were equivocal. Leukemic lines were established in C3Hf/Lw and C3Hf/Fg strains through inoculation of virus into prospective parents in the neonatal period. The frequencies and ages at death from lymphocytic leukemia through 4 generations of C3Hf/Gs mice revealed no loss of virulence of virus. Apparently, low-leukemic and high-leukemic lines may be established simply through foster-nursing of litters. There was no evidence to support the concept for transmission of virus through germinal cells. The results reported here concerning the pattern for congenital transmission of Moloney virus stand in sharp contrast to the patterns for transfer of potentialities to develop leukemia in the high-leukemic strains of mice, AKR, C58, F and C3Hf/Fg.

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Plaque Formation by Myxovirus Parainfluenza Type 2 in Grivet Monkey Kidney Cells. (27046)

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The plaque technique developed by Dulbecco(1) provides a method for relatively precise analysis of infectivity of virus preparations. This technique also lends itself to isolation of pure lines of virus. In many instances plaque morphology has been used to differentiate between virus strains and types(2,3). Deibel has reported plaque formation with parainfluenza type 3 virus in stable human amnion cells(4). Marston and Vaughan have reported a plaque method for parainfluenza type 3 virus assay wherein the plaques are located by the hemadsorption technique(5). Hsiung has reported that the plaque technique

may allow detection of some myxoviruses, although cytopathic changes are not apparent in fluid cultures of the virus(6). This report describes adaptation of the plaque technique for parainfluenza type 2 virus in monolayers of grivet monkey kidney cell cultures.

Materials and methods. Viruses. The strain of myxovirus parainfluenza type 2 used throughout these studies was obtained from Dr. Robert M. Chanock and has been propagated in grivet monkey kidney cell cultures in this laboratory. The strain has been freed of contaminating simian viruses. All of the virus samples described here were obtained from infected fluids of grivet monkey kidney cell cultures or purified preparations made from these infected fluids.

Tissue culture. Trypsinized grivet monkey kidney cells were obtained by digestion of the cortical tissue with 0.25% trypsin (Wilson 1:300) overnight at 5°C. The trypsinized cells were harvested by centrifugation and suspended in growth medium (0.5% lactalbumin hydrolysate, 0.025% yeast extract, Hanks' salt solution, and 2% calf serum). The cells were planted in Smith bottles at a concentration of 250,000 cells/ml and 25 ml/bottle. The bottles were incubated at 36-37°C. The medium was removed on the fourth or fifth day and replaced with medium 199 containing 2% calf serum. The cell cultures were ready for plaque tests 7 days after planting.

Agar overlay medium. a) Two per cent agar (Difco, granular). The agar is prepared as required as a 2% (W/V) solution. The agar is sterilized by autoclaving for 30 minutes at 120°C. It is removed from the autoclave, cooled to 45°C, and kept at that temperature until used.

b) A 4.3X concentrate of the nutrient medium is made up as follows.

	ml
Autoclaved demineralized water	300
Eagle's amino acids (100X)*	43
Hanks' salt solution 10X, without glucose, low calcium**	430
Glucose (100 g/l)	43
Glutamine (29 g/l)	43
Sodium bicarbonate (10%)	72.5
Penicillin	430,000 units
Streptomycin	430,000γ
Autoclaved demineralized water to make 1 liter	

c) All components of the medium are sterilized by filtration through Selas 02 or ultrafine glass filters. It is important that this concentrate be prepared by adding each of the ingredients in the order listed. The medium is adjusted to pH 7.5 ± 0.05 with sodium hydroxide.

d) The final overlay medium is prepared by mixing 30 ml of the medium concentrate per every 100 ml of 2% agar at 45°C. Two and twenty-five one hundredth (2.25) ml of neutral red (0.1% W/V) is added for each 130 ml of overlay. It is essential that all materials be thoroughly mixed.

Virus seeding. The medium is removed from each of the cell culture bottles. The cell sheet is washed once with low calcium Hanks' (containing glucose), adjusted to pH 8.0. Cultures are seeded with 0.5 ml of the appropriate virus dilution. Hanks' balanced salt solution at pH 8.0 is used as the virus diluent. The bottles are placed on a level surface in a 36-37°C incubator for 1.5 hours. At the end of incubation period, they are removed from the incubator and 25 ml of final overlay medium pipetted into each bottle in such fashion that the agar does not impinge on the cell sheet directly. The bottles are placed cell sheet down at room temperature until the agar hardens. They are then placed (inverted) in an incubator at 36-37 C. All the plaque cell cultures are covered with aluminum foil to avoid the photodynamic destruction of the cell sheet by neutral red. The plaques are countable and are read in 10-12 days.

Results and discussion. Incubation time. By the method described, excellent plaque formation was obtained with parainfluenza type 2 virus. Typical plaques are shown in Fig. 1. Plaques were not easily discernible 8 days after seeding. By the tenth day of incubation, the plaques were readily visible and could be counted with ease. The number of plaques per bottle usually increased by the twelfth day and the plaques grew somewhat in size. Typical data indicating numbers of plaques per bottle at 10 and 12 days of inclubation are given in Table I.

* Obtained from Microbiological Associates as a sterile product.

** Anhydrous calcium chloride, 0.7 g/liter.

FIG. 1. Plaques of myxovirus parainfluenza type 2 in grivet monkey kidney cell cultures.

TABLE I. Plaque Counts at 10 and 12 days.

Sample No.	10th Day No. of plaques/bottle	12th Day No. of plaques/bottle
1	89, 109	107, 128
2	68, 95	109, 81
3	45, 45	49, 54
4	21, 13	21, 12
5	25, 35	24, 35
6	100, 164	128, 167
7	113, 92	132, 134
8	6, 5	8, 7
9	12, 11	13, 12

Correlation of plaque assay with roller tube hemadsorption assay. The plaque assay, in general, showed good correlation in magnitude with infectivity titers of parainfluenza type 2 virus measured by the hemadsorption endpoint(7). These data are outlined in Table II.

TABLE II. Comparison of Plaque Assay with Roller Tube Hemadsorption Assay.*

Sample No.	PFU/0.2 ml	$TCID_{50}$/0.2 ml
1	1.8×10^6	5.0×10^6
2	$.5 \times 10^5$	1.0×10^5
3	$.6 \times 10^8$	2.5×10^8
4	2.0×10^6	5.0×10^6
5	$.6 \times 10^6$	1.6×10^7
6	$.7 \times 10^5$	1.6×10^6
7	1.1×10^7	1.6×10^7
8	$.6 \times 10^7$	1.6×10^7
9	1.5×10^6	2.5×10^6

* Hemadsorption assays were performed in grivet monkey kidney cell cultures. 0.5 log dilutions were employed. A 0.1% suspension of guinea pig erythrocytes was used. Incubation with red cells for 20 min at 4°C and 2 hr at room temperature.

We are indebted to Dr. Louis Potash for hemadsorption assays.

Proportionality on dilution.† An analysis of the proportionality on 10-fold dilution was made for a series of 58 samples. A 10-fold increment should yield a theoretical ratio of 0.1 or 10%. Geometric mean ratio for the 58 samples analyzed was estimated to be 0.105 or 10.5% with 95% confidence limits of 9.1% and 12.1%. This did not differ significantly from expectation. Individual values ranged from 2.4% to 20.4%.

Summary. A method has been described for plaque formation of parainfluenza type 2 virus in grivet kidney monolayer cell culture. The method has shown utility in the assay of virus infectivity. The plaquing technique should also be useful for cloning of this virus for genetic studies.

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