Plaque Formation by Infectious Canine Hepatitis (ICH) Virus. (24712)

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It is generally agreed that ability of an animal virus to form plaques on a susceptible cell monolayer parallels its ability to be cytopathogenic for this cell. Although several years have elapsed since infectious canine hepatitis (ICH) virus was shown to multiply in dog-kidney tissue culture and to cause characteristic cytopathic changes(1), plaque production with this virus has not been reported. Attempts have been made without success. This communication describes production of plaques by ICH virus derived from either dog or swine kidney cultures.

Materials and methods. The *ICH virus* used was isolated in our laboratories as previously described(2). Two preparations were employed for plaque assay: one representing 37th passage of virus in dog-kidney tissue culture, and the other 30 transfers in swine kidney(3). *Cultivation and titration.* Dog and swine-kidney cultures were prepared by method of Dulbecco and Vogt(4) in either stationary tubes (1 ml) or Povitsky bottles (100 ml). Dog kidney cells were also grown in 50 mm Petri dishes (5 ml). Tubes were used for serial transfer of the virus, and bottles for preparation of large pools. Growth medium consisted of Earle's basal solution(5) containing lactalbumin hydrolysate and 15 or 20% calf serum. Just before inoculation, tubes and bottles were renewed with mixture 199(6), supplemented for dog-kidney tubes, with 2% horse serum. Virus titrations were carried out in dog kidney tubes and were terminated after 7 days at 37°C. Fifty % end-point tissue culture doses (TCD_{50}) titers were calculated by Reed and Muench formula(7). *ICH immune serum.* This was prepared in foxes as described earlier(1). Immune and normal sera were inactivated at 56°C for 30 minutes before use in the neutralization test. *Serum neutralization.* To one set of serial 10-fold dilutions of virus in glucosol(8) were added equal volumes of glucosol; a second set received equal volumes of 1:16 dilution of normal dog serum, and a third set equal volumes of 1:16 dilution of ICH immune fox serum. After incubation in a 37°C water bath for 1 hour, each serum-virus mixture was inoculated into 3 culture tubes at 0.1 ml/tube. These were incubated at 37°C and observed for 7 days. TCD_{50} titers were calculated as indicated above.

Results. Plaque production. Plates of primary passage dog-kidney monolayers were washed once with 3 ml phosphate buffer saline solution (PBS)(9). Appropriate dilutions of ICH virus of either dog- or swine-kidney origin, made in Eagle's medium(10) fortified with 1% calf serum, were added to the monolayers in 1 ml amounts. The plates were then incubated at 37°C for 3 hours to permit virus attachment, at which time they were overlayed with 7 ml of a medium consisting of 5% calf serum, 1% agar and 1:40,000 neutral red in Eagle's medium. Incubation was continued at 37°C in an atmosphere of 5% CO_2 in air. The plaques appear sometime between fifth and tenth day (Fig. 1). Under the same conditions, equally successful results were obtained with second passage dog kidney cells.

Reproducibility and precision of assays. To determine validity of the plaque method for assay of ICH virus, several virus stocks of either dog- or swine-kidney origin were plated on different days, using 2 or 3 dilutions/assay. Results are given in Table I. A linear relationship was obtained between number of plaques and viral concentration, over a relatively wide range of dilutions. This suggests that a plaque can arise from infection with a single virus particle. Reproducibility and precision obtained in these experiments seem also to be as good as those seen with other animal viruses(4,11,12,13) and bacteriophage(14).

Plaque identification. The plaque producing virus was identified as ICH by tube and plaque-assay neutralization with known specific serum.

Comparison of tube and plate assays. Com-

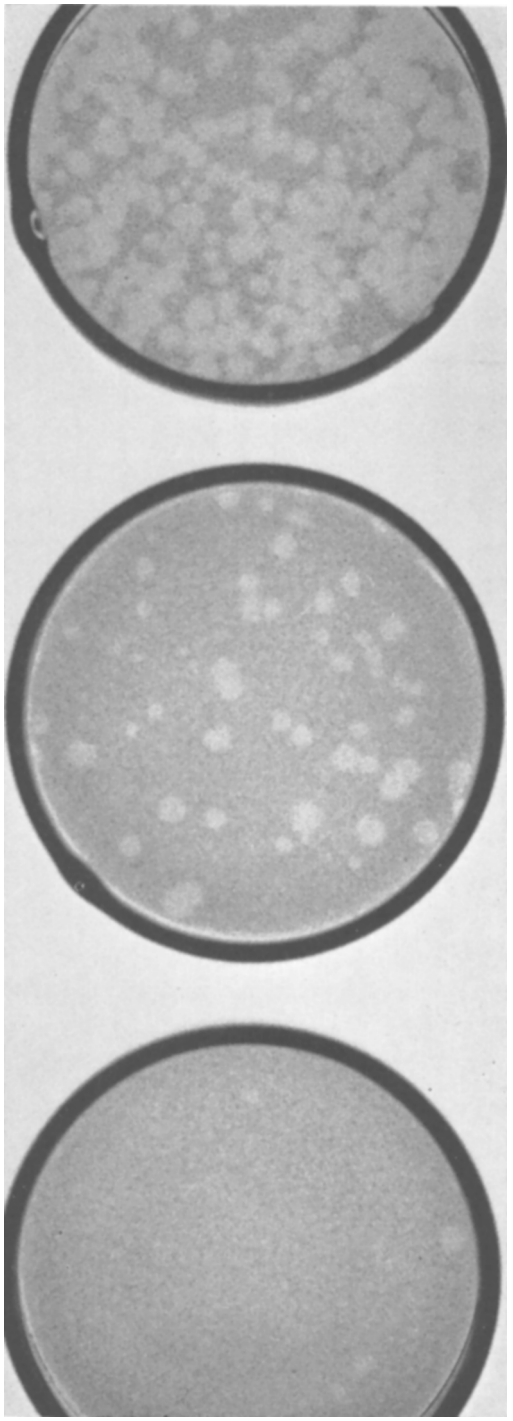

FIG. 1. Plaques of ICH showing effects of dilution.

parative titrations of various virus stocks were carried out by plaque assay and by standard dog-kidney tube method. Results are shown in Table II. Under conditions of these experiments, it appears that plaque assay is more sensitive than tube method.

TABLE I. Representative Experiments Demonstrating Reproducibility of Plaque Count and Its Response to Dilution.*

Virus stock	Assay No.	Dilution	Plaque count	Titer of stock
A	1	$1/10^5$	33,38	3.6×10^6
		$1/10^6$	5,1	$3.0 \times$ "
	2	$1/10^5$	30,32	$3.1 \times$ "
		$1/10^6$	3,4	$3.5 \times$ "
B	1	$1/10^5$	48,32	$4.0 \times$ "
		$1/10^6$	3,3	$3.0 \times$ "
	2	$1/10^5$	69,57,49	$5.8 \times$ "
		$1/10^6$	2,3,4	$3.0 \times$ "
C	1	$2/10^4$	51,46,69	$2.7 \times$ "
		$5/10^5$	6,16,20	$2.8 \times$ "
		$1/10^6$	3,7,0	$3.0 \times$ "
D	1	$5/10^6$	59,78,82	1.5×10^7
		$1/10^6$	17,22,12	$1.7 \times$ "

* Experiments with same stocks were done on different days.

Discussion. Under appropriate conditions ICH virus of either dog- or swine-kidney culture origin can form plaques on monolayers of 1st or 2nd passage dog kidney cells. Moreover, our data justify the use of the plaque method for assay of the virus. It also supports the concept that the ICH plaque, like plaques formed by other animal viruses studied (11,12,13), can be initiated by a single infective particle. The seeming discrepancy between titers obtained by tube and plate methods, may be due to the fact that tube titrations were routinely terminated on 7th day.

It has not yet been possible to determine relative plating efficiency of swine-kidney and dog-kidney grown virus on both dog- and

TABLE II. Comparative Titrations of ICH Virus Stocks by Plaque Assay and by Standard Dog-Kidney Tube Method.

Virus stock	Dog-kidney tube assay: Infective particles/ml*	Plaque titer: PFP/ml†
1	2×10^7	2.9×10^8
2	2×10^6	5.8×10^6
3	$2 \times$ "	1.4×10^7

* One ID_{50} corresponds, on the average, to 0.7 tube infective particle.

† PFP = plaque forming particle.

swine-kidney monolayers because of the difficulty in keeping primary swine cultures for the long time needed for plaque production. However, work on this problem is being continued.

Summary. Plaque formation by infectious canine hepatitis virus was demonstrated on dog kidney monolayers with both dog and swine kidney grown virus. A plaque-derived virus clone was identified as ICH by stationary tube neutralization test as well as by plaque assay. Under conditions of the study, assay by plaque plates appears to be a more sensitive titration method than by stationary tissue culture tubes.

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Experimental Aminonucleoside Nephrosis (II): Effect of Adrenalectomy on Fluid Retention of Aminonucleoside Nephrosis.* (24713)

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In young immature rats, aminonucleoside nephrosis is characterized by proteinuria, hypoproteinemia, hypercholesterolemia, increased rate of secretion of aldosterone, and fluid retention of which 50% is in the form of ascites(1-3). To appraise the relationship of hyperaldosteronism to fluid retention, the ascites produced by aminonucleoside nephrosis was measured in adrenalectomized rats receiving maintenance doses of corticosterone and maintenance or increased doses of either desoxycorticosterone or aldosterone. The results strongly suggest that in aminonucleoside nephrosis an increased rate of production of aldosterone is one, but not necessarily the only, causative factor in gross fluid retention.

Methods. Immature rats of Long-Evans strain, weighing 90-100 g, were used and fed a diet of Purina Fox Chow. Following adrenalectomy the animals were maintained on 1% sodium chloride in drinking water. Four days after operation the saline solution was replaced by tap water and maintenance therapy with aldosterone and corticosterone was started. After 4-5 days of maintenance therapy, nephrosis was induced by single daily subcutaneous injections of 6-dimethyl-aminopurine 3-amino-d-ribose at dose of 1.75 mg in 0.2 ml of water/rat/day for 8 consecutive days. Six days following last injection of the nephrotoxic drug, the animals were killed by bleeding from inferior vena cava. Development of nephrosis was established on the basis

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