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Alterations in Growth of Strain L Cells Exposed to Smoke Gases.* (27405)

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The purpose of this investigation was to observe the effect of smoke and certain non-smoke gases on growth of cells in culture. Our interest in this subject was stimulated by the over-all problem of cancer of the lung and by the fact that only limited studies on the effects of smoke on single cells have been published. Cigarette smoke bubbled through a culture of certain pathogenic bacteria in a test tube killed the bacteria(1). The interfering action of cigarette smoke on the ciliated mucous secreting respiratory epithelium *in vitro* has also been established(2). The particulate phase of cigarette smoke over and above the chemistry of smoke has been implicated in the interference of normal functioning of respiratory epithelium(3).

The present series of studies was undertaken further to elucidate the direct action of smoke on living cells.

Materials and methods. Strain L cells designated 929-421-303, obtained from Dr. W. R. Earle, were maintained in 2 cc of medium NCTC-109, with 10% horse serum in B-15 Bellco Flasks with the pH adjusted to 7.4. The flasks were seeded with 50,000 cells/ml. The doses of smoke used were 0.5 cc, 1.0 cc, and 1.5 cc at N.T.P. After preparation the flasks were allowed to remain stationary in the incubator at 37.5°C. At the end of 24 hours the cells had adhered to the glass, and were well spread out, but had not completed their first division cycle. Smoke was collected under sterile conditions in a syringe from a modified Phipps and Bird smoking apparatus. A sterile side arm was installed and the smoke was collected directly at its source. The flasks were inverted and the prescribed

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volume of smoke was introduced into each flask. The cells were exposed to smoke for 1-2 seconds, the flasks were then re-inverted, and the fluid medium allowed to wash over the cells. The flasks were then gently agitated until all smoke was absorbed by the medium. In the 3 doses of smoke used there was no change in pH of the medium. It had been determined that volumes of smoke larger than 1.5 cc caused a definite pH change with the resulting acidity causing the cells to cytolyze.

The flasks were returned to the incubator and examined periodically with an inverted microscope.

Smoke from several sources was used. This included smoke from the combustion of cigarettes, cigarette tobacco, cigarette paper and onion skin paper. Tobacco wrapped cigarettes were also used. Control cultures were treated in exactly the same manner as the experiments except that they received similar doses of sterile air. The effect of non-smoke gases as illuminating gas and carbon monoxide was also studied under similar conditions.

In the first part of the study the smoke and other gases were injected 24 hours after seeding of the culture. In each experiment 6-8 flasks were used at each smoke or gas dose with an equal number to serve as controls. After 48-52 hours the cells were trypsinized and counted in a hemocytometer chamber.

In the second part of the study smoke gases were injected 48 hours after seeding of the cultures and 48 hours later half of the control and treated cultures were subcultured. In each experiment 10-12 flasks were used at each smoke dose. Cell counts were accomplished at 24-hour intervals on representative cultures for the 7-day period of each experiment.

Results and discussion. The results of the first part of the study are shown in Table I. Whereas the non-smoke gases had no significant effect on growth of L cells in culture, as indicated by the similarity of the numerical populations of both experimental and control cultures, the smoke gases had decided deleterious effects. This is indicated by the inhibition of growth in all the experimental cultures. The cigarette paper and onion skin paper smoke produced a significant lowering of the population with all doses used.

TABLE I. Effect of Gases on Growth of Strain L Cells.

Gas	% growth relative to control Means and stand. dev. .5 cc	1.0 cc	1.5 cc
Cigarette smoke	75 ± 9	43 ±14	26 ±12
Cigarette tobacco only smoke	70 ±17	31 ± 9	26 ±12
Tobacco wrapped cigarette smoke	81 ± 7	60 ±16	19 ± 6
Cigarette paper smoke	16 ± 2	18 ± 3	15 ± 3
Onion skin paper smoke	26 ± 7	31 ± 8	24 ± 6
Carbon monoxide			98 ±13
Illuminating gas			108 ±23

It is evident from the figures presented that the recovery of cells exposed to cigarette smoke occurs readily, except at high concentrations, whereas the recovery of cells exposed to smoke from cigarette paper and onion skin paper is at best marginal at all concentrations.

The findings of the second part of the study are shown in Fig. 1. The smoke was injected 48 hours after seeding of the culture in contrast to 24 hours as was done in the first part. The cell population 48 hours after the injection of smoke rose higher when the cells were exposed to the gas in the logarithmic phase of their growth. It is evident from Fig. 1 that after injection of 1.5 cc of cigarette smoke, there is satisfactory recovery of the subcultured cells, but after injection of a similar volume of either cigarette paper or onion skin paper smoke, recovery is poor.

Periodic microscopic examination of the cultures revealed that those cultures which received non-smoke gases showed no cellular changes and were comparable cytologically to control cultures. Certain unusual characteristics of the "smoked cells", however, were observed. These included the tendency of the cells to round up and leave the surface of the glass. They became granular and occasionally pyknotic. The severity of these reactions was related to the size of the smoke dose, increasing with the volume of smoke.

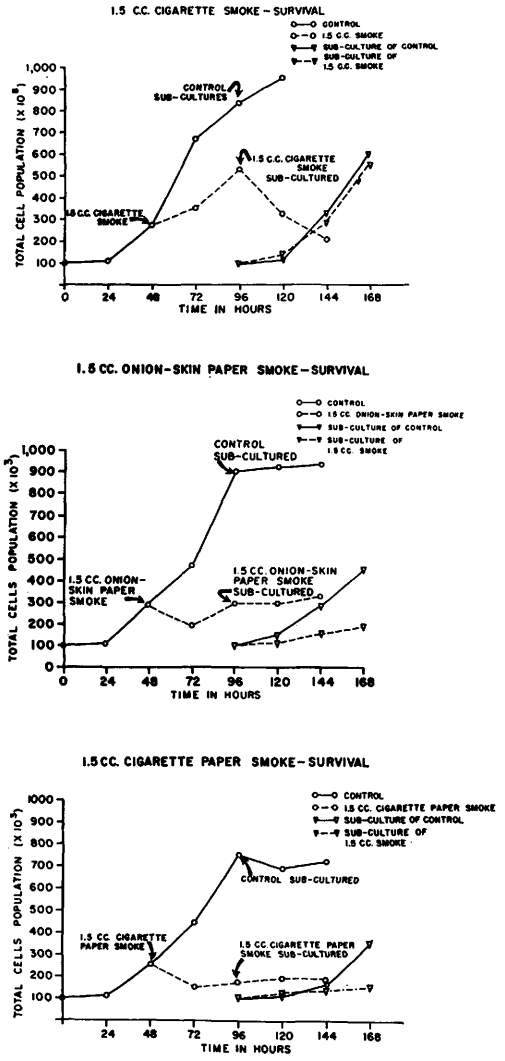

FIG. 1. Survival studies on Strain L cells exposed to 1.5 cc of cigarette smoke, 1.5 cc of onion skin paper smoke, or 1.5 cc of cigarette paper smoke.

It was also noted that particulate matter found in smoke varied per unit amount according to the source and volume of the smoke. Smoke from paper contained more particulate matter than smoke from cigarette tobacco or from the whole cigarette. The experimental changes appeared to be related in part to the amount of particulate matter in the medium after exposure. In recent investigations of the effect of cigarette smoke on ciliated epithelium(3), the results indicated impedance of mucus flow and ciliary action. This work suggested that the particulate phase of cigarette smoke may be of significance.

Perfusion chamber studies of the immediate effect of smoke and non-smoke gases on cell morphology are being reported(4).

Summary. Under the conditions of this study it appears that there is a "toxic" effect of smoke gases on strain L cells in culture. This results both in morphological changes in the cells and in alterations in growth curves as indicated by the reduction of cell populations. The smoke from the combustion of either cigarette paper or onion skin paper appears to be more "toxic" than that from the combustion of a cigarette or cigarette tobacco alone. The effect of cigarette or other smoke gases may result in part, at least, from the particulate matter present in smoke gases.

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Excretion of Urinary 5-HIAA in Dog Pellagra.* (27406)

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It is generally accepted that serotonin synthesis is related to tryptophan intake. The major pathway of tryptophan metabolism leads to kynurenin → 3-OH-kynurenin → 3-OH-anthranilic acid and → nicotinic acid (1,2). The minor pathway leads to 5-OH-

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