Discussion. Serologic tests for Mycoplasma in many instances leave much to be desired. Although sensitive tests have been developed through the years for a number of Mycoplasma the majority require tedious laboratory techniques. At present the most sensitive test for detection of *M. pneumoniae* antibodies is the immunofluorescence test of Liu(6). This is an application of the anti-globulin procedure. It is a very sensitive and specific test but requires frozen sections of lungs of infected embryos, and the numbers of manipulations required in this system are usually large. The anti-globulin procedure described herein should be applicable for detection of *M. pneumoniae* antibodies. It may also be useful in determining antigenic similarity. In cross agglutination tests, common antigenic components of other species of Mycoplasma were detected.

Summary. Application was made of the anti-globulin procedure for detection of antibody to 3 Mycoplasma of avian origin. The test was specific, and antibodies were indicated at higher titers.

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Propagation of Encephalomyocarditis Virus in Swine Embryo Kidney Cells.* (29319)

H. ROUHANDEH, R. R. CHRONISTER AND M. L. BRINKMAN
(Introduced by H. A. Wenner)
Section of Virus Research, Department of Pediatrics and Department of Microbiology, School of Medicine, University of Kansas, Kansas City, Kansas

Encephalomyocarditis (EMC) virus has been recovered from tissues of naturally-infected pigs by Murane *et al*(1) and from pigs fed mouse-passaged EMC(2). In the course of our work we found that EMC virus produced cytopathic effect (CPE) on swine embryo kidney (SEK) cells. Reported herein are some quantitative data on the effects of EMC virus on SEK cells.

Materials and methods. Kidneys from fetal swine were removed aseptically and dispersed with trypsin by the method of Youngner(3) and suspended in medium LG(4) plus 10% calf serum. Test tubes (16 × 150 mm) received 1 ml each of a 0.20% cell suspension and were stoppered and incubated on a slant at 37°C. Petri plates (60 mm) received 8 ml each of 0.20% cell suspension and were kept in humidified atmosphere of an incubator continuously flushed with a mixture of 3% CO_2 and 97% air. Sheets developed in both tubes and plates in 5-8 days. Mouse L-cells were prepared as previously described by Rouhandeh(5). Tubes received 2 ml each of cell suspension.

EMC virus was supplied by American Type Culture Collection as a mouse-brain suspension. The virus was serially passaged 40 times in L-cells in our laboratory. It was plaque-purified by 3 serial isolations, and plaque-purified pools were prepared in L-cells. These stocks averaged 2-4 × 10^8 plaque-forming units (PFU) per ml in L-cells and in mice.

Tubes containing SEK cell sheets were washed twice, each time with 2 ml of phosphate-buffered saline (PBS)(6) and then inoculated with 0.1 ml each of L-cell passaged virus containing approximately 1,000 PFU

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per ml. Following 1 hour adsorption at 37°C tubes received 1.5 ml each of high-cystine altered Eagle's maintenance ($hcAE_M$) medium(7) and then were incubated at 37°C in a roller drum. Cultures were checked daily for CPE; when CPE was complete virus was harvested by freezing and thawing and used to make subsequent passages in SEK cells.

SEK cells grown on cover slips in Leighton tubes were inoculated with 0.1 ml containing 1,000 PFU of SEK passaged (8 serial passages) EMC virus. Control tubes received PBS. At intervals of 24, 28, and 48 hours cover slips were removed and fixed in ethyl alcohol and ether, then stained with acridine orange (AO)(8).

For plaque assays on SEK and on L-cells sheets were washed twice, each time with 4 ml PBS; plates received 0.3 ml each of 10-fold dilutions of virus propagated in tubes as described above. Following 1 hour adsorption at 37°C plates were overlaid with 6 ml $hcAE_M$ medium containing 0.9% agar. When agar had solidified plates were incubated in CO_2 atmosphere. Following 5-7 days incubation for plaques on SEK and 3-5 days on L-cells, a second agar overlay containing 0.01% neutral red was added.

The Reed-Muench method(9) was used for calculation of tube and mouse infectivity titers. Mouse LD_{50} titers were calculated from experiments in which suckling mice were inoculated intracerebrally with 0.02 ml of serial 10-fold dilutions of supernatant from 8th passage of EMC virus in SEK cells. Titers were based on mortality of mice; typical signs of paralysis were observed in infected mice.

Anti-EMC serum was kindly supplied by Colonel E. L. Buescher, Walter Reed Institute, Washington, D. C.; some was also prepared in our laboratory by immunizing rabbits.

Results. L-cell propagated virus stocks of EMC produced distinctive CPE in SEK cells within 24 hours through 8 serial passages tested; CPE was complete in 48-72 hours. In AO-stained preparations the cytoplasm and nucleoli of uninfected cells were various shades of orange and red-orange, and the nuclei were apple-green. As CPE progressed in infected preparations, typical orange stain decreased; at 48 hours post infection the orange-staining material had almost completely disappeared, and only green-staining nuclei were visible.

TABLE I. Plaque Formation by Encephalomyocarditis Virus in Swine Embryo Kidney Tissue Culture Cells.

Exp No.	Virus conc	No. of plaques	Mean ± S.E.*
1	10^{-4}	Confluent	—
	10^{-5}	36, 42, 28, 18, 32	31.2 ± 2.6
	10^{-6}	8, 5, 3, 2, 0, 1	3.8 ± 1.2
2	10^{-4}	Confluent	—
	10^{-5}	28, 32, 12, 14, 19	21.0 ± 1.0
	10^{-6}	4, 2, 1, 3, 1	2.2 ± 1.0

* S.E. = standard error.

TABLE II. Comparison of Swine Embryo Kidney Passaged Encephalomyocarditis Virus Titers in Swine Embryo Kidney Tissue Culture Cells and Suckling Mice.

	Titer $\log_{10}$ (infective units/ml)		
	Tissue culture		Suckling mice
Exp No.	PFU	$TCID_{50}$	LD_{50}
1	7.2	6.5	5.0
2	7.5	7.1	5.5
3	6.5	6.3	5.8
4	7.5	6.8	5.2
5	6.8	6.2	5.0

EMC virus produced plaques 1.5 mm in diameter on SEK cells. Plaque counts given in Table I indicate that the number of plaques produced was proportional to concentration of virus in inoculum. Virus stocks treated with anti-EMC sera produced no or few plaques. In Table II are given results from experiments comparing titers of 8th SEK-passage of EMC in SEK tissue cultures by plaque method and tube titration with mouse infectivity titration. The tissue culture system was more sensitive than mouse assay.

The effect of passage of EMC virus in SEK cells on plaque size and relative efficiency of plating (e.o.p.) is shown in Table III. Plaque titers of EMC passaged 40 times in L-cells and titered in both L-cells and SEK cells are compared to those of SEK-passaged (1st and 8th passages) EMC in both SEK and L-cells. Titers of L-cell grown virus were about 50 times higher in L-cells than in SEK cells. Plaque diameters on L-cells following passage in SEK cells were 1.5 mm, as com-

TABLE III. Effect of Passage of Encephalomyocarditis Virus in Swine Embryo Kidney Cells on Plaque Size and Relative Efficiency of Plating in Homologous and Heterologous Systems.

EMC virus stock	Mean plaque dia (mm) in 5 days		Titered in		Relative e.o.p.*
	L-cells	SEK cells	L-cells	SEK cells	
L-cell passaged	4.0	1.5	4.0×10^8	8.0×10^6	0.02
1st passage in SEK cells	1.5	1.5	2.0×10^7	1.2×10^7	0.6
8th passage in SEK cells	1.5	1.5	2.5×10^7	1.6×10^7	0.64

* Relative e.o.p. was calculated by dividing the plaque titers of EMC in SEK cells by plaque titers in L-cells.

pared to 4 mm plaque diameters of L-cell grown virus on L-cells. Passage of EMC on SEK cells increased relative e.o.p. on SEK cells 30-fold as a result of one passage and 32-fold after 8 passages. Apparently a variant of EMC was obtained as a result of passage in SEK cells.

The hemagglutinating activity of SEK-passaged EMC (8 passages) was tested by the method of Horvath and Jungeblut(10) using sheep red blood cells; a titer of 256 was found.

Summary and conclusions. L-cell grown EMC virus produced CPE on SEK cells through 8 serial passages tested. A comparison of infectivity of SEK-passaged EMC in SEK tissue cultures and in suckling mice showed both tube titration and plaque method more sensitive than mouse assays. Modification of EMC virus following passage in SEK cells was manifested in lower titer and smaller plaques on L-cells and in its pathogenicity for mice. SEK-passaged (8 passages) EMC virus agglutinated sheep erythrocytes. SEK provides an easily obtainable primary cell system in which biological properties of EMC virus may be studied.

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