Colorado Tick Fever Virus: Growth in a Mosquito Cell Line

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Two strains of Colorado tick fever virus grew in Singh's *Aedes albopictus* cells. In one of three experiments, virus growth continued for 7 weeks.

Although Colorado tick fever (CTF) virus will grow in primary tissue cultures of its vector, *Dermacentor andersoni* (11), this and six other tick-borne viruses failed to grow perceptibly in an insect cell line (*Antheraea eucalypti*) that supported significant growth of certain culicine-borne arboviruses (12). Other reports on the susceptibility of various established insect cell lines to infection with arboviruses (1, 6, 8-10) indicate that the ability of these cells to support viral growth reflects the natural vector relationships of the virus. In general, culicine-borne viruses, particularly those of antigenic group B, grow readily but tick-borne viruses grow poorly or not at all in insect cells. Because host specificity of a virus is not always maintained in tissue explants or cell cultures (3, 4), the preceding generalization must be considered tentative and exceptions documented. This note describes such an exception.

Two strains of CTF virus, differing in passage history and pathogenicity, were used: SS-18, in its 6th mouse passage, and Florio-2, in its 66th mouse passage. The latter is unique in its ability to kill adult mice after intracerebral injection. Origins of these strains were previously given (11). A stock of SS-18 virus was prepared by isolating a plaque from an African green monkey kidney (Vero) cell monolayer and transferring it to a solution of equal parts physiological saline and normal rabbit serum (PS-NRS). A stock Florio-2 virus was prepared as a 10% suspension of infected suckling mouse brain in PS-NRS.

The mosquito cell line established by Singh (7) from *Aedes albopictus* was obtained in its 49th passage from W. Downs and S. Buckley of the Yale Arbovirus Research Unit. Monolayers, representing the 51st to 56th passages, were prepared as stationary cultures in 2-oz (56.7 g) glass prescription bottles or in 2-dr screw-cap vials. Cultures were initiated from trypsinized cells suspended in medium in concentrations sufficient to produce monolayers in 2 to 4 days (5×10^4 to 10×10^4 cells/ml). Bottles received 5 ml and vials received 1 ml of cell suspension. Cultures were

incubated at 27 or 32 C and medium was changed weekly.

Cell-culture medium consisted of lactalbumin hydrolysate with Hanks salts (HLH medium; Grand Island Biological Co., Grand Island, N.Y.), to which was added (per ml): 20% heat-inactivated fetal calf serum, 10 mg of bovine plasma albumin V (Armour Pharmaceutical Co., Kankakee, Ill.), 100 units of penicillin G (Na salt), 100 μ g of streptomycin sulfate, and 40 μ g of neomycin sulfate. This was adjusted to pH 7.0 and sterilized by pressure filtration. In one experiment, Singh's medium (7) was used.

Cultures, after becoming monolayers, were inoculated by replacing their medium with virus stocks diluted 10^{-3} or 10^{-5} in fresh medium. In two experiments, the inoculum was not removed for 7 days, at which time medium was changed; in a third, it was allowed to adsorb at 32 C for 2 hr and was then decanted; the cultures were then washed once and overlaid with uninfected medium. Controls contained corresponding dilutions of virus in medium without cells.

Virus in mosquito cell cultures was titrated by plaque assay in Vero cell monolayers or by intracerebral inoculation of 2 to 4-day-old mice. Calculation of LD_{50} was by the method of Reed and Muench (5). Immediately after introduction of virus into mosquito cell cultures, samples from control vessels were stored at -65 C. Twenty-four hours later, at intervals for the first week, and weekly thereafter, two cultures were frozen at -65 C. Later these cultures were thawed, their cells were ruptured by shaking with glass beads, and contents of duplicate cultures were pooled for virus assay. Controls were similarly treated, except that they were less frequently sampled and were not shaken with glass beads. Virus identity was confirmed in 4-week samples by plaque-reduction neutralization test in Vero cell monolayers.

In each experiment, replication of CTF virus in the mosquito cells was evident (Table 1). This was greatest during the 3rd and 4th weeks after

TABLE 1. *Growth of Colorado tick fever virus in Aedes albopictus cell cultures*

Time after inoculation	Strain SS-18 ^a (incubated at 27 C)	Strain Florio-2 ^b (incubated at 32 C)	Strain SS-18 ^c (incubated at 32 C)
Day 0 ^d	1.0×10^4	8.9×10^3	2.0×10^2
1	1.0×10^4	1.1×10^4	8.0×10^1
3	2.0×10^4	2.0×10^5	1.6×10^2
5		2.0×10^5	7.1×10^2
Week 1	4.5×10^5	5.0×10^4	1.3×10^3
2	7.0×10^5	1.6×10^5	
3	2.1×10^6	$>1.3 \times 10^6$	8.0×10^3
4	4.0×10^6	$>8.9 \times 10^5$	8.9×10^3
5	1.5×10^6		
6	3.5×10^6		
7	9.5×10^5		

^a Plaque-forming units per milliliter; HLH medium; inoculum not decanted.

^b Suckling mouse LD₅₀/milliliter; Singh's medium; inoculum not decanted.

^c Suckling mouse LD₅₀/milliliter; HLH medium; inoculum decanted after 2 hr.

^d Values for day 0 obtained from controls; all others from cultures.

FIG. 1. *Growth of Colorado tick fever virus (strain SS-18) in Aedes albopictus cell cultures; titrated in Vero cells. Virus was not recovered in controls (HLH culture medium, without cells) subsequent to day 14.*

inoculation. Controls yielded no more virus than that inoculated and for no longer than 14 days (Fig. 1). Maximal virus increase (2.2 to 2.6 logs or greater) occurred in both strains when cultures were allowed to remain in contact with inoculum for 1 week, regardless of medium used or temperature of incubation. In one experiment, cultures continued to yield virus for 7 weeks in amounts nearly 100-fold higher than that inoculated. Cytopathogenic effect of CTF virus on mosquito cells was not seen when infected cultures were compared with uninfected ones.

This is the first report of growth of a known tick-borne virus in insect cells in vitro. To our knowledge, it is also the first evidence that CTF virus will grow in any arthropod host system other than that of ticks. Attempts to demonstrate CTF virus propagation after parenteral inoculation of a variety of insects, including mosquitoes, have failed (2), which indicates that, in comparison to other arboviruses so tested, this virus is limited in its ability to infect alien arthropod hosts. Whether the present results reflect an alteration of cell susceptibility to virus through long-term in vitro cultivation or an inherent property of the whole organism remains to be investigated.

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LITERATURE CITED

- Converse, J. L., and S. C. Nagle, Jr. 1967. Multiplication of yellow fever virus in insect tissue cell cultures. *J. Virol.* 1: 1096-1097.
- Hurlbut, H. S., and J. S. Thomas. 1960. Experimental host range of the arthropod-borne viruses in arthropods. *Virology* 12:391-407.
- Ignoffo, C. M. 1968. Specificity of insect viruses. *Bull. Entomol. Soc. Amer.* 14:265-267.
- Luria, S. E. 1953. *General virology*. John Wiley and Sons, Inc., New York.
- Reed, L. J., and H. Muench. 1938. A simple method for estimating fifty per cent endpoints. *Amer. J. Hyg.* 27:493-497.
- Řeháček, J. 1968. The growth of arboviruses in mosquito cells in vitro. *Acta Virol.* 12:241-246.
- Singh, K. R. P. 1967. Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.). *Curr. Sci.* 36:506-508.
- Singh, K. R. P., and S. D. Paul. 1968. Multiplication of arboviruses in cell lines from *Aedes albopictus* and *Aedes aegypti*. *Curr. Sci.* 37:65-67.
- Singh, K. R. P., and S. D. Paul. 1968. Susceptibility of *Aedes albopictus* and *Aedes aegypti* cell lines to infection by arbo and other viruses. *Indian J. Med. Res.* 56:815-820.
- Sutor, E. 1966. Growth of Japanese encephalitis virus in Grace's continuous line of moth cells. *Virology* 30:143-144.
- Yunker, C. E., and J. Cory. 1967. Growth of Colorado tick fever (CTF) virus in primary tissue cultures of its vector, *Dermacentor andersoni* Stiles (Acarina: Ixodidae), with notes on tick tissue culture. *Exp. Parasitol.* 20:267-277.
- Yunker, C. E., and J. Cory. 1968. Infection of Grace's *Antheraea* cells with arboviruses. *Amer. J. Trop. Med. Hyg.* 17:889-893.