Plaque Assay of Measles Virus on *Erythrocebus patas* Monkey Kidney Monolayers.* (23943)

G. D. HSIUNG, ANNA MANNINI, AND JOSEPH L. MELNICK**
Section of Epidemiology and Preventive Medicine, Yale University School of Medicine

Since the first isolation of measles virus in human and rhesus kidney cells by Enders and Peebles(1), the virus has been grown in human amnion and cancer cells and in chick embryos(2-6). During our investigation of the viral susceptibility of kidney cells from the African red grass monkey (*Erythrocebus patas*)(7,8), it was found that measles virus could grow readily in patas cultures and produce distinct plaques with clear centers and sharp boundaries.

Materials and methods. Measles virus† used was the Edmonston strain which had been through 23 passages in human kidney culture(1), 1 passage in HeLa culture, and 7 passages in Hep-2 cells(4). Measles antiserum: pre- and post-inoculation sera were obtained from monkeys immunized with a single injection of measles virus grown in Hep-2 cell culture. Paired sera from measles patients were obtained from Greenland.‡ Bottle cultures of monkey kidney monolayers were prepared and overlaid as described previously (7,9). The neutralization test was performed by mixing equal amounts of virus containing approximately 50-100 plaque forming units (PFU) with 0.5 ml of serum at different dilutions. The mixture was incubated at room temperature for one hour, and 0.2 ml of the mixture was inoculated per bottle.

Results. Fig. 1 and 2 show the plaques produced by measles virus at 3 tenfold dilutions in patas bottle cultures, 6 and 10 days after seeding. Small but distinct plaques were obtained as early as 5-6 days after inoculation. After the size of a plaque increased to 2-3 mm in diameter it became very distinct. Titration of measles virus in patas cells by the plaque method and also by CPE (cytopathogenic effect) in tubes is shown in Table I.

Measles plaques were also obtained in rhesus bottle cultures but only if the cell sheets were in excellent condition. Even so, the plaques were not as clear, and the boundaries were not as distinct as those shown on patas monolayers (Fig. 3). In simultaneous tests of cells grown out under identical conditions, plaque counts in rhesus cultures were about 10% of those in patas cultures. Quantitative aspects of the propagation of measles virus in a variety of cell lines including patas cells will be reported by Black. A study of the sequence of cytological changes correlated with

TABLE I. Titration of Measles Virus by Plaque Formation and by Cytopathogenic Effect (CPE).

Days after inoc.	PFU/0.1 ml inoculum — Dilutions of tissue culture fluid: 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	CPE* — Dilutions of tissue culture fluid: 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	0	0	0	0
3	0	0	0	0	0	4	1	0	0	0
6	> 100	59	7	0	0	4	4	2	0	0
7	>1000	133	21	2	0					
10	c	s-c	26	5	0	4	4	4	1	0
14						4	4	4	4	2

* No. of tube cultures showing CPE. Four cultures inoculated at each dilution.
c = confluent; s-c = semiconfluent.

* Aided by grant from Nat. Fn. for Infantile Paralysis.

** Present address: Dept. of Epidemiology, Baylor Univ. Col. of Medicine, Houston, Texas.

† Measles virus and immune serum kindly supplied by Dr. Francis L. Black.

‡ Obtained through courtesy of Dr. Biggo E. Bech of the Serum Inst., Copenhagen, Denmark.

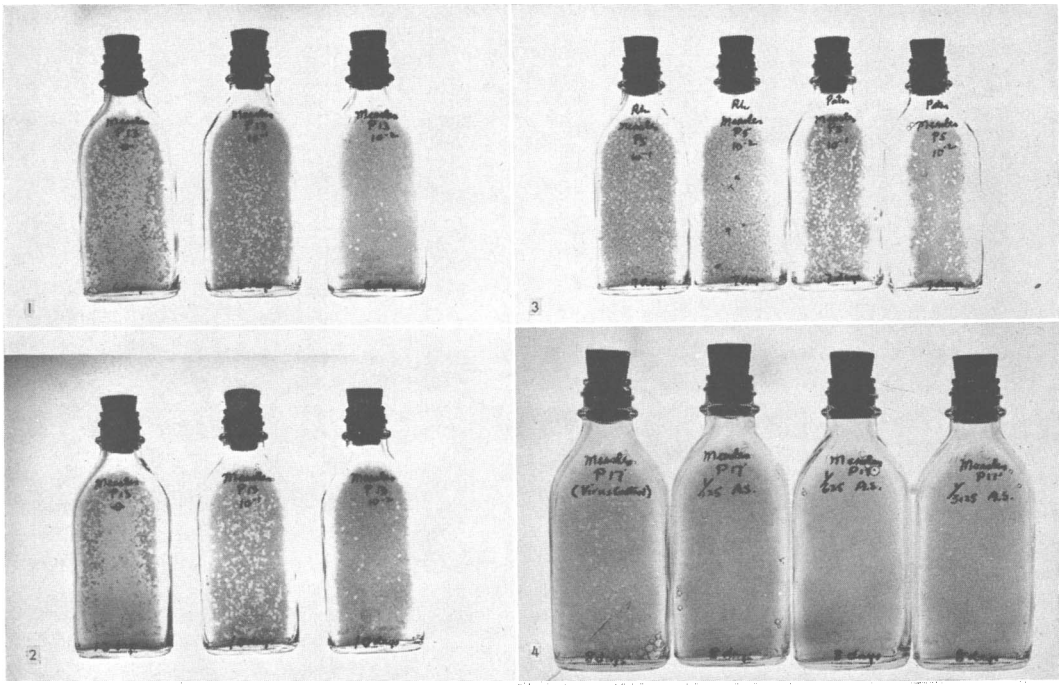

FIG. 1. Quantitative titration of measles virus (13th passage in patas cultures) 6 days after seeding serial 10-fold dilutions, .1 ml/3-oz bottle.

FIG. 2. As Fig. 1 but 10 days after seeding.

FIG. 3. Plaque formation of measles virus in rhesus cultures (2 bottles at left) and patas cultures (2 bottles at right), 7 days after seeding.

FIG. 4. Neutralization test by plaque method. Bottle on left contains challenge dose of measles virus (17th patas passage). Next 3 bottles contain 0.2 ml of virus-serum mixture, at serum dilutions of 125, 625, and 3125, respectively. The virus was neutralized completely at the 2 lower dilutions, and there was 90% plaque reduction at the highest dilution.

the growth cycle of measles virus in patas cells will be published separately, together with Ruth Kleinfeld.

Neutralization of measles virus (both the 4th and 17th passage of measles virus in patas cells) by specific measles monkey antiserum indicated that the patas-passaged measles virus and the Hep-2 passaged measles virus are still antigenically the same. The neutralization end point was obtained on the 7th day of incubation. After 4 passages in patas cells, the virus was neutralized by antisera obtained from 2 immunized monkeys (Table II). The titer of antiserum obtained from monkey 8628 was 1:400 when tested by the conventional tube method, using Hep-2 cells; however, 80% plaque reduction was obtained at a serum dilution of 1:3125. Fig. 4 illustrates another test by the plaque reduction method, with the same serum versus the 17th patas-passaged virus.

TABLE II. Neutralization of Measles Virus in Its 4th Patas Passage by the Plaque Method.

Measles immune monkey No.	Control, PFU per bottle	PFU in presence of serum dilutions: 1:5	1:25	1:125	1:625	1:3125
8628	57		0	0	0	11
9526	57	0	0	0	41	49

Neutralization of the patas-passaged measles virus was also tested with human serum samples. Table III gives a typical result of a measles neutralization test. The sera were heated at 56°C for 30 minutes before use. No neutralizing antibody was observed in the serum sample taken prior to symptoms, but neutralization occurred at 1:25 of the second serum sample, obtained from the patient one day after the rash appeared. Five days later the serum titer increased to 1:625, after which it fell slightly during the next month.

Discussion. Although cytopathic changes

TABLE III. Plaque Neutralization Test of Measles Virus by Serum Obtained in Course of Measles Infection.

Date of serum	Phase of disease	No. plaques expected in absence of serum	PFU/.1 ml in presence of serum						
			Undil.	1:5	1:25	1:125	1:625	1:3125	1:15625
9/30	Prior to clinical symptoms	43	20	46	40				
10/ 3	One day after rash	43	0	1	5	24	29	52	
10/ 8	Convalescence	43				0	10	24	48
11/10	"	43		0	0	0	32		

All serum samples were heated at 56°C for 30 min.

of measles (multinucleate giant cells with intranuclear inclusions) were observed in patas cultures 2 days after infection with large doses of virus, with small doses 2-3 weeks were necessary before the final reading could be made. With the plaque method, final results were obtained at the end of one week after inoculation and, in addition, there was no need for changing the medium, as was necessary for the tube method. Quantitative results were easily achieved by counting the number of the distinct small plaques.

Foamy agents(10) have been confused with measles virus because of their characteristic cytopathic changes in rhesus monkey kidney cultures in fluid medium. However, measles-like plaques have not been observed with the foamy agents under agar in rhesus cultures, although the cell sheets were destroyed by the agents. Foamy-like virus has not been recovered from the patas cultures which we have used in our studies over the past 3 years(7,8), except recently from 2 patas monkeys which had been kept in the stock colony for 10 months or longer, and the possibility exists that they might have acquired their infections from other monkeys in the colony.

Summary. Measles virus produces tiny but distinct plaques with sharp boundaries, in bottle cultures prepared from kidneys of African red grass monkeys (*Erythrocebus patas*). As with other viruses, quantitative assays and neutralization tests with measles virus may now be carried out by the plaque technic.

1. Enders, J. F., Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.

2. Baker, R. F., Rapp, F., Grogan, E. A., Gordon, I., *Bact. Proc.*, 1957, M42, 76.

3. Dekking, F., McCarthy, K., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 1.

4. Black, F. L., Reissig, M., Melnick, J. L., *ibid.*, 1956, v93, 107.

5. Milovanovic, M. V., Enders, J. F., Mitus, A., *ibid.*, 1957, v95, 120.

6. Ruckle, G., *J. Immun.*, 1957, v78, 330.

7. Hsiung, G. D., Melnick, J. L., *ibid.*, 1957, v78, 137.

8. ———, *Ann. N. Y. Acad. Sci.*, 1958, in press.

9. ———, *J. Immun.*, 1957, v78, 128.

10. Rustigian, R., Johnston, P., Reihart, H., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 8.

Received March 7, 1958. P.S.E.B.M., 1958, v98.

Extrapituitary Interaction between Pitressin and ACTH.* (23944)

PAUL C. ROYCE† AND GEORGE SAYERS

Department of Physiology, Western Reserve University School of Medicine, Cleveland, O.

Pitressin increases the level of ACTH in the blood(1). It has been assumed that this effect is mediated solely by the action of vasopressin on the adenohypophysis(2). The following studies were undertaken to determine whether this assumption is correct.

Methods. Adrenal ascorbic acid depletion

* Aided by research grant from U. S. Public Health Service.

† National Science Fn. Postdoctoral Fellow.