ISOLATION OF ENTEROVIRUSES FROM THE "NORMAL" BABOON (*PAPIO DOGUERA*)[1]

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ABSTRACT

FUENTES-MARINS, R. (Southwest Foundation for Research and Education, San Antonio, Texas), A. R. RODRIGUEZ, S. S. KALTER, A. HELLMAN, AND R. A. CRANDELL. Isolation of enteroviruses from the "normal" baboon (*Papio doguera*). J. Bacteriol. **85:** 1045–1050. 1963.—In a study designed to determine whether the baboon (*Papio doguera*) would be suitable for use as a model of human virus infections, the normal enterovirus flora was determined. Five agents were isolated from 101 stool samples: four from the African group and one from the African-African group. None of the stool samples from the Domestic group of animals was found to contain any agents. On the basis of their biological characterizations (animal source, cytopathic effect, plaque formation, and antigenic relationships), the isolates were separated into two groups. Accordingly, prototype strains AA153 and A13 were designated for the African-African and African groups, respectively. These viruses have not been found to be related to any known human viruses. Their relationship to organisms isolated from other animals, especially primates, awaits study. The failure to detect latent viruses in preparations of baboon kidney cell cultures suggests a relatively "clean" animal which may be used with safety in preparation of vaccine for human administration.

The widespread occurrence of enteroviruses in the intestines and other tissues of various animal species is now well-recognized (Hsiung and Melnick, 1958; Kalter, 1960). The relatively high incidence of viruses native to the commonly used primate cells (*Macaca mulatta* and *M. philippinesis*) emphasizes the need for investigations into the usefulness of other primate tissues for cultivation of human viruses. This need assumes greater significance when it is recognized that current vaccines, both killed and live, are developed in rhesus (*M. mulatta*) kidney cells containing agents of unknown capabilities. Furthermore, the lack of a characteristic cytopathic effect (CPE) makes recognition of these agents difficult, time-consuming, and expensive.

Hsiung and Melnick (1957) and, more recently, Kalter et al. (1962) studied the susceptibility of baboon (*Papio doguera*) kidney cells (BKC) to enteroviruses; a similarity in virus sensitivity of rhesus and baboon kidney cells was observed. To evaluate further the usefulness of the baboon as an experimental model of human disease, studies of its enteric viral flora were completed.

This report provides data on the enterovirus flora of normal baboons. A preliminary characterization of the isolates is presented.

MATERIALS AND METHODS

Baboons. The following groups of animals were employed. African, animals born in Africa and maintained in captivity at the Southwest Foundation for Research and Education (SFRE). African-African, recently captured animals in minimal contact with man (less than 2 weeks); these animals are part of the stocks maintained at SFRE Primate Research Station, Darajani, Kenya, East Africa. Domestic, a group of animals inbred in this country for over 30 years.

Preparation and inoculation of baboon kidney cells. Kidneys were obtained and processed according to procedures considered standard in this laboratory (Kalter and Hillis, 1961). However, 5% calf serum resulted in the more rapid production of BKC monolayers which were subsequently

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maintained on either Medium 199 or 0.5% lactalbumin hydrolysate supplemented with 1.0% calf serum in Earle's basal salts solution.

Culture tubes were inoculated with either 0.1 or 0.2 ml of a 10 to 20% stool suspension and observed for the development of a CPE. All specimens were passaged at least three times before being discarded as negative.

Preparation of antiserum. Rabbits were inoculated intravenously with 1.0 ml and intraperitoneally with 5.0 ml of undiluted, infected BKC virus preparations. On days 3 and 5, 1 ml of virus suspension was given intravenously to each rabbit. A final 1.0-ml intravenous dose of antigen was given to each animal 7 to 10 days later, and they were bled 7 to 10 days thereafter. In addition, specific serum containing antibodies to one or the other prototype virus was obtained from a number of baboons.

RESULTS

Isolations. As seen in Table 1, five agents were isolated from 101 stool samples tested. Four viruses were obtained from the African group and one from the African-African group of animals. It will be noted that none of the stool specimens in the Domestic group has, as yet, been found to contain any agents.

The isolates were separated into two groups on the basis of animal source and CPE production. All isolates from the African group were identical and unrelated to the one isolate from the African-African group. Accordingly, prototype strains AA153 and A13 were designated for the African-African and African groups, respectively, and their biological characteristics determined. Titers for both viruses are shown in Table 2.

Cytopathic effects. Small foci of degeneration, characterized by rounding of the cells, were observed in unstained cultures 24 hr after inoculation with AA153 virus. The CPE progressed until all cells were detached from the glass surface and, in hematoxylin- and eosin-stained preparations, intranuclear inclusion bodies were demonstrable at 24 hr. These inclusions were homogenous, acidophilic, spherical or oval, and separated from the nuclear membrane by a clear space or halo (Fig. 1).

TABLE 1. *Number of virus isolations from baboon stool samples*

Source of sample	No. of specimens tested	No. of viruses isolated
African	31	4
African-African	40	1
Domestic	30	0
Total	101	5

TABLE 2. *$TCID_{50}$ per ml of representative enterovirus isolates*

Isolate no.	Passage in BKC				
	P_3	P_5	P_6	P_7	P_8
A13	—	5.03	—	6.2	5.2
AA153	—	3.7	3.3	3.2	3.2

A13 virus produced diffuse areas of degeneration at 24 hr, which consisted of rounding and contracting cells with a tendency to form clumps. This degeneration progressed slowly until the entire monolayer was destroyed. Stained cells were round or stellate and joined by protoplasmic processes (Fig. 2). No inclusion bodies or elementary bodies were observed.

Antigenic relationships. Table 3 demonstrates the specific neutralizing antibody response of pooled rabbit serum to each prototype virus. Low-titered antiserum was obtained in both groups of animals, but cross reactions between the two prototype stains were not observed. This lack of antigenic relationship was substantiated by the presence of neutralizing antibodies to A13 in four baboons (two African and two Domestic) and antibodies to AA153 in three different baboons (Domestic; Table 4).

The probable failure of enteroviruses to invade and thus produce antibodies is in agreement with the finding of other investigators (Hull, *personal communication*). Approximately 20% of the 37 animals tested contained antibodies to one or the other baboon virus. It was of interest to note that three of the Domestic animals had antibodies to AA153, although no isolation has been made from these animals (Table 5).

Relationship to known human enteroviruses. Antisera prepared against human enteroviruses failed to disclose any antigenic relationship between these baboon agents and the polioviruses, Coxsackie B 1-6 and A 9, ECHO 1-22 (ECHO 17, 20, and 21 not tested as yet), or the adenoviruses. The relationship of these agents to various enteroviruses of primate origin has not been determined.

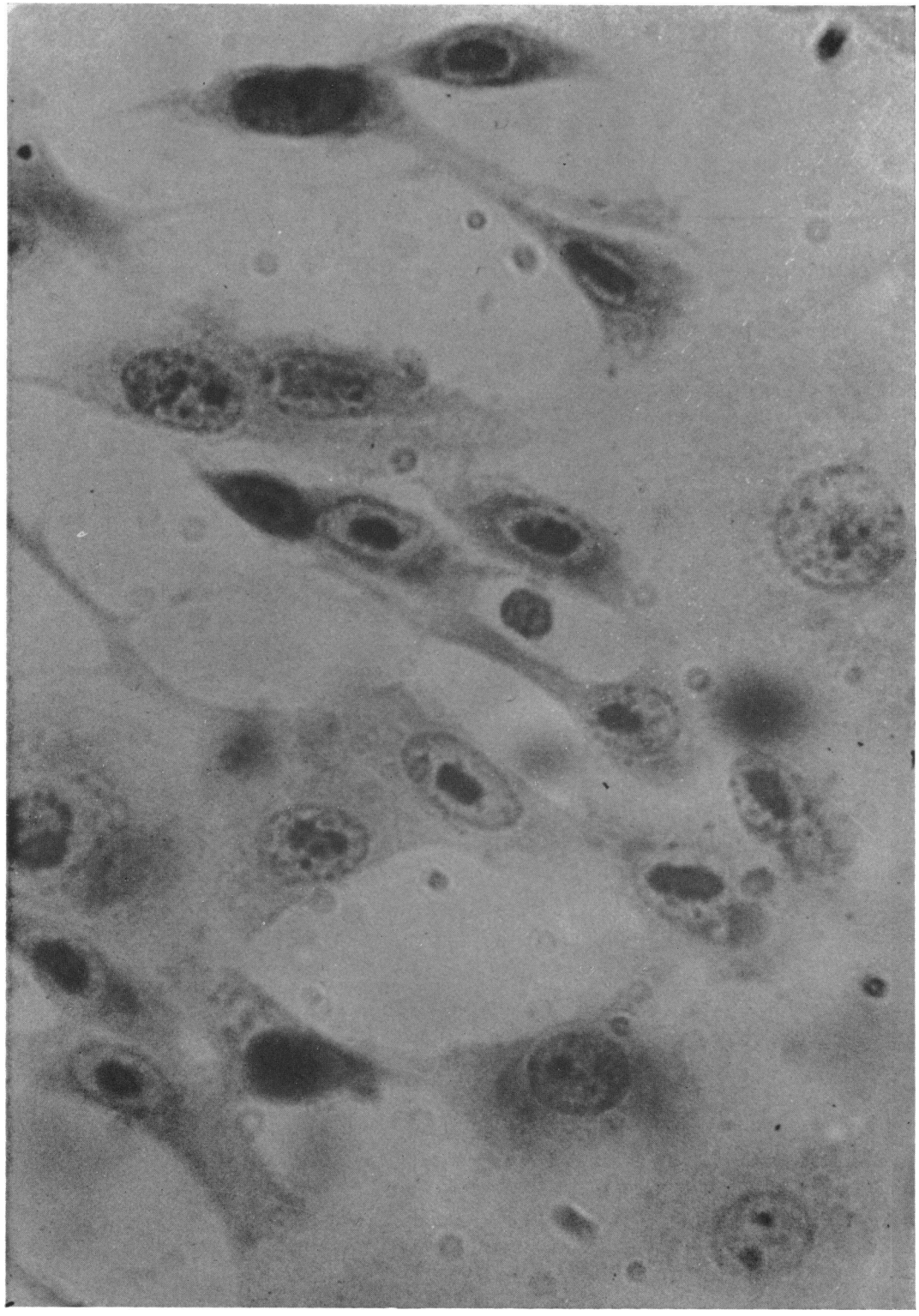

FIG. 1. *Cytopathic effect in AA153. Hematoxylin- and eosin-stained preparation showing intranuclear inclusion bodies and characteristic cytopathic effect.*

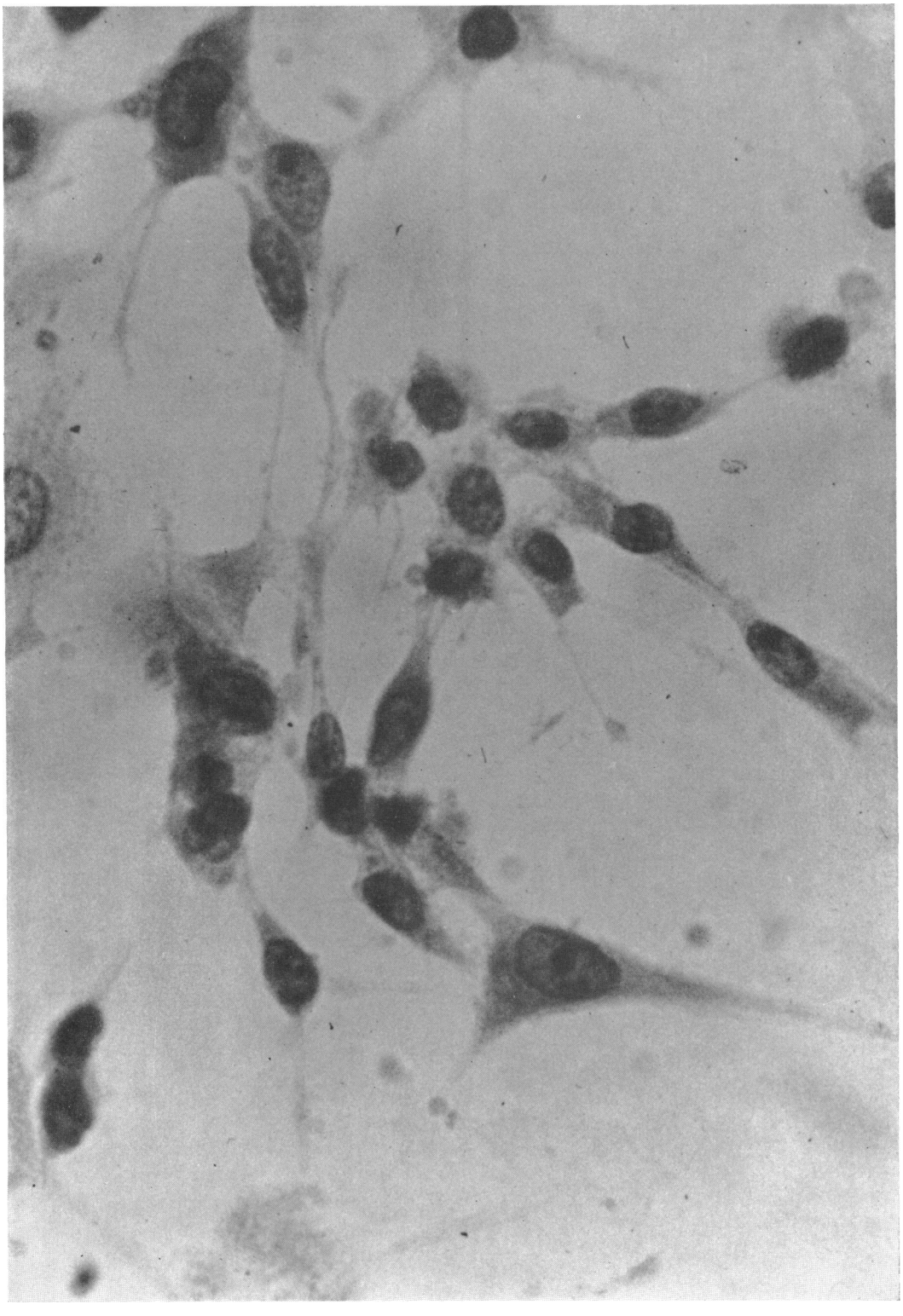

FIG. 2. *Cytopathic effect in A13. Hematoxylin- and eosin-stained preparation showing characteristic cytopathic effect. No inclusion bodies are demonstrable.*

Miscellaneous characteristics. To ascertain the relationship of these viruses to other known agents, various biological characteristics were investigated Neither prototype strain was inactivated by treatment with ether or chloroform, thus ruling out lipid as an essential component. No hemagglutination was observed at room temperature and at 37 C with erythrocytes derived from type O humans, baboons, sheep, guinea pigs, and chickens. In addition, A13 and AA153 failed to produce infection in young adult (3 weeks old) and suckling (less than 24 hr old) mice. CPE was observed in monkey kidney cell (MKC) preparations but not in HeLa cells. Heating at 56 C for 1 hr did not destroy the two prototypic strains. Bromodeoxyuridine as used by Cooney, McLaren, and Bauer (1962) failed to inhibit the synthesis of infective A13 but showed a slight inhibition of AA153.

Latent viruses. Uninoculated baboon kidney cells from 25 to 30 different baboons have been kept under continuous observation for the presence of latent agents. Such "normal" or uninoculated tissues have thus far failed to indicate the presence of a native virus even though primary, secondary, and tertiary serial passages of original baboon kidney cells were observed until the cells degenerated. Repassage of this degeneration material into fresh BKC monolayers failed to demonstrate an agent capable of producing CPE. It should be emphasized that SV40 (vacuolating virus) does produce a detectable CPE on BKC (Hsiung and Melnick, *personal communication*).

TABLE 3. *Antibody response of rabbits to prototype strains*

Virus	Rabbit antiserum	
	A13	AA153
A13	1:40	Negative
AA153	Negative	1:200

TABLE 4. *Specificity of baboon sera to prototype baboon isolates*

Virus	Baboon serum no.						
	A2	A96	D7	D21	D22	D37	D40
A13	+	+	+	−	−	+	−
AA153	−	−	−	+	+	−	+

TABLE 5. *Number of baboons with antibodies to prototype isolates*

Source of baboon serum	No. of sera tested	No. with antibodies	
		153	13
African	22	0	2
Domestic	15	3*	2*
Total		3	4

* See Table 4.

Discussion

The two baboon viruses isolated and described here have not been found to be related to any known human viruses. Their relationship to organisms isolated from other animals, especially primates, awaits study. This becomes exceedingly difficult because of the numerous agents that have been isolated and either poorly defined or not studied in detail.

Perhaps of greater importance is the failure to detect latent viruses in preparations of BKC used in these studies. These findings suggest a relatively "clean" animal which may be used with safety in preparation of vaccines for human administration. This failure to detect latent viruses in uninoculated baboon kidney cells has also been indicated by Melnick (*personal communication*), although an opposing point of view has been expressed by Rosanoff (*personal communication*). It is obvious that a new cell line is needed for development of human vaccines currently produced in rhesus monkey cells. The continued isolation of viruses from MKC vaccine preparations emphasizes this need (Cox, 1961).

The baboon kidney cell has been found to be highly sensitive to human enteroviruses (Kalter et al., 1962). It has the added advantage of indicating the presence of SV40 virus. Thus, a highly sensitive host cell and indicator system is available for production of vaccines that are currently prepared in rhesus kidney cells.

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