Cigarette Smoke: The Effect of Residue on Mitochondrial Structure

Abstract. *Cigarette smoke residue is ciliatoxic to* Tetrahymena pyriformis. *Principal sites of activity are the mitochondria of the cell. The internal membranes of the mitochondria are degraded with time, correlating well with loss of ciliary activity and cell death.*

There have been numerous investigations into the effect of various agents on respiratory cilia. Of particular interest have been the ciliastatic or ciliatoxic agents in cigarette smoke (*1*). Although components of both the gaseous and particulate phase are known to retard or inhibit ciliary activity in the respiratory passages of mammals and similarly to inhibit the ciliary activity of *Paramecium* (*2*), nothing is known about specific sites of activity of these agents on the cell. To further examine this question, we conducted a series of experiments on the ciliated protozoan, *Tetrahymena pyriformis*.

Cells were grown in 150 ml of 2 percent proteose peptone in liter flasks. Residue from mainstream cigarette smoke was collected through intermittent suction on Whatman No. 1 filter paper (*3*) and suspended in 15 ml of a 24-hour logarithmic growth culture (approximate density, 100,000 cells per milliliter) in a 250-ml flask. The residue from one cigarette was employed for each 15 ml of cells. Filter paper plus residue was added to the culture and agitated continuously. Samples were taken every 7 minutes for examination under phase microscopy and fixation for electron microscopy. Fixation was carried out for 20 minutes in a mixture of osmium tetroxide and glutaraldehyde (*4*). Cells were dehydrated in alcohol and embedded in Epon 812 (*5*). Sections were cut on a Porter Blum MT-1 microtome with diamond knives, stained with lead citrate and uranyl acetate, and examined with a RCA EMU 3E microscope.

The first appearance of alteration of cell activity was observed after about 28 minutes exposure to the residue (*6*). At that time there was a reduction of ciliary beat, and cell motility was considerably reduced. By 35 to 45 minutes, ciliary beat was very slow in most of the cells, and later ciliary activity ceased. This was followed by rounding of the cells and eventual ciliary loss.

The most striking alteration in cell structure was in the mitochondria. Normal mitochondria in *Tetrahymena* consist of tubular inner membranes (cristae) enclosed in a continuous outer membrane. After exposure to the residue for 7 minutes (Fig. 1), the tubular arrangement (*T*) of the inner mitochondrial membrane became altered, with the appearance of a few shelflike mitochondrial cristae (arrows) characteristic of higher cells. Alteration of the inner microtubular network increased with continued exposure, and after 42 minutes (Fig. 2) most of the inner tubular membranes were destroyed. The granular matrix visible in normal mitochondria was not altered in mitochondria exposed to residue for 7 minutes. However, significant reduction in the matrix was visible after prolonged exposure to residue (Fig. 2). The outer mitochondrial membrane appeared to be unaffected by the residue, and mitochondrial swelling generally observed

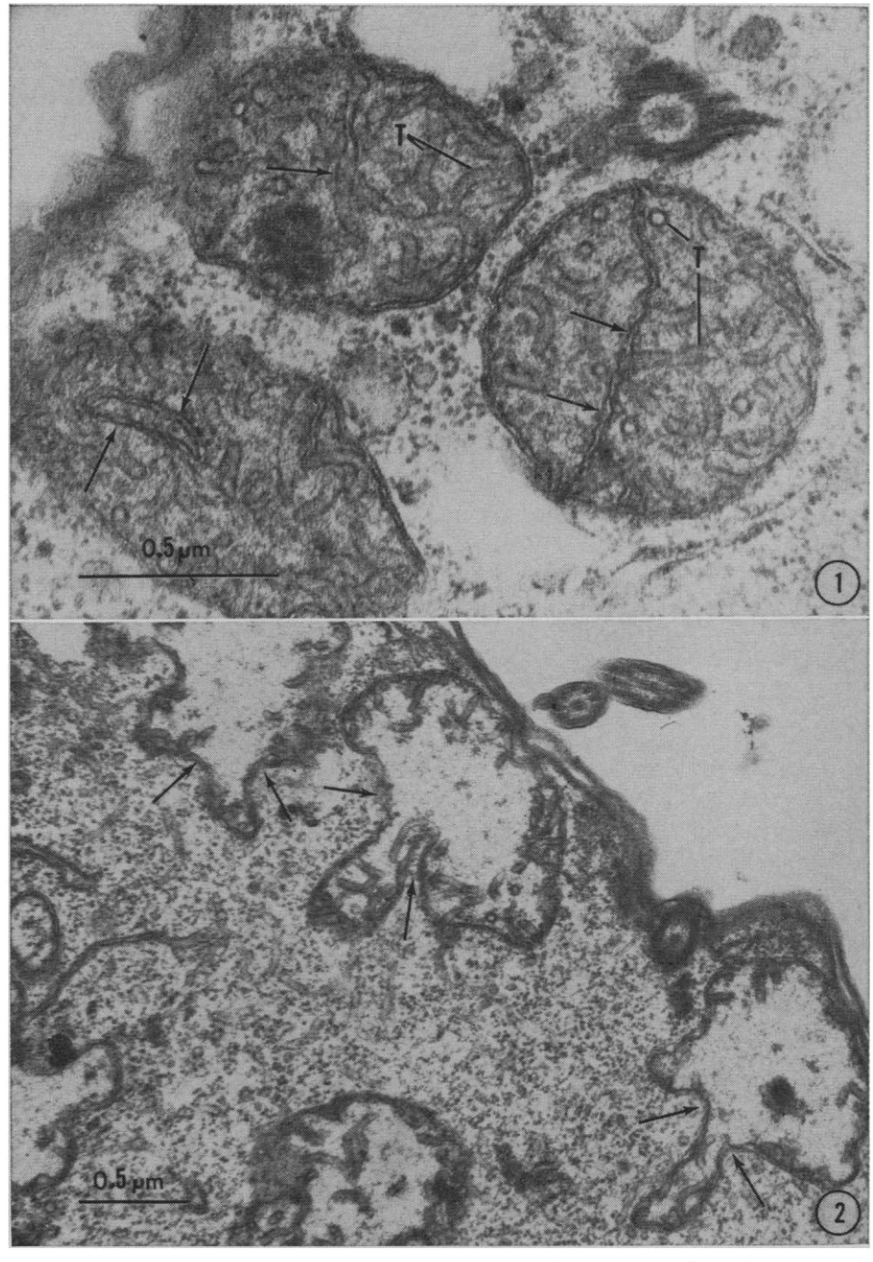

Fig. 1. Mitochondria from cells exposed to residue for 7 minutes. Shelflike (arrows) and tubular (*T*) cristae are indicated. Fig. 2. Mitochondria from cells exposed to residue for 42 minutes. Infolding of the mitochondrial membranes is indicated (arrows).

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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References and Notes

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3. For collection of residue, a 250-ml Erlenmeyer flask fitted with a two-hole rubber stopper was employed. Two bent glass tubes were inserted into the flask through the stopper. The cigarette was attached to one end of a tube, the end inside the flask being wrapped with filter paper. Suction was applied to the second tube, and residue collected on the filter paper.
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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

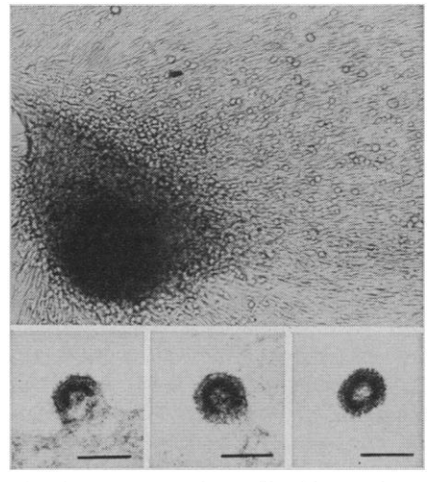

Fig. 1. Human embryo fibroblast culture containing a focus of cell transformation induced by the feline fibrosarcoma virus; photomicrograph of unstained culture (× 100). C-type virus particles in various stages of development observed in this culture are shown in the bottom row; the bars shown represent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