fects were obtained with 6-mercaptopurine and thioguanine in combination with 5-bromodeoxyuridine, 4-thiodeoxyuridine, 5-acetoxyuracil and 6-azauracil plus urethan. 5-Bromodeoxyuridine is notable for the magnitude of the ratio of maximum tolerated dose to minimum effective dose.

1. Nathan, H. C., Bieber, S., Elion, G. B., Hitchings, G. H., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 796.

2. Elion, G. B., Singer, S., Hitchings, G. H., *J. Biol. Chem.*, 1954, v208, 477.

3. Hitchings, G. H., In Rhoads, ed., *Antimetabolites and Cancer,* Washington, A.A.A.S., 1955, 231.

4. ———, *Am. J. Clin. Nutr.*, 1955, v3, 321.

5. Elion, G. B., Bieber, S., Nathan, H., Hitchings, G. H., *Cancer Res.*, 1958, v18, 802.

6. Dutton, R. W., Dutton, A. H., Vaughan, J. H., *Biochem. J.*, 1960, v75, 230.

7. O'Brien, T. F., *Fed. Proc.*, 1961, v20, 27.

8. Elion, G. B., Bieber, S., Hitchings, G. H., *Acta Unio Intern. Contra Cancrum,* 1960, v16, 605.

9. Calne, R. Y., *Transplantation Bull.*, 1961, v28, 65.

10. Uphoff, D. E., Pitkin, L., *Blood,* 1962, v20, 113.

11. McLaren, A., *Transplantation Bull.*, 1961, v28, 99.

12. Dameshek, W., Schwartz, R., *Trans. Assn. Am. Physicians,* 1960, v73, 113.

13. Dameshek, W., *Blood,* 1962, v20, 119.

14. Fishman, M., *J. Exp. Med.*, 1961, v114, 837.

15. Bieber, S., Nathan, H. C., Hooper, D. C., *Blood,* 1962, v20, 111.

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Susceptibility of Baboon (*Papio doguera*) Kidney Cells to Human Enteroviruses. (27784)

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The enterovirus flora of numerous animal species has been extensively investigated. Similarly, certain animal tissues have served as substrates for propagation of many viruses (1,2,3). The relative high incidence of native viruses in monkey kidney cell (*Macaca mulatta*) preparations suggested the need for studies on additional host cells for production of poliovirus vaccines and for allied viral investigations(4). The phylogenetic relationship of the baboon (*Papio doguera*) to the more commonly used laboratory primates recommends this animal for such purposes.

Hsiung and Melnick compared the susceptibility of kidney cell cultures of 13 monkey species and 4 baboon species to various enteroviruses(5). The use of various animal kidney cells as a "differential medium" for enterovirus isolation and identification was suggested.

In an attempt to develop a model animal system more closely related to man, preliminary investigations comparing the baboon kidney cell (BKC) and monkey kidney cell (MKC) for their propagative properties of known enteroviruses were undertaken. The ability of these 2 cell systems to isolate unknown enteroviruses from stool samples was also investigated.

Materials and methods. Preparation of kidney cell cultures. Kidneys were obtained from young, adult baboons by nephrectomy or by sacrificing of the animal. The kidneys were placed in Hanks' Balanced Salt Solution (BSS) maintained at 4°C, previously adjusted to pH 7.4-7.5 by addition of 7.5% $NaHCO_3$. After removal of the capsule, the kidney was minced and trypsinized in 0.25% trypsin containing 100 units penicillin and 100 μg streptomycin per ml. The trypsinization was performed by either the overnight method of Bodian(6) or the more rapid procedure recently reported by Wallis and Melnick(7). The cells, 3.0×10^5 per ml in 0.5%

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lactalbumin enzymatic hydrolysate, plus 5.0% calf serum in Hanks' BSS containing antibiotics, were then introduced into screw capped tubes and incubated at 37°C. The growth medium was changed after 3-4 days and confluent monolayers were obtained 5-7 days after the initial planting. At that time the monolayers were washed twice with Hanks' BSS and maintenance medium consisting of 199 or 0.5% lactalbumin enzymatic hydrolysate with 1.0% calf serum in Earle's BSS and this medium was added to the cell culture. *Test specimens.* The enteroviruses were originally obtained from the CDC, Atlanta, Ga. and were representative prototypic strains. The 3 avirulent polioviruses were supplied through the courtesy of Dr. Albert Sabin. A large number of fecal samples containing enteroviruses (as determined in MKC) was provided by Dr. Andrew Fodor, CDC, Atlanta, Ga. Of these fecal specimens, 75 were selected at random and together with another 25 stool samples obtained from presumably normal individuals, were individually prepared as 10-20% suspensions in Hanks' BSS.

Cytopathic effect (CPE) was determined by introducing either 0.1 or 0.2 ml per tube of test material to BKC and MKC monolayers. When a 3 or 4 + CPE was observed, the tubes were stored in a –20°C freezer until such time as a second passage was carried out. All specimens were passaged at least 3 times before discarding as negative. *Neutralization tests.* Antiserum prepared either in rabbits or monkeys was used at dilutions of 1:5 or 1:10. An anti-poliovirus pool was used by combining 3 dilutions (1:5) of each specific poliovirus antiserum resulting in a dilution of 1:15. No attempt was made to identify specifically the poliovirus isolates. Other enteroviruses were identified without the use of pooled antiserums.

For seroidentification 0.3 ml of 100 TCD_{50} of each virus was added to 0.3 ml serum dilution, then 2 monolayer tubes were each seeded with 0.2 ml of the virus-serum mixture. Tests were concluded when the control tubes (virus plus normal serum) indicated a 3 or 4 + CPE.

Results. Titration of prototype enteroviruses in BKC. Prior to determining their $TCID_{50}$ all enteroviruses were passaged at least twice in BKC, harvesting when maximal (3+ or 4+) CPE was observed. The results of titrating this BKC passaged material in BKC and in MKC, expressed as the log $TCID_{50}$ per 0.1 ml, are shown in Table I. The original MKC titers are also given for comparison. A good correlation between BKC and MKC sensitivities existed in most instances.

TABLE I. Enterovirus Titrations of BKC Passaged Material in BKC and MKC.

Entero-virus	Original titer MKC†	Titer		Titer $BKCP_2$ in MKC
		$BKCP_2$	$BKCP_3$	
Polio 1[1]	7.0	7.0	6.5	6.5
2[2]	7.0	5.5	6.5	5.5
3[3]	7.0	6.0	6.0	5.5
1[4]	6.5	5.5	5.5	5.5
2[4]	6.5	5.5	5.0	5.5
3[4]	7.0	6.5	5.5	5.5
ECHO 1	6.5	1.5	6.5	5.0
2	6.0	neg.	neg.	neg.
3*	5.0	2.5	>7.0	2.5
5*	7.5	3.0	>7.0	4.5
6	7.0	4.0	>7.0	4.5
7*	7.5	6.5	>7.0	6.5
8*	5.0	5.5	6.5	4.5
9	6.5	6.0	>7.0	5.5
10*	6.0	3.5	3.5	5.5
11*	6.5	6.0	>7.0	5.0
12*	6.5	6.5	>7.0	6.5
13	6.5	4.5	>7.0	3.0
14	4.3	neg.	1.0	neg.
15	5.0	5.5	6.0	3.5
16	5.5	5.0	4.5	4.5
17*	4.5	5.0	6.0	4.5
18	4.0	5.0	6.0	4.0
19*	5.5	5.5	>7.0	5.0
20	6.0	4.0	7.0	3.5
21	4.0	neg.	neg.	neg.
22	5.5	3.5	6.0	5.5
23	5.5	neg.	6.4	neg.
24	5.0	neg.	5.0	4.5
25	4.0	4.5	>7.0	4.5
26	4.5	1.5	>7.0	4.0
27	4.5	4.0	>7.0	4.0
Cox. A9	7.0	4.5	>7.0	5.5
Cox. B1	5.0	4.5	5.0	2.5
B2	7.0	3.0	2.5	3.0
B3	6.0	4.0	4.5	2.5
B4	6.0	3.5	3.5	3.0
B5	5.5	4.0	5.5	2.0
B6	5.5	4.0	5.5	3.5

* Hemagglutinate type O RBC's at 22°C.
† Log $TCID_{50}$/0.1 ml.
[1] Mahoney [2] MEF 1 [3] Saukett [4] Sabin

Certain major discrepancies did occur, primarily as an inability of several viruses to pro-

TABLE II. Isolation and Identification of Viruses from Human Stool Samples in MKC and BKC.

Source of sample	Viruses isolated	No. specimens tested	No. isolated in MKC	No. isolated in BKC
Clinical patients	Poliovirus		50	45
	Coxsackie*	75	8	8
	Unidentified†		17	16
			75	69
"Normal"	Unidentified†	25	2	7
		100	77	76

* 5 Coxsackie B5, 1 Coxsackie B3.
† Not polioviruses types 1, 2 or 3, Coxsackie viruses B1-6, A9 or ECHO 9.

liferate in BKC. This was most apparent with the following viruses: ECHO 2, 14, 21, and to a certain extent with the Coxsackie B group of viruses. Proliferative differences extended from complete failure to grow to that of differences in titer of one to several logs (Table I). Whether or not these differences are real or merely a problem of adaptation is not known. Changes, as observed by titrating third passage material, ($BKCP_3$) are given to indicate differences that were observed.

SV_{40} virus was found to produce a characteristic CPE when placed on BKC monolayers. This is in agreement with the independent findings of Hsiung and Melnick (personal communication).

Hemagglutinating data were also found to demonstrate the known ability of various enteroviruses to agglutinate erythrocytes (type O human cells) following proliferation in BKC. It will be noted that the hemagglutinating capacity is similar to that reported for enteroviruses grown in MKC preparations.

Isolation of enteroviruses from stool samples. Good agreement was obtained using both cell systems, *i.e.*, BKC and MKC, for isolating enteroviruses from the stool (Table II). The majority of specimens contained polioviruses, and 90% isolation agreement was obtained in the 2 test systems. Of the 8 Coxsackie viruses isolated, both BKC and MKC preparations demonstrated identical results, *i.e.*, 7 Coxsackie B5 and 1 Coxsackie B1 virus. Seventeen unidentified agents were detected by MKC and 16 with BKC. While these agents have not yet been identified, they are not poliovirus types 1-3, Coxsackie viruses B1-6, A9 or ECHO 9.

Of interest was the finding of agents in the "normal" stool samples. While not totally unexpected, the relatively large number detected by the BKC system was surprising. These agents have not been completely identified, but they too are not polioviruses 1-3, Coxsackie viruses B1-6, A9 or ECHO 9.

Discussion. Our results agree closely with those of Hsiung and Melnick(5) and substantiate their findings that there is little significant difference in the capacity of BKC and MKC to propagate enteroviruses. Additional studies are required to clarify discrepancies in susceptibilities reported here. Although Hsiung and Melnick described a number of variations in susceptibility of different species of primate studied, the reasons for these differences are not apparent(5). It is possible that enteroviruses in some cases have a greater capacity to adapt after several passages in one species kidney cell in deference to another cell species.

The requirement of "clean" susceptible cell lines for cultivation of viruses is made imperative by the presence of extraneous viruses in MKC. Cultivation and differentiation of the numerous presently known viruses as well as for isolation of presumably yet undiscovered viral agents also requires a variety of readily available sensitive cells. It is probable that the baboon kidney contains "normal" viral flora, but these have not been encountered. In contrast, Tobin has described the various viruses encountered by employing MKC(8). As expected, several baboon enteroviruses have been isolated and are described elsewhere(9).

If additional studies continue to substantiate this similarity in susceptibilities between the two kidney cell preparations, a substitute donor, *i.e.*, the baboon would be available. In view of the resistance of at least one virus (vacuolating virus, SV40) to formalin(10) and its apparent widespread presence in the currently used formalized poliomyelitis vaccines(4), this need for other host tissues, especially one capable of indicating the presence of SV_{40} virus, may become a real factor in future vaccine productions. Currently the

baboon kidney cell is being considered in our laboratory as a possible substrate for poliovirus vaccine production.

Summary. Baboon (*Papio doguera*) kidney cells demonstrate a susceptibility to human enteroviruses, in general, similar to that of the rhesus monkey. Its relative freedom from "native" viruses and ability to demonstrate the presence of SV_{40} virus suggests a more widespread application to virological studies and vaccine production.

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1. Hsiung, G. D., Melnick, J. L., *Ann. N. Y. Acad. Sci.*, 1958, v70, 342.
2. Kalter, S. S., *Bull. Wld. Hlth. Org.*, 1960, v22, 319.
3. Madin, S. H., Andriese, P. C., Darby, N. B., *Am. J. Vet. Res.*, 1957, v18, 932.
4. Cox, H. R., *Bact. Rev.*, 1961, v25, 383.
5. Hsiung, G. C., Melnick, J. L., *J. Immunol.*, 1957, v78, 137.
6. Bodian, D., *Virology*, 1956, v2, 575.
7. Wallis, C., Melnick, J. L., *Texas Reports Biol. and Med.*, 1960, v18, 670.
8. Tobin, H. O'H., *Bull. Wld. Hlth. Org.*, 1960, v22, 431.
9. Fuentes-Marins, R., Rodriguez, A. R., Kalter, S. S., Hillis, W. D., Hellman, A., *Bact. Proc.*, 1962, 136.
10. Gerber, P., Hottle, G. A., Grubbs, R. E., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 205.

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Hypoxanthine Dehydrogenase in the Developing Chick Embryonic Kidney. (27785)

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Although several investigations(1,2,3) have established the pattern of uric acid excretion in the allantoic fluid of chick embryos, no studies have been reported on the development of enzymes which accomplish the synthesis of uric acid in embryonic tissues. Morgan(4) showed that embryonic liver did not perform the reaction. Adult avian kidney was shown by Edson *et al.*(5) to be an active site of uric acid synthesis, and recently this reaction was found to be catalyzed by a DPN dependent hypoxanthine dehydrogenase(6). It, therefore, seemed of interest to study the changes in hypoxanthine dehydrogenase in renal tissue during the embryonic period.

At the 5th day of incubation of eggs, uric acid appears in the allantoic fluid. The mesonephric kidney of the embryo functions from the 5th to 12th day as the excretory organ(7). Between the 12th and 15th day the metanephric kidney, which becomes the adult avian kidney, rapidly develops and becomes functional. Both mesonephric and metanephric tissues thereafter perform secretory and excretory functions in the chick embryo, although involution of the mesonephros begins after the 15th day(8). In this paper the specific activity of hypoxanthine dehydrogenase in the mesonephric and metanephric renal tissue is reported for the period of incubation from the 5th to the 21st day.

Materials and methods. Diphosphopyridine nucleotide (DPN) was purchased from Pabst Laboratories and hypoxanthine from Nutritional Biochemical Co. Rhode Island Red eggs obtained from a local farm were incubated at 37.8° in a constant temperature wooden incubator whose humidity was kept at approximately 60%. To insure optimal growth conditions, the eggs were rotated every 24 hours. Morphological development was classified according to the stages proposed by Hamburger and Hamilton (9) for normal development of the chick embryo. The average incubation time quoted by these authors for each stage was used, so that the morphological development is expressed as "corrected incubation time." After varying periods of incubation, the eggs were candled

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