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THE ISOLATION OF ENTEROVIRUSES FROM THE NORMAL BABOON (PAPIO DOGUERA)

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FOREWORD

This report was prepared at the USAF School of Aerospace Medicine by the following personnel:

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ABSTRACT

Two viruses isolated from the normal baboon are described. These two agents have not been found to be related to any known human viruses. One virus was isolated from the African-African group (animals recently captured in minimal contact with man, i.e., less than two weeks) and the other agent was isolated from the African group (animals born in Africa and maintained in captivity at the Southwest Foundation for Research and Education). These two agents are distinct and show no evidence of cross reactions. Their biologic characterization is described.

This technical documentary report has been reviewed and is approved.

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Colonel, USAF, MSC
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THE ISOLATION OF ENTEROVIRUSES FROM THE NORMAL BABOON (PAPIO DOGUERA)

1. INTRODUCTION

The widespread and frequent occurrence of viruses in the intestines and other tissues of various animal species is now well recognized (3, 6). This relative high incidence of viruses native to the commonly used primate kidney cells *(Macaca mulatta* and *Macaca philippinensis)* 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, killed and live, are developed in rhesus monkey *(Macaca 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 (3) and more recently Kalter et al. (8) have 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 further evaluate 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.

2. MATERIALS AND METHODS

Baboons

The following groups of animals were employed: (1) Africans (A)—animals born in Africa and maintained in captivity at the Southwest Foundation for Research and Education (SFRE); (2) African-Africans (AA)—animals recently captured in minimal contact with man (less than 2 weeks);[1] (3) Domestics (D)—a group of animals inbred in this country for more than 30 years.

Preparation and inoculation of baboon kidney cells

Kidneys were obtained and processed according to procedures considered standard in this laboratory (7). However, 5% calf serum resulted in the more rapid production of BKC monolayers which were subsequently maintained on either 199 or 0.5 lactalbumin hydrolysate, supplemented with 1.0% calf serum in Earle's BSS.

Culture tubes were inoculated with either 0.1 ml. or 0.2 ml. of a 10-20% stool suspension and observed for the development of a cytopathic effect. All specimens were passaged at least 3 times prior to being discarded as negative.

Preparation of antiserum

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

[1]These animals are part of the stocks maintained at SFRE Primate Research Station, Darajani, Kenya, East Africa.

TABLE I

Number of virus isolations from baboon stool samples

Source of sample	Number specimens tested	Number viruses isolated
Africans	31	4
African-Africans	40	1
Domestics	30	0
Total	101	5

3. RESULTS

Isolations

As seen in table I, five agents were isolated from 101 stool samples tested. Four viruses were obtained from the African group and one from the African-African group. It will be noted that none of the stool specimens in the domestic group contained 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, a prototype strain—i.e., AA 153 and A 13—was designated for the African-African and African groups, respectively, and their biologic characteristics were determined. Titers for both viruses are shown in table II.

Cytopathogenic effects

Twenty-four hours after inoculation with AA 153 virus, small foci of degeneration, characterized by rounding of the cells, were observed in unstained cultures. The CPE progressed until all cells were detached from the glass surface and intranuclear inclusion bodies were demonstrable in preparations of hematoxylin and eosin stain after 24 hours. These inclusions were homogeneous, acidophilic, spherical or oval, and were separated from the nuclear membrane by a clear space, or a halo (fig. 1).

TABLE II

$TCID_{50}$ per milliliter of representative enterovirus isolates

Isolate number	Passage in BKC				
	P_3	P_5	P_6	P_7	P_8
A 13	—	5.03	—	6.2	5.2
AA 153	—	3.7	3.3	3.2	3.2

A 13 virus produced diffuse areas of degeneration at 24 hours, 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 III demonstrates the specific 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 strains were not observed. This lack of antigenic relationship was substantiated by the presence of neutralizing antibodies to A 13 in 4 baboons and antibodies to AA 153 in 3 different baboons (table IV).

The probable failure of enteroviruses to invade and thus produce antibodies is in agreement with the finding of other investigators (5). Approximately 20% of the 37 animals tested contained antibodies to one baboon virus or the other. It was of interest to note that 3 of the domestic animals had antibodies to AA 153, although no isolation has been made from these animals (table V).

Relationship to known human enteroviruses

Antiserums prepared against human enteroviruses have 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

FIGURE 1

H and E stained preparations of intranuclear inclusion bodies produced by AA 153.

TABLE III

Antibody response of rabbits to prototype strains

Virus	Rabbit antiserum	
	A 13	AA 153
A 13	1:40	negative
AA 153	negative	1:200

as yet), or the adenoviruses. The relationship of these agents to various enteroviruses of primate origin has not been determined.

Miscellaneous characteristics

To ascertain the relationship of these viruses to other known agents, various biologic characteristics were investigated. Neither prototype strain was inactivated by treatment with ether or chloroform, thus ruling out lipid

FIGURE 2

H and E stained preparations of the protoplasmic processes of A 13.

TABLE IV

Specificity of baboon serums to prototype baboon isolates

Virus	Baboon serum number						
	A 2	A 96	D 7	D 21	D 22	D 37	D 40
A 13	+	+	+	−	−	+	−
AA 153	−	−	−	+	+	−	+

TABLE V

Number of baboons with antibodies to prototype isolates

Source of baboon serum	Number serums tested	Number with antibodies	
		AA 153	A 13
Africans	22	0	2
Domestics	15	3*	2*
		3	4 Total

*See table IV.

as an essential component. No hemagglutination was observed at room temperature or at 37° C. with erythrocytes derived from type O humans, baboons, sheep, guinea pigs, or chickens. In addition, A 13 and AA 153 failed to produce infection in young adult mice (3 weeks old) and in suckling mice (less than 24 hours old). CPE was observed in MKC preparations but not in Hela cells. Heating at 56° C. for one hour did not destroy the two prototypic strains. Bromodeoxyuridine failed to inhibit the synthesis of infective A 13 but showed a slight inhibition of AA 153.

Latent viruses

Uninoculated baboon kidney cells have been continuously kept under 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 passage 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 SV_{40} (vacuolating virus) does produce a detectable CPE on BKC (4).

4. DISCUSSION

The isolation and some characterization of two baboon viruses are described. These two agents have not been found to be related to any known human enteroviruses. Their relationship to organisms isolated from other animals, especially primates, awaits study. This study becomes exceedingly difficult because of the numerous agents that have been isolated, poorly defined, or that have not been 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 (9), although an opposing point of view has been expressed by Rosanoff (10). 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 (1).

The baboon kidney cell has been found to be highly sensitive to human enteroviruses (8). It has the added advantage of indicating the presence of SV_{40} virus. Thus, a highly sensitive host cell and indicator system is available for production of vaccines that are currently prepared in rhesus kidney cells.

REFERENCES

1. Cox, H. R. Oral poliomyelitis vaccine. Bact. Rev. 25:383-388 (1961).
2. Hsiung, G. D., and J. L. Melnick. Comparative susceptibility to kidney cells from different monkey species to enteric viruses (poliomyelitis, Coxsackie, and ECHO groups). J. Immun. 78:137-146 (1957).
3. Hsiung, G. D., and J. L. Melnick. Orphan viruses of man and animals. Ann. N. Y. Acad. Sci. 70:342-361 (1958).
4. Hsiung, G. D., and J. L. Melnick. Personal communication.
5. Hull, R. N. Personal communication.

6. Kalter, S. S. Animal "orphan" enteroviruses. Bull. WHO 22: 319-337 (1960).

7. Kalter, S. S., and W. D. Hillis. Procedures for routine laboratory diagnosis of virus and rickettsial diseases. USAF School of Aerospace Medicine Report No. 62-10, Nov. 1961.

8. Kalter, S. S., W. D. Hillis, A. Hellman, R. A. Fuentes-Marins, A. R. Rodrigues, and M. T. Werthessen. The susceptibility of baboon (*Papio doguera)* kidney cells to human enteroviruses. Fed. Proc. 21:458 (1962).

9. Melnick, J. L. Personal communication.

10. Rosanoff, E. I. Personal communication.

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