after insult with other noxious agents did not occur. In fact, there seemed to be increased inward folding of the outer mitochondrial membrane (Fig. 2, arrows). It was at this time that reduction of ciliary beat and cell movement was most pronounced.

In cells exposed to residue for as long as 70 minutes, when ciliary loss and cell death were imminent, remnants of the inner tubular network persisted. These tubules are primarily along the periphery of the mitochondria. While most mitochondria of a cell were similarly affected by the residue, some were unaltered after as much as 70 minutes. Thus it may be that only those mitochondria which were functionally active were altered by residue. The gaseous phase (*7*), although also ciliatoxic, did not seem to cause breakdown of the inner mitochondrial membranes. It did, however, cause swelling of the mitochondria.

The ciliatoxic effects of cigarette smoke have been demonstrated in experimental animals from a number of different phyla (*1*), such as respiratory epithelium of humans, rabbits, rats, and frogs, chick trachea tissue cultures, gill cilia of the clam, and *Paramecium* (*2*). Thus a similar effect in *Tetrahymena* was expected. Several investigators have attributed major ciliatoxic effects to the gaseous phase of cigarette smoke. For example, another ciliated protozoan, *Paramecium aurelia*, tolerated nicotine concentrations above those found in cigarette smoke. However, the ciliatoxic effect of the gas phase was almost as great as that of whole smoke, suggesting that the toxic component resides primarily in the gaseous phase of cigarette smoke (*1*). Similar conclusions have been drawn from studies on mammalian respiratory cilia (*8*). Other investigators have found that the volatile, acidic, and phenolic fractions were the most toxic to clam gill cilia (*1*). Some of this fraction may reside in the particulate matter.

Contrary to many of these observations we have found what appears to be a twofold effect, depending on whether the particulate or gaseous phases were employed. The general disruption of internal mitochondrial structure associated with the particulate phase of cigarette smoke would undoubtedly block energy production for ciliary activity. However, the gaseous phase, considered by others to be more toxic, causes mitochondrial swelling without the concomitant breakdown of inner mitochondrial structure. These questions are being investigated to specifically identify mitochondrial toxic factors and correlate them with ciliatoxicity.

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6. In each of several experiments in which cell changes were followed with the light microscope, alterations with time were shifted by 5 to 15 minutes. This time variation was comparable in the four experiments examined with the electron microscope. Since all residue was not recovered on the filter paper it appears that mitochondrial damage is a function of residue concentration when all other factors (cigarette brand, burn time, and length) are constant. This has been substantiated by physiological studies in which continuous suction is applied and all residue is recovered. Under such conditions time is less of a variable. It should be noted that there appears to be substantial variation in time when different cigarette brands are compared.
7. Mainstream smoke was filtered through a Gelman type A glass fiber filter (efficiency 98 percent for particles as small as 0.05 μm). The remaining gaseous phase was bubbled through a cell culture.
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Feline Leukemia and Sarcoma Viruses: Susceptibility of Human Cells to Infection

Abstract. *Human embryonic cells are highly susceptible to infection with feline leukemia and sarcoma viruses. These viruses were propagated in human cultures without antigenic modification or loss of infectivity for cat or human cells. Virus stocks contained at least 10^6 infectious units of virus per milliliter for human cells. Virus present in 10^{-6} dilution of virus stock replicated to detectable amounts as early as 7 days after virus infection. The feline sarcoma virus induced morphological transformation of human cells.*

Feline leukemia and sarcoma are caused by C-type RNA viruses similar to those which cause the naturally occurring leukemia and sarcomas in mice and chickens (*1*). The feline leukemia virus (FeLV) readily replicates in cultures of feline and canine embryonic cells without causing any visible effects (*2*, *3*), whereas the feline fibrosarcoma virus (FSV) (*4*) induces foci of cell transformation (*5*) in these cultures.

Jarrett (*6*) and O'Connor and Fischinger (*7*) found that human embryonic cells support the growth of FeLV in vitro. We have recently found that cultured human embryonic cells are extremely susceptible to infection with newly isolated field strains of leukemia and sarcoma viruses of the cat. The leukemia and sarcoma viruses thus propagated in human cells are fully infectious for human, dog, and cat embryonic cells.

In this study FSV was derived from a 5½-year-old Siamese male cat with naturally occurring fibrosarcoma (*4*). Virus stocks used in this study were prepared as partially purified concentrates of tumors (*8*, *9*) induced in cats and dogs with cell-free preparations of the virus. In addition, virus stocks were prepared in cultures of feline embryonic fibroblasts (FEF) with FSV propagated in cats and dogs. Cells and fluids of infected cultures showing confluent

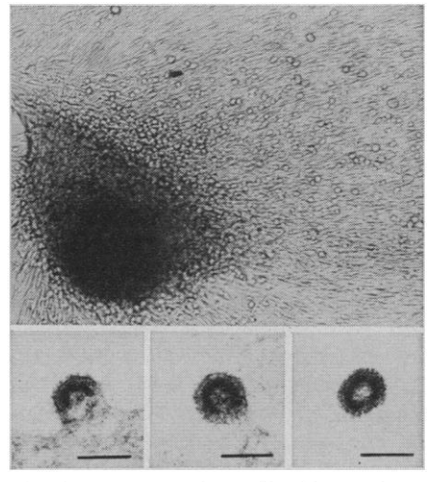

Fig. 1. Human embryo fibroblast culture containing a focus of cell transformation induced by the feline fibrosarcoma virus; photomicrograph of unstained culture (× 100). C-type virus particles in various stages of development observed in this culture are shown in the bottom row; the bars shown represent 100 nm.

transformation were treated with ultrasound and clarified.

Stocks of FeLV were similarly prepared as partially purified concentrates of naturally occurring feline lymphosarcoma, clarified pleural fluid of an affected cat, and clarified fluid of infected FEF cultures (*3*).

Monolayer cultures of whole human embryonic fibroblasts (WHE), FEF, and canine embryonic kidney were prepared with primary or secondary cells that had been stored in liquid nitrogen (*10*). Growth medium consisted of Eagle's minimum essential medium supplemented with glutamine (2 mmole/liter), fetal bovine serum (5 percent heated at 56°C for 30 minutes), tryptose phosphate broth (5 percent), and antibiotics [penicillin (250 unit/ml), streptomycin (250 μg/ml), and fungizone (25 μg/ml)].

Cells dispersed with trypsin were suspended in fresh growth medium (10^5 cells per milliliter), planted in disposable plastic petri dishes, immediately infected with virus dilutions, and incubated at 37°C in a humidified incubator flushed with 5 percent CO_2 in air. Culture medium was replaced every 3 days. The cells were dispersed with trypsin and serially transferred into screw-capped bottles every 5 to 6 days.

At intervals after inoculation, virus replication was determined by the following tests: (i) cultures were examined microscopically for the presence of transformed cells, (ii) cell antigens were collected and tested, in complement-fixation tests (*3, 11*), against serums from dogs bearing tumors induced with feline fibrosarcoma virus and containing complement-fixation antibodies to feline sarcoma virus, (iii) the release of virus from cells was tested by labeling with tritiated uridine (*12*), and (iv) thin sections of cells were examined for virus particles by electron microscopy (*13*).

Human cells infected with FeLV appeared to be morphologically normal. On the other hand, infection with FSV from tumors and from tissue culture caused the appearance of microscopically visible foci of cell transformation, first evident about the 15th day after virus inoculation (Table 1; Fig. 1). Cell transformation was produced with different stocks of FSV. Budding and mature C-type particles were observed in infected cells as early as 6 days after virus inoculation (Fig. 1). This correlated well with results of labeling with [^{3}H]uridine, which also showed that by 6 days virus was released in high titers into the culture medium. Infected cultures also contained viral antigens detectable in complement-fixation tests with the dog antiserum (Table 1).

Table 1. The susceptibility of human embryo cultures to feline sarcoma and leukemia virus infection and virus-induced cell transformation. Virus stocks used in experiment 2 were different from those used in experiment 1. Radioactivity is measured as counts per minute in the peak fraction (1.16 g/cm^3 in sucrose gradients); 0 indicates no radioactive peak with an actual count of less than twice the background count of 25.35 per minute.

Virus	Time after inoculation (days)				
	Experiment 1			Experiment 2	
	10	17	24	6	13
			Foci		
FeLV	0	0	0	0	0
FSV	0	+	+	0	0
FSV	0	+	+	0	0
None	0	0	0	0	0
			C-type particles		
FeLV	+	+	+	+	+
FSV	+	+	+	+	+
FSV	+	+	+	+	+
None	0	0	0	0	0
			Radioactivity (count/min)		
FeLV	300	300	500		2500
FSV	950	900	1000		8750
FSV	4200	1050	2700		7765
None	0	0	0		0
			Complement-fixation titer		
FeLV	2	2	2	<2	4
FSV	>4	>4	>4	4	>4
FSV	>4	>4	>4	2	>4
None	<2	<2	<2	<2	<2

Foci of cell transformation induced by FSV in human embryonic cultures consisted of discrete aggregates of round cells which increased in size to form macroscopically visible clumps. Crisscross refractile fibroblasts and scattered round cells were observed around these foci (Fig. 1). Feline leukemia virus and FSV propagated in human cultures were infectious for human, feline, and canine embryo cultures.

A parallel assay of FSV in human and feline embryo cultures showed that human cells we used were approximately 10 times less susceptible to transformation than cat cells (Table 2). On the 7th day after virus inoculation, C-type virus particles were present in large numbers in all human and feline cultures inoculated with stock diluted up to 10^{-6}. Again the tritiated uridine technique gave parallel results. However, the complement-fixation for antigen detection was not a sensitive technique for the demonstration of virus present in cultures (Table 2).

Serial passage of cell-free culture fluids of infected human and cat cultures into like cultures gave the following results. Cell transformation was produced in cultures inoculated with undiluted stock and 10^{-2} dilutions of virus stock. C-type virus that was not cytopathogenic was detected in cultures inoculated with stock diluted 10^{-3} to 10^{-6}.

The antigenic characteristics of sarcoma virus propagated in human cells were studied by a focus-neutralization test in which a FSV-neutralizing antiserum obtained from a dog carrying a FSV-induced fibrosarcoma was used (*4, 11*). The mixtures of virus and serum, containing 10^3 FEF focus-forming units of virus per milliliter, were incubated at room temperature for 1 hour and then inoculated into FEF and WHE cultures in 0.2-ml amounts. These cultures were observed for foci. This serum reduced the capacity of FSV to transform human cells tenfold and completely neutralized the transformation of feline embryonic cells by a FSV stock grown in human cells. Thus no obvious antigenic change occurred in one passage of FSV through human cells.

Under experimental conditions, avian and murine C-type RNA tumor viruses cross species barriers usually without production of infectious virus (*9, 14*). Most field strains of avian and

Table 2. Comparative susceptibility of human and feline embryonic cells to infection with feline sarcoma virus and associated feline leukemia virus. Cultures were examined electron microscopically 7 days after virus inoculation. The cultures continued to be positive on subsequent days tested. Cultures were labeled with uridine 13 days after inoculation. The reciprocal of complement-fixation titer is given; antigens were collected for CF test 12 days after virus inoculation.

Virus dilution	Cell transformation (No. foci dish)		C-type particles		Release of [^{3}H]uridine labeled virus		Complement-fixation titer	
	Human	Feline	Human	Feline	Human	Feline	Human	Feline
Undiluted	80	TNTC	+	+			>4	>4
10^{-2}	4	50	+	+			>4	2
10^{-3}	0	3	+	+	3952		2	<2
10^{-4}	0	0	+	+	6157	1324	<2	<2
10^{-5}	0	0	+	+	2746	1846	<2	<2
10^{-6}	0	0	+	+	905	1774	<2	<2
Control	0	0	0	0	0	0	<2	<2

murine leukemia viruses fail to cross species barriers and seldom infect human cells (*15*). However, recent studies indicate that some laboratory-adapted strains of murine leukemia and sarcoma viruses may infect and propagate in human cells (*15, 16*).

Our studies and others (*6, 7*) indicate that newly isolated field strains of feline leukemia and sarcoma viruses readily infect and propagate in human cells. In addition, we have observed that FSV induces morphological transformation in WI-38 diploid human embryonic lung cultures, similar to that observed in WHE cultures. Antigenic and host-range modifications of FSV do not occur in one passage in WHE cells. The fact that FSV transforms human cells and the accompanying FeLV replicates to demonstrable levels within 7 days, even when diluted to 10^{-6}, demonstrates the extreme susceptibility of human cells to the naturally occurring tumor viruses of the domestic cat.

Dog cells are extremely susceptible to the cell-transforming effects of FSV. They support the growth of feline leukemia and sarcoma virus as well as cat and human cells do (*5–7*). It is noteworthy that the feline C-type viruses are oncogenic in vivo in other hosts; for example, Rickard (*17*) found that FeLV produces lymphosarcoma in dogs and Dienhardt and Thielen (*17*) found that their strain of FSV produces sarcoma in the marmoset. Feline leukemia virus has been found in salivary glands of leukemic cats (*18*). It is therefore conceivable that human cells that are quite susceptible to infection in vitro may have some degree of susceptibility in vivo, such as when the virus is introduced parenterally through a bite or scratch by a viremic cat with or without clinical symptoms of leukemia. Although there is no evidence to implicate feline leukemia and sarcoma viruses in human cancer, further studies are necessary to determine the possible occurrence of some horizontal spread of cancer by this mode.

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13. The cells were pelleted and fixed in 2 percent glutaraldehyde in cacodylate buffer, *p*H 7.2. They were then postfixed in Dalton's chrome-osmium, dehydrated in methanol and propylene oxide, and embedded in Epon-Araldite. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with a Hitachi HU 11-E electron microscope.
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Blood Concentrations of Acetaldehyde and Ethanol in Chronic Alcoholics

Abstract. *Fifteen adult male alcoholic volunteers were studied before, during, and after a 10- to 15-day period of experimentally induced intoxication. Blood acetaldehyde concentrations ranged from 0.11 to 0.15 and from 0.04 to 0.08 milligrams per 100 milliliters when blood ethanol concentrations ranged from 1 to 400 milligrams per 100 milliliters after consumption of bourbon or grain ethanol, respectively. No dose or dose-time relationships were found between blood ethanol concentrations and blood acetaldehyde concentrations during any phase of this study.*

The biological concomitants of acetaldehyde metabolism have been stressed as potentially important factors in the determination of many pathophysiological processes associated with alcohol addiction (*1*). It has been demonstrated that a number of unique effects induced by acetaldehyde are not caused by ethanol alone (*2*), and that acetaldehyde causes significant effects on biotransformation and metabolism of catechol and indole amines (*3–5*). Demonstration that acetaldehyde competitively inhibits 5-hydroxyindoleacetaldehyde oxidation to 5-hydroxyindoleacetic acid (*4*) explains, in part, the mechanism of the increased formation of 5-hydroxytryptophol and methoxyhydroxyphenylglycol (*5*) in man during alcohol ingestion. Another consequence of competitive inhibition may be an accumulation of intermediary aldehydes derived from biogenic amines that would react with the intact amines to form Schiff bases which, in turn, may give rise to various alkaloids such as tetrahydroisoquinoline and tetrahydropapaveroline. Thus it has been suggested that the addictive properties of ethanol may be related, in part, to the concentration of acetaldehyde generated during ethanol metabolism and to the subsequent formation of tetrahydroisoquinolines or tetrahydropapaverolines (*6*).

The rate of acetaldehyde metabolism is quite rapid (*7*), and blood acetaldehyde concentrations are relatively low after short-term administration of ethanol to man (*8*). However, no serial determinations of acetaldehyde concentrations have been reported after long-term administration of high-dosage eth-