Biological Properties of a Syncytia-Forming Agent Isolated from Domestic Cats (Feline Syncytia-Forming Virus)[1] (35167)

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(Introduced by M. S. Silverman)

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It has been a common experience among tissue culturists that adult feline cells do not propagate well *in vitro*. In our laboratory, the cultivation of cells derived from normal and neoplastic tissues from adult cats met with consistent failure. Feline cells, after approximately 21 days in culture began to deteriorate, showing a characteristic cytopathic effect distinguished by the formation of large syncytia. A syncytia-forming agent has been isolated from such cultures and shown to be myxovirus-like (1).

In this communication we report *in vitro* characteristics of a similar agent isolated from cells of a feline specimen, and the incidence of a like agent in tissues of 66 cats obtained from three counties of California during the first quarter of 1969.

Methods and Materials. Normal and neoplastic tissues from adult cats were obtained from veterinary practitioners in Los Angeles, Alameda, and Contra Costa counties of California. Tissues were taken at surgery or necropsy and transported to the laboratory in iced medium.

Cultivation of cells. Each tissue was minced to a very fine suspension and seeded into Falcon 250 flasks with minimum essential medium (MEM), (Eagle) or L15 medium (Leibovitz) containing 10–20% fetal calf serum (FCS). Penicillin and streptomycin were added at 100 and 50 units/ml, respectively. The cultures were incubated at 37° until a monolayer of cells had grown out from the tissue fragments, usually in 3 days. The fragments were then released from the surface by gentle agitation, carefully aspirated off and discarded. The monolayer was fluid changed, omitting antibiotics, and then returned to the incubator. Cultures were fed at 3 to 4-day intervals.

Virus isolation. The prototype agent employed for these experiments was isolated from a culture of an osteosarcoma obtained from an adult cat at autopsy. Syncytia were observed after the cells were in culture for 21 days. Infected cells and fluid were frozen-preserved in ampoules at —70°.

Assay. A cell line, passages 3 to 6, derived from a normal whole feline fetus was used for assay of virus. Infected cells were stained with Giemsa at 3- to 4-days postinfection and designated positive or negative for the presence of syncytia. End-point dilutions were read and the titers were calculated using the method of Reed and Muench (2).

Host range determination. Cell lines of various animal species were grown to near confluency in MEM with 10% FCS. The monolayers were washed three times with MEM and inoculated with 0.5 ml of undiluted stock virus/Falcon 250 flask or 0.1 ml/tube. After 1.5-hr adsorption at 37°, maintenance medium (MEM with 2% FCS) was added and the cultures returned to the incubator. Cultures were observed for syncytia formation and cytopathic effects (CPE) daily for 10 days with fluid changes at 3- to 4-day intervals. Coverslip preparations were stained with Giemsa.

Serology. Neutralizing antisera to simian foamy viruses, types 1 through 7, were generously provided by Dr. Paul B. Johnson, Uni-

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versity of Louisville, Louisville, Kentucky. Human convalescent serum to human respiratory syncytia virus was obtained from Dr. J. Van Kirk, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. Antisera against mumps, herpes simplex, and adenovirus type 2 were obtained from the American Type Culture Collection. Neutralization tests were carried out in a human foreskin fibroblast cell line, (Hs 3OF) at the fourth and sixth passage. An equal volume of virus suspension containing approximately 100 $TCID_{50}$ was mixed with appropriate dilutions of each serum and held at room temperature for 1 hr. One ml of the serum–virus mixture was inoculated into each of 2 tissue culture tubes. Tests were read after Giemsa staining on the fifth day. Control cultures received a virus–normal fetal calf serum mixture.

Electron microscopy. Cell monolayers fixed with 2.5% glutaraldehyde were removed from flasks by scraping and were pelleted. Cell pellets were treated with 1% chrome osmium and stained with uranyl acetate before embedding in Epon. Thin sections were stained with uranyl acetate and lead citrate prior to their electron microscopic examination.

Preparations studied by negative staining with phosphotungstic acid were as previously described. The electron microscope used was a Siemens 101.

Results. Field incidence. The incidence of feline syncytia-forming virus (FeSFV) in tissues from adult feline residents of Los Angeles, Alameda, and Contra Costa Counties of California was approximately 90% during the first 4 months of 1969; 44 of 48 cat tissues received for culture was destroyed with typical CPE (Table I). In no instance was there a history of infectious disease in the animals. During the same time period, fetal tissues obtained after hysterectomies had an FeSFV infections rate of only 16%. Newborn kittens from a few hours to a few days old carried the agent in as high a frequency as adult animals.

Syncytia formation, extensive degeneration and eventual destruction of the culture occurred in cells cultured for approximately 21 days (fluid changed at 3–4-day intervals, but not subcultured). Frequent subpassages prolonged the life of such cultures, but all were eventually destroyed. The agent isolated from one culture was characterized. For determining incidence, the appearance of typical CPE was used as indicative of FeSFV infection.

TABLE I. Incidence of Feline Syncytia-Forming Virus (FeSFV) in Cultured Cells from Domestic Cats.

	Types of tissues cultivated		
		Adult	
	Fetal	Normal	Tumor
No. of tissue donors	18	38	10
No. with normal growth	15	1	1
No. with CPE[a] and syncytia	3	37	9

[a] Cytopathic effect.

Transmission. Fluid harvested from cultures showing extensive syncytia formation and CPE was passed through a 0.45-μ Millipore filter and put onto feline bone marrow cells (Fc6BM). The cultures were prepared for electron microscopy when the CPE was almost complete (usually 21 days). Figure 1 shows virus particles budding from the surface of a cell as well as free in the lumen of a syncytium. The virus particles are morphologically similar to those previously described (1, 4, 6). Unlike the C-type oncogenic viruses, which also bud from the cell surface, FeSFV has a completed nucleoid at the time budding occurs. The characteristic surface spikes also distinguish this virus from the C-type viruses.

Host range in cultured cells. Table II lists the cells used, their source and morphological type. All domestic feline cells (*Felis catus*) tested were susceptible to FeSFV. The cells derived from normal feline fetuses, Fc9WF (whole fetus), Fc3Th (thymus), Fc6FBM (bone marrow), Fc3Tg (tongue) displayed syncytia with 10 to 15 nuclei (Fig. 2). The culture, F1B, derived from a peripheral lymph node of a lymphomatous adult cat was

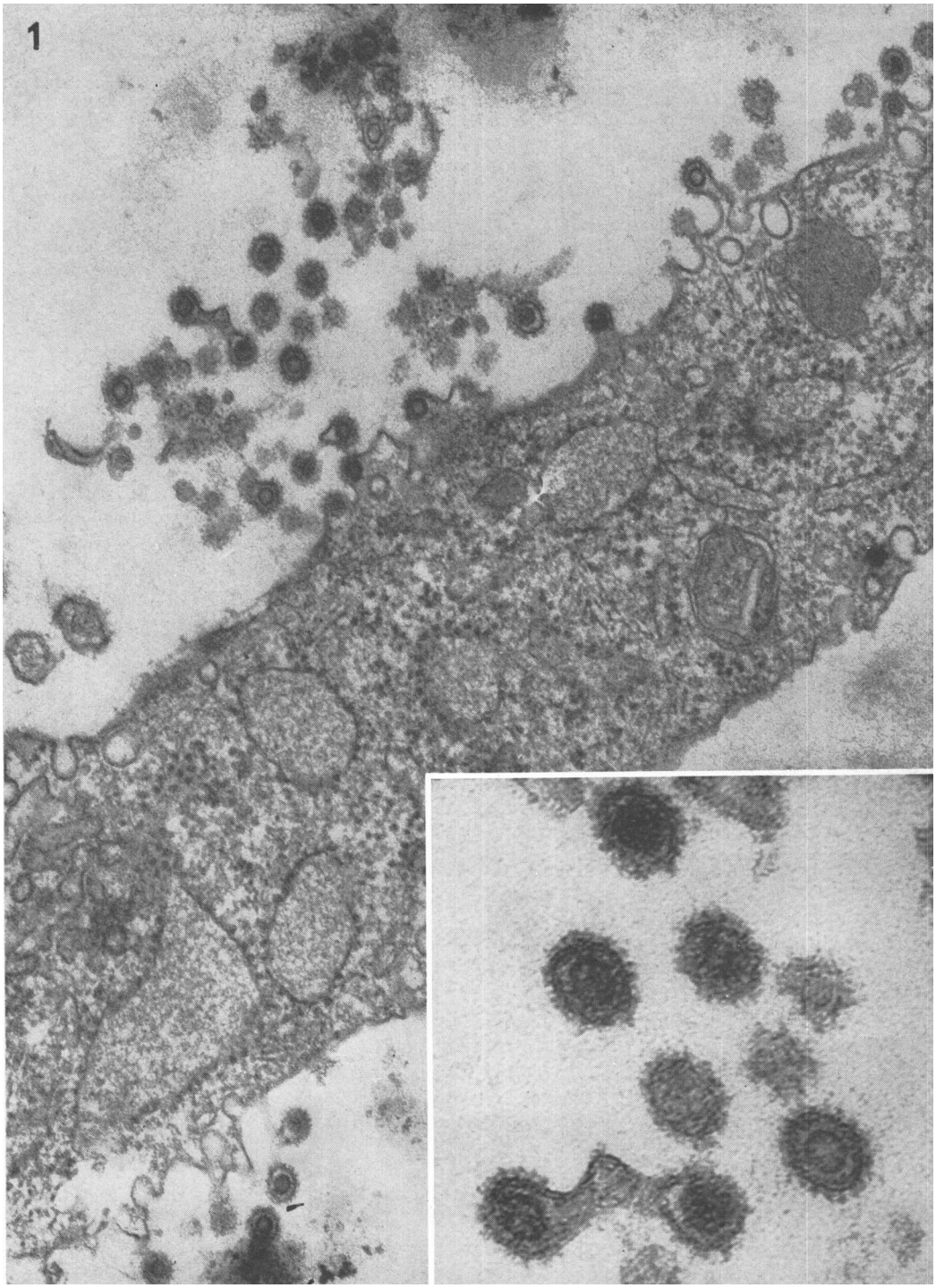

FIG. 1. Feline fetal bone marrow cells infected with feline syncytia-forming virus. Virus particles are shown budding from the cell surface and free in the lumen of a syncytium. Distinct spikes are present on the surface of the virion; the nucleoid is completed at the time of budding and release. The virion is approximately 65 mμ and the nucleoid approximately 36 mμ in diameter. 60,000×; insert 165,000×.

TABLE II. Host Range of Feline Syncytia-Forming Virus (FeSFV) in Cultured Cells.[a]

Species	Cell line	Cell type	CPE observed	Syncytia formed	Post-infection (days)
Feline	Fc 9 WF	Fibroblastic	+	+	3
	Fc 3 Tg	Fibroblastic	+	+	3
	Fc 3 Th	Fibroblastic	+	+	3
	F1 B	Fibroblastic	+	+	3
	Fc 6 BM	Fibroblastic	+	+	3
Human	Hs 5 tumor	Fibroblastic	+	+	4
	Hs 45 foreskin	Fibroblastic	—	+	3
	Hs 52 thymus	Fibroblastic	—	+	3
	Hs 30 foreskin	Fibroblastic	+	+	3
	Hep 2	Epithelial	—	—	10
Bovine	EBTr	Mixed	—	+	4
Dolphin	DbK	Mixed	—	+	4
Monkey	Vero	Epithelial	—	—	10

[a] All cells except F1B, Hep 2, and Vero were initiated in this laboratory. F1B was initiated in his laboratory by Dr. John Riggs, California State Public Health Laboratories, Berkeley, California; Hep 2, from the American Type Culture Collection and Vero was obtained from Dr. J. Hardy, University of California, Berkeley, California.

also susceptible. It is interesting that this culture has been shown to be releasing C-type virus particle, and there is no apparent interference between FeSFV and the C-type virus (8).

Kidney cells of the dolphin, *Delphinus bairdi*, (Db1K) at passage 3 showed syncytia 4 days after exposure to FeSFV (Fig. 2C). A bovine trachea cell line (EBTr) was also susceptible to the virus. Two established cell lines, an African green monkey (Vero) and a human (Hep 2) were not susceptible.

Low passages of several human fibroblast-like cell lines recently initiated in this laboratory were susceptible to FeSFV, some lines showing syncytia only, while others were destroyed during the interval observed. There was no correlation between susceptibility and the source (normal or neoplastic) of the tissues.

Interferon induction. Fluids harvested from feline cultures infected with FeSFV and showing complete CPE were assayed for the presence of interferon. None was detected in fluids from either the F1B or Fc3Th cell lines, at a dilution of 1:4, using the plaque-reduction method with vesicular stomatitis virus as indicator. The procedures described by Wagner *et al.* (3) were used.

Growth, stability, and ether sensitivity. The virus grew to maximum titer in feline cells, yielding usually 5×10^4 $TCID_{50}$/ml. Various freeze–thaw and sonication regimens were tested for increasing the infectivity titer. The results were disappointing; the virus was very unstable and a large loss of titer resulted. Best preservation and storage of FeSFV was obtained when 10% dimethylsulfoxide was added to the suspension prior to the freezing at —70°.

When 1×10^4 $TCID_{50}$ in 1 ml of virus was shaken with an equal volume of ether, infectivity was no longer detectable.

Neutralization tests. No neutralization of FeSFV could be detected with any of the seven types of simian foamy virus antisera tested. Antisera to mumps, herpes, and adenovirus type 2 did not neutralize the virus. Human convalescent serum to respiratory syncytial virus (Long) delayed development of CPE by FeSFV at dilutions of 1:20, 1:50, and 1:100, a possible nonspecific effect.

Discussion. FeSFV appears to be a widespread agent among domestic cats of California. The incidence of FeSFV in cats other than those reported here is unknown. Howev-

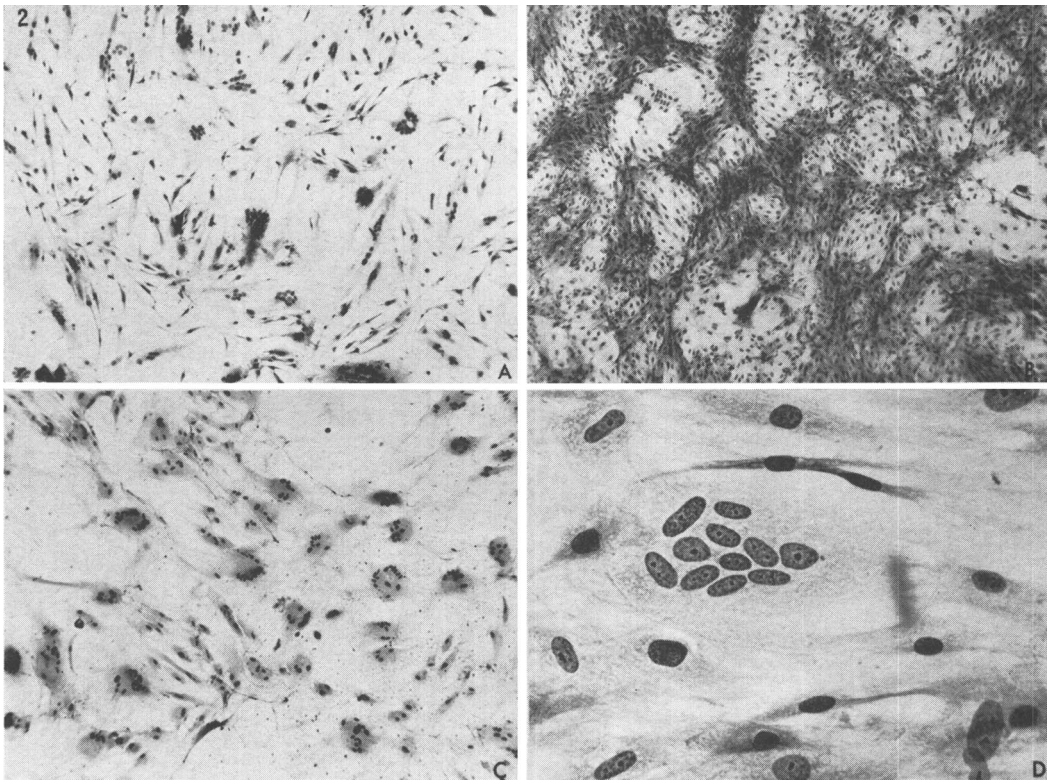

FIG. 2. Giemsa stained cells infected with Feline syncytia-forming virus (FeSFV): (A) fetal cat cells 3 days postinfection (PI); (B) feline cell line, F1B, 3 days PI; (C) dolphin cells, 4 days PI; (D) human foreskin fibroblast cells 3 days PI.

er, the agent has been isolated from cats in New York State by Dr. C. G. Rickard, Cornell University (personal communication), a cat from Ohio (4), and in cats in Scotland by Dr. W. F. H. Jarrett, University of Glasgow, Glasgow, Scotland (personal communication).

In each of the above instances, studies on feline RNA tumor viruses led to the observation of the syncytia-forming agent. Fabricant *et al.* (5) reported the agent in all of 12 cats with induced urolithiasis, in 1 of 3 unobstructed cats, and in 1 of 5 untreated control cats. These findings suggest that the incidence of FeSFV is high among debilitated and diseased cats.

A similar agent has been described by Malmquist *et al.* (6) in cultured lymphocytes from lymphomatous cattle. Both the bovine agent, and the feline, reported here, were difficult to transmit with cell-free fluid, indicating a strong cell-association. Electron micrographs of the bovine agent (6) revealed budding particles morphologically similar to the FeSFV as described by Riggs *et al.* (1), Fabricant *et al.* (5), the feline agent isolated by McKissick and Lamont (4) and the present FeSFV (isolated by this laboratory).

The sensitivity to ether, together with its cytoplasmic maturation site and its morphology, as described by Riggs *et al.* (1) indicate a myxovirus.

Feline rhinotracheitis virus (FeRV) produced giant cells and inclusion bodies in feline kidney cells (7), whereas we observed, as did Riggs *et al.* (1), that FeSFV does not produce inclusion bodies in cultures feline fibroblasts nor in the feline F1B cells. FeRV does not produce a cytopathic effect in established human cell lines (Chang liver and conjunctiva) (7), and interestingly in these studies FeSFV did not grow in two established cell lines, the human Hep 2 and the

Vero. However, it grows in a wide range of cultured cells which includes primary human cells.

There is a lack of information on the biological activity and the field incidence of FeSFV. The virus is slightly pyrogenic in normal cats (8) and grows very slowly in tissue culture. Consequently, it has probably been overlooked. The very high prevalence of subclinical infection in adult domestic cats of California and its presence in all lymphomatous cats tested make it worthy of further study.

Summary. Tissues obtained from adult cats were destroyed, when cultured, by a slow-growing syncytia-forming virus which was transmitted to cultured cells of several species, including human. Ninety percent of the cats obtained were infected. The agent is myxovirus-like, but does not induce interferon production.

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