid changed every 2 days (days #2, 4, 6, 8 in some, and 1, 3, 5, 7 in others) unless cells had degenerated in interim.

† In T-30 flasks containing 420 × 10^4 cells, and 5 ml fluid. Culture fluid changed on day #1, 3, 5 (6) unless cells had degenerated in interim.

‡ No cytopathogenic effect at the time indicated.

HeLa cell cultures. In subsequent experiments, APC viruses of types 1-6 were carried through 5 serial passages in KB cells, and herpes simplex and vaccinia viruses through 3 passages, with rapid production of cytopathogenic effects in all passages. Isolations of all 3 types of poliomyelitis and of several types of APC viruses from field specimens have also been made in KB cells.

Equivocal results were obtained with 4 additional viruses. a) Rabies virus produced cytopathogenic changes in the primary inoculation, but not in the second passage, and no virus could be recovered from the latter. b) The SV_1 strain of APC(6) similarly produced cytopathogenic changes on primary inoculation, and not in second passage. However, the second passage material contained virus, demonstrable by cytopathogenic effect in monkey kidney; and on continued passages through the KB cell, virus was detectable after the fourth passage, indicative of continuing viral multiplication in the absence of significant cytopathogenic effect. c) Both strains of mumps virus produced cytopathogenic effects in both the first and second passage, but did not cause the death of the culture. Cellular degeneration and multiplication proceeded simultaneously throughout the period of test. There is no information as to whether the elaboration of virus in such cultures (*cf.* also SV_1 and yellow fever in this group) reflected the production of virus by only a portion of the cells, which degenerated

TABLE II. Evidence of Viral Multiplication in KB Cell.

Virus	Viral assay of original inoculum*		Viral activity* of 2nd passage harvest				
	Tissue culture	Animal or egg inoc.	Day on which harvested	Estimated dilution of original inoculum in harvest†	Complement fixing titer of antigen	Tissue culture titer	Animal or egg inoculation†
Poliomyelitis (Mahoney)	7.5		1	$1:10^4$		8.5	
Herpes simplex		4.0	2	"			4.8
Vaccinia		‖	3	"		>2.5	
APC, type 1	7.0		3	$1:2 \times 10^5$	1:256	9.5	
" " 3	8.0		1	$1:2 \times 10^6$	1: 32	8.0	
" " 4			2	$1:5 \times 10^5$	1:128	8.0	
Mumps (mouse)		4.5	5	$1:10^8$			>4.0
LCM		5.0	5	$1:10^9$			5.5
EMC		8.6	6	$1:10^6$			>8.0
Yellow fever		6.0	6	$1:10^9$			2.5
APC (SV_1) (6)	6.5		6	$1:2 \times 10^6$		2.5	
Rabies		7.5	6	$1:10^9$			<1.5§
GD VII		7.0	6	"			<1.5§
APC (DC) (7)		‖	6	$1:10^8$	<1:4	<1.0	
" (Type 8) (8)		‖	5	"	"	1.5	
Coxsackie A9	7.0±		5	$1:5 \times 10^8$		ca. 1.5	
Influenza (type B)		7.0	6	$1:10^8$			<1.5§
Idem		3.0	6	"			<1.5§
Mumps (egg)		7.5	5	$1:10^7$			<1.5§

* Logarithm of infectious doses per ml.

† Based on amounts of inoculum introduced into flask, and dilution effected by repeated fluid changes, on the assumption that up to 10% of fluid may have been left each time medium was changed as indicated in footnotes of Table I.

‡ Intracerebrally in adult mice, except for (a) mumps virus, inoculated intracerebrally in suckling mice, and (b) influenza virus, inoculated into allantoic sac of egg embryos.

§ Negative in highest concentration tested.

‖ Original inoculum not titered.

in consequence, or whether even the cells which showed no significant cytopathogenic changes were participating in the elaboration of virus. When the second passage material of the mumps cultures was tested intracerebrally in suckling mice, only the culture of the mouse-derived strain proved infectious; and neither strain was now infectious in eggs. In a following experiment, mumps virus was passaged 5 times. In each passage there was a 5% inoculum, the fluid was changed every 2 days, and the harvest for sub-inoculation was collected on the 6th day. The cytopathogenic effect varied markedly and irregularly for individual passages, from no demonstrable change in the 2nd and 3rd passage, to approximately 50-75% degeneration in the 1st and last passages. The 5th passage harvests were estimated to represent a 10^{-15} dilution of the original inoculum. As in the previous experiment, when the 5th passage material was inoculated intracerebrally in suckling mice, only the culture of the mouse strain was infectious; and neither strain could now produce either infection or demonstrable virus in eggs. There was thus definite multiplication of the mouse-adapted mumps strain; but the only indication that the egg-adapted strain had propagated was the partial and irregular cytopathogenicity. d) Although yellow fever virus produced no significant cytopathogenic effect in either passage, the second passage material proved infectious on intracerebral inoculation in mice. In a second experiment, after 5 passages through the KB cell, estimated to represent a 10^{-15} dilution of the original inoculum, the undiluted culture material produced infection on intracerebral inoculation in mice. As with the mumps strains, the cytopathogenic effects in these serial passages were irregular and incomplete.

The other viruses studied, including mouse

encephalomyelitis (Theiler GD VII), 2 other APC strains (type 8 and strain DC), a Coxsackie virus, and influenza type B, produced no cytopathogenic effects in either passage, and except for a small amount of residual virus in the second passage of the APC type 8 and Coxsackie A9, there was no evidence of viral multiplication. In subsequent attempts with higher titer inocula, type 8 APC has been established in serial passage. It should be pointed out that the DC strain grows poorly in all cell types tested in tissue culture, including HeLa cells(7).

Summary. 1) A human epithelioid carcinoma cell in tissue culture (strain KB) has been found susceptible to a number of viruses, including poliomyelitis, herpes simplex, vaccinia, a number of APC strains, LCM and EMC. Marked cytopathogenic effects were produced in 1-7 days, and viral multiplication was shown by complement fixation, tissue culture, or animal titration of the second passage material. 2) Only partial and irregular cytopathogenic effects were obtained with mumps, yellow fever, and the SV_1 strain of APC virus, but with apparently continuing elaboration of virus on repeated passage. Both an egg-adapted and a mouse strain of mumps virus were found to have lost their infectivity for eggs after several culture passages. 3) Coxsackie virus (strain A9), influenza type B, one APC type, and GD VII had no cytopathogenic effect on this cell, and there was no evidence of viral multiplication. Rabies virus was cytopathogenic in the first passage only; and the harvest of the second passage was not infectious on intracerebral inoculation in mice.

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Comparative Absorption of Vitamin B_{12} Analogues by Normal Humans. II. Chloro-, Sulfato-, Nitro- and Thiocyanato- *vs* Cyanocobalamin. (22264)

CHARLES ROSENBLUM, RICHARD S. YAMAMATO, RUTH WOOD, DAVID T. WOODBURY, KUNIO OKUDA, AND BACON F. CHOW.

Merck Sharp & Dohme Research Laboratories, Rahway, N. J., and the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

The first(1) paper of this series reported results of comparative oral absorption studies of chlorocobalamin and cyanocobalamin employing the urinary excretion method of Schilling(2). These tests were interpreted as indicating the superiority of cyanocobalamin over chlorocobalamin for alimentary absorption by humans. Results of oral tolerance tests with humans, and of fecal excretion measurements with rats, confirming the low chlorocobalamin absorption, are reported herein. In addition, similarly inferior absorptions of sulfato-, nitro- and thiocyanatocobalamins are reported, based upon urinary and fecal excretion studies in humans, and upon fecal excretion by rats.

Methods. Materials. The preparation and properties of the radioactive and normal chlorocobalamins and cyanocobalamins employed in these experiments have been described(1). Sulfatocobalamins were obtained photochemically as for chlorocobalamin except that irradiation was performed in 0.01 M H_2SO_4 solution. Samples of the nitroco-