

used as a substrate.

1. Glock, G. E., in *Biochemists' Handbook*, C. Long, ed., E. and F. Spon, Lond., 1961, p345.
2. Kirkman, H. N., *J. Biol. Chem.*, 1962, v237, 2364.
3. Greiling, H., Kisters, R., Hoppe-Seylers *Z. physiol. Chem.*, 1965, v341, 172.
4. Colowick, S. P., Goldberg, E. B., *Bull. Res. Council, Israel*, 1963, v11A4, 373.
5. Salas, M., Vieula, E., Sols, A., *J. Biol. Chem.*, 1965, v240, 561.
6. Levy, H. R., *Biochem. Biophys. Res. Comm.*, 1961, v6, 49.

7. Beutler, E., Collins, Z., *Scand. J. Haemat.*, 1965, v2, 343.

8. Betke, K., Baltz, A., Maas, U., *Z. Kinderheilk.*, 1960, v84, 226.

9. Isselbacher, K. J., in *Metabolic Basis of Inherited Disease*, Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., eds., McGraw-Hill, N. Y., 1960, p208.

10. Beutler, E., Baluda, M. C., Donnell, G. N., *J. Lab. Clin. Med.*, 1964, v64, 694.

Received February 7, 1966. P.S.E.B.M., 1966, v122.

Susceptibility of Cultured Renal Cells from Different Species of Subhuman Primates to Simian Virus 40.* (31223)

RICHARD N. USHIJIMA, F. STUART SHININGER, AND CHARLES E. GARDNER
(Introduced by A. W. Frisch)

Department of Pathology, Oregon Regional Primate Research Center, Beaverton, and Department of Bacteriology, University of Oregon Medical School, Portland, Oregon

While many animal viruses are capable of infecting cultured renal cells derived from a broad spectrum of animals, limitations as to host specificity are well recognized. In an early *in vitro* study, Kaplan(1) found that poliovirus induced a cytopathic effect (CPE) in some subhuman primate cells but not in others. Cultured renal cells from rhesus (*Macaca mulatta*) and crab-eating (*M. cynomolgus*) monkeys were found to be susceptible while those from the capuchin monkey (*Cebus capucina*) were not. Similar observations have been made by Sweet and Hilleman(2) with Simian Virus 40 (SV40), a papovavirus, which was cytopathic to cultured renal cells from African green monkey (*Cercopithecus aethiops*) but not from rhesus except after a prolonged incubation period(3). Based on these differences, Hsiung *et al*(4) suggest that virus host range may be another criterion to aid in the grouping of primates. This article will contribute additional information to that previously reported(4) on those subhuman primates whose kidneys are either refractive

or susceptible to CPE when infected *in vitro* with SV40.

Materials and methods. Trypsinized kidney cells from the different species of primates were grown in medium 199 (Hanks' base) with 0.05% yeastolate and 10% calf serum. All media used throughout the experiment contained 100 µg/ml of streptomycin and 100 U/ml of penicillin. The SV40, initially obtained from Dr. Fred Rapp, was prepared and titrated in primary African green monkey (AGM) kidney cultures. The infective volume for monolayer tube cultures was 0.2 ml of 10-fold serial dilution of stock virus.

Cells grown in Falcon disposable flasks (No. 3017) were exposed for 2 hr to $16^{6.7}$ TCID₅₀ of the virus and then washed 5 times with 4-ml volumes of maintenance medium before incubation. All infected and control cultured cells were maintained in medium 199 (Earle's base) supplemented with 3% calf serum. The cultures were retained for 35 days with medium changes made at 5- to 6-day intervals.

Results. As shown in Table I, cultured renal cells from different species of animals demonstrated varying degrees of susceptibil-

* Publication No. 149 from Oregon Regional Primate Research Center, supported in part by Grant FR 00163 of Nat. Inst. Health.

TABLE I. Effect of SV40 on Cultured Renal Cells from Different Species of Primates.

Species of animals	No. of animals	Days of incubation		
		First culture showing CPE	Last culture showing CPE	Mean titer TCID ₅₀ /0.2 ml
<i>Cercopithecus aethiops</i> , African green	2	5	14	10 ^{7.7}
<i>Cercopithecus mitis</i> , Sykes' monkey	2	14	30*	10 ^{6.8}
<i>Cercocebus atys</i> , sooty mangabey	2†	8	23	10 ^{6.0}
<i>Papio cynocephalus</i> , yellow baboon‡	1	7	18	10 ^{6.2}
<i>Papio</i> sp., baboon§	2	10	20	10 ^{5.0}
<i>Macaca nemestrina</i> , pigtail macaque	2	0	0	
<i>Lagothrix</i> sp., woolly§	1	0	0	
<i>Saimiri sciurea</i> , squirrel	3	0	0	
<i>Tamarinus nigricollis</i> , black and red tamarin	1	0	0	
<i>Galago crassicaudatus</i> , greater bushbaby	2	0	0	
<i>Perodicticus potto</i> , potto	1	0	0	
<i>Nycticebus coucang</i> , slow loris	3	0	0	
<i>Loris tardigradus</i> , slender loris	1	0	0	
<i>Lemur mongoz</i> , mongoose lemur	2	20	0	
<i>Lemur catta</i> , ring-tailed lemur	1	0	0	

* Incubated for a total of 45 days with no additional increase in titer.

† One animal contaminated with a latent virus.

‡ Thirty-day cultures began to indicate presence of a viral contaminant. Endpoint of 18 days used in the calculation.

§ Species unknown. Records from where animal trapped not available.

|| In 10⁻¹ dilution tubes only.

ity to SV40. CPE was first observed in the AGM kidney cultures 5 days after infection with the lowest dilution of the virus. In the higher dilutions, the last tube showing CPE was observed 14 days after infection.

The cells from Sykes' monkey (*Cercopithecus mitis*) with 72 chromosomes(5), closely related to AGM with 60 chromosomes, required 14 days for the initial indication of CPE and 30 days for a demonstrable CPE with the lowest infective dose. Other tube cultures incubated for an additional 15 days did not develop CPE. Whereas the titer of the stock virus was calculated as 10^{7.7} TCID₅₀/0.2 ml on AGM cells, the titration with Sykes' monkey kidney cells was nearly 10-fold less. Microscopically, the course of infection between AGM and Sykes' monkey cells was indistinguishable.

Cells from sooty mangabey (*Cercocebus atys*), yellow baboon (*Papio cynocephalus*) and the unknown baboon species were also found to be receptive to the lytic action of SV40. Although the initial CPE was observed after a shorter incubation period in these animals than in the Sykes, the susceptibility ratio was less as evidenced by the titration index of the stock virus. Vacuoles were seen in several infected cells throughout the culture

but were not as extensive as those observed in infected AG or Sykes' monkey kidney cells.

The unclassified viral agent which was detected in 30-day-old cultures may have affected the endpoint titration for the yellow baboon. Since 3 of 4 control tubes of the yellow baboon kidney cultures began to show evidence of viral infection at 30 days, the 18-day endpoint was taken as the last tube showing CPE induced by SV40 infection.

Cultured kidney cells from pigtail macaque (*Macaca nemestrina*), woolly monkey (*Lagothrix* sp.), squirrel monkey (*Saimiri sciurea*), black and red tamarin (*Tamarinus nigricollis*), greater bushbaby (*Galago crassicaudatus*), potto (*Perodicticus potto*), slow loris (*Nycticebus coucang*), slender loris (*Loris tardigradus*), and ring-tailed lemur (*Lemur catta*) exposed to SV40 did not undergo degeneration within the 35-day incubation period. Only cells from the mongoose lemur (*Lemur mongoz*) infected with 10⁻¹ dilution of the stock virus showed signs of infection and then only after the 20th day. Vacuolated cells progressively increased in the monolayer from the 30th day.

Neutralization tests of fluid medium from CPE flask cultures against SV40 antiserum

did not reveal the presence of other viruses capable of infecting AGM kidney cells.

Discussion. Of all the primates tested, it is evident that the renal cultures from the African green monkey are still the most suitable indicator cell to titrate SV40. The data in Table I show that cultures from the Sykes' monkey require a longer incubation period and are less sensitive to the lytic properties of the virus. It would be of interest to compare the degree of susceptibility to SV40 of other species within the genus *Cercopithecus*. These would include *C. mona* with 66 chromosomes and *C. cephus* with a tentative count of 54 chromosomes(5). In this regard, Sweet and Hilleman(2) have reported that SV40 was highly cytopathic to cells from the patas (*Erythrocebus patas*) monkey, an animal closely related to *Cercopithecus*(6).

The sooty mangabey, from Guinea Coast of West Africa, and the baboons, also from Africa, exhibited a similar degree of sensitivity. Steinbaugh and Melnick(7) reported that kidney cells from the dog-faced baboon (*Papio doguera*) infected with SV40 produced plaques under an overlay medium. Easton (3) noted that normal rhesus kidney cells ultimately degenerated from about 60 days after infection. It therefore appears that at least a qualitative similarity exists in susceptibility to the virus by cultured renal cells of the *Cercopithecinae* with the African animals being more sensitive. Although the infected pigtail macaque cells were not retained for longer than 35 days, SV40 was present in a 10^{-5} dilution of the culture fluid taken on the 30th day of infection.

As to whether cultured cells from the other animals were able to synthesize SV40 has not been fully investigated except for cells from the tamarin, slow loris, ring-tailed lemur and bushbaby. Samples of 30th-day culture fluid from infected cells of these 4 species of animals that were diluted to 10^{-1} and added to BSC-1 cells failed to induce CPE. It is interesting to note that infected cultures derived from rabbit, mouse and porcine(8), as well as chick embryo and HeLa cells(9), continued to release infectious virus for a considerable length of time after infection.

Of the two species of animals from Mada-

gascar tested, only the mongoose lemur showed sensitivity. This may be a species specific phenomenon not characteristic of the other lemurs. For example, Hsiung and Melnick(10) have found that kidney cells of the yellow baboon were resistant to CPE by ECHO type 1 virus, whereas cells from the drill baboon were highly susceptible.

At the moment precautions must be taken not to utilize cellular degeneration imposed by a viral infection as a definitive criterion for primate classification. Conclusions may be arrived at only when all species in a given genus are adequately tested, but on the information available at present, it appears that primatologists may be able to include viral susceptibility as another basis to reinforce and expand the present system of primate classification.

Summary. Kidney cells from several different species of subhuman primates were cultured and infected with SV40. The cells were observed for 35 days, and the course of infection was studied. Cells from *Cercopithecus mitis*, *Cercocebus atys*, *Papio cynocephalus* and an unknown species of baboon were susceptible to the lytic effect of SV40 but were not quantitatively as sensitive as those from *Cercopithecus aethiops*.

The technical assistance of Beverly Cole is acknowledged.

1. Kaplan, A. S., *Virology*, 1955, v1, 377.
2. Sweet, B. H., Hilleman, M. R., *Proc. Soc. Exp. Biol. and Med.*, 1960, v105, 420.
3. Easton, J. M., *J. Immunol.*, 1964, v93, 716.
4. Hsiung, G-D., Black, F. L., Henderson, J. R., in *Evolutionary and Genetic Biology of Primates*, ed., Buettner-Janusch, J., Academic Press, 1964, v11, 6.
5. Bender, M. A., Chu, E. H. Y., *ibid.*, 1963, v1, 292.
6. Simpson, G. G., *Bull. Am. Museum Nat. Hist.*, 1945, v85, 181.
7. Stinebaugh, S. E., Melnick, J. L., *Virology*, 1962, v16, 348.
8. Black, P. H., Rowe, W. P., *Proc. Soc. Exp. Biol. and Med.*, 1963, v114, 721.
9. Eddy, B. E., Grubbs, G. E., Young, R. D., *ibid.*, 1962, v111, 718.
10. Hsiung, G-D., Melnick, J. L., *J. Immunol.*, 1957, v78, 137.

Received February 8, 1966. P.S.E.B.M., 1966, v122.