Cytologic and Cytochemical Effects on Primary Mouse Kidney Tissue and Lung Organ Cultures after Exposure to Whole, Fresh Smoke and Its Gas Phase from Unfiltered, Charcoal-filtered, and Cigar Tobacco Cigarettes

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SUMMARY

A model system using a smoking machine suitable for short- and long-term studies on the effects of whole cigarette smoke and its gas phase on tissue and organ cultures is described. Results obtained so far showed no significant differences between cell damaging activity of puffs from whole fresh smoke and from those of the gas phase. However, while puffs from unfiltered cigarettes evoked rapid destruction of mouse kidney tissue and lung organ cultures, such damage was absent after the smoke passed through a charcoal filter. On the other hand, cultures exposed to smoke from charcoal-filtered cigarettes disclosed a temporary increase in the mitotic index over that of control cultures. Puffs from cigarettes made up from cigar tobacco produced less and somewhat different damage from that observed after exposure of cultures to smoke from unfiltered cigarettes.

INTRODUCTION

There is a great need for further exploration of the role of cigarette smoking in the causation of bronchogenic carcinoma in man. Surprisingly enough, most experimental studies in this area are not concerned with the biologic effects of cigarette smoke itself, but with those of cigarette smoke condensates or their components (19). Since these preparations have different physical and chemical characteristics from those of cigarette smoke per se, an extrapolation of results obtained with such preparations on animals or on in vitro systems to the question of a causal relationship between cigarette smoking and human lung cancer is open to criticism. More directly related to this specific problem are experimental investigations on the effect of cigarette smoke itself and of its interaction with the respiratory tract and other tissues.

Within the last 10 years a few investigators have focused their attention on development of inhalation procedures, and some fruitful information has been gained from systematic chronic inhalation studies carried out in animals with cigarette smoke alone (2, 10, 14, 15, 18), or in combination with other factors, such as influenza virus (5, 8, 12). Furthermore, chronic inhalation studies with a newly developed smoking machine, in which mice alternatively inhale individual puffs of fresh cigarette smoke and fresh air, disclosed a greater frequency and wider spectrum of tumors in the exposed mice than in control mice, not only after inhalation of fresh unfiltered whole smoke, but also after inhalation of the gas phase of cigarette smoke (11).

Thus, investigation of the sequential effects of whole cigarette smoke and particularly of its gas phase on cells and tissues in in vitro systems appeared of great interest. It also was important to find out whether effects observed in such in vitro systems will or will not be in accordance with the results obtained in the whole animal. In the present experiments, cell and organ cultures were exposed to various types of fresh smoke, and early changes in growth, RNA, and DNA synthesis following the treatment are described. An exploration of the influence of smoke on intracellular nucleoprotein metabolism seemed especially promising because it has been reported that cigarette smoke has alkylating activity (20). Alkylating agents are known to interact with cell metabolites, such as nucleic acids, and thereby cause damage, mutation, and/or malignant transformation of cells (1, 20).

MATERIALS AND METHODS

For the bioassay of the effect of cigarette smoke and its gas phase, kidney tissue and lung organ cultures were utilized. Preparation of both types of cultures was done under sterile conditions. Monolayers of primary Snell's mouse kidneys were prepared by the same method employed in our previous studies with influenza virus (9). Kidneys from mice 8–10 days old were removed aseptically and placed into Eagle's medium to avoid drying of tissues. After removal of the capsules, the tissue was cut into small pieces of 2–3 mm and twice trypsinized with fresh 0.25% trypsin in phosphate-buffered saline (approximately 5 ml for 10 kidneys). After the second trypsinization, the suspension was centrifuged for 5 minutes at 1500 rpm, the supernatant discarded, and the tissue resuspended in Eagle's medium with 10% calf serum; 1.5-ml quantities of the
Organ cultures of embryonic mouse lung were made according to instructions given personally by Dr. I. Lasnitzki, with the modification that the cultures were kept at 37°C in a CO2 incubator.

The lungs from approx. 17-day-old mouse embryos were aseptically removed and cut into 1—2-mm pieces, carefully avoiding tearing or squeezing of the lungs. Sterile lens paper strips (30 x 6 mm) moistened with a drop of medium holding 3—4 of the small lung pieces were transferred to a stainless steel micromesh grid (20 x 20 x 3 mm) kept in a small Petri dish. Eagle’s medium (3—4 ml) containing 20% calf serum was added gently, avoiding direct contact between lung explants and medium. However, about 2.5 mm of the lens paper hanging over each end of the grid were immersed in the medium. In order to obtain good lung organ cultures with a minimum of necrosis it is important that all steps are carried out very rapidly.

Three different types of cigarettes were used, a cigarette without a filter (X), the same type of cigarette with an activated charcoal filter (Z), and a cigarette made of cigar tobacco (U). For a better comparison between the effects of unfiltered and charcoal-filtered cigarettes, X and Z cigarettes of exactly the same length, the same paper, and the same packing of tobacco were prepared.

The smoking machine constructed for the bioassay of the effect of cigarette smoke on cells in tissue and organ cultures is illustrated in a photograph (Fig. 1) and a schematic drawing (Fig. 2). The machine consists of 4 separate compartments (volume of each compartment = 680 ml) in which either mice, tissue cultures, or organ cultures can be placed and exposed to puffs of fresh cigarette smoke at intervals selected at will. In each of the 4 compartments of the smoking machine a Petri dish without cover containing a kidney monolayer 3—4 days old, or a lung organ culture was placed as closely as possible to the opening of the cigarette holder so that a puff of fresh smoke from the lighted cigarette could pass immediately over the cultures. By means of a vacuum pump and a cam, a puff of smoke was drawn from each lighted cigarette into each of the 4 compartments; each compartment was then evacuated and air was allowed to flow in. Since the air enters the compartment from the side (Fig. 2) instead of by the direct route which is provided for the smoke itself, it is not known how much air reaches the cultures themselves. However, the fact that the atmosphere in the compartments and directly over the cultures becomes rapidly free of smoke before the next puff of smoke is drawn into the chamber would suggest that air also reaches the cultures. Each culture was exposed only once to a number of puffs. In order to find out whether there was a difference in the biologic effect of puffs of smoke derived from the first half of the cigarette (B) from that of the second half (E), cultures were compared which had been exposed to either one of them. In trial experiments which were done to determine the proper dose, it was noted that the same number of puffs from the second half of unfiltered cigarettes and from cigar tobacco cigarettes evoked much more cell damage (2—3 times) than that observed after puffs from the first half. Therefore, in the present study cultures were exposed to about double the number of puffs from the first half and compared with cultures exposed to half the number of puffs from the second half of unfiltered and cigar tobacco cigarettes. Each puff volume was 5 ml for 5 seconds duration, and the interval between each puff was 15 seconds. This relatively small volume applied within 5 seconds was chosen instead of the standard 35 ml for 2 seconds duration to avoid unspecific shock of the cultures by a rapid exposure to a large amount of smoke. Each comparison between the effects of X, Z, and U cigarettes was carried out on cultures exposed to cigarette smoke in the same compartment. For the production of the gas phase of the fresh cigarette smoke, the cigarettes were placed in front of a special filter holder containing a Cambridge Filter disc (N.9-86 Phipps and Bird, Richmond, Virginia). Each cigarette gave from 20 to 22 puffs when smoked to a butt length of 23 mm. After exposure to cigarette smoke, media were removed from tissue and organ cultures as soon as possible and replaced by fresh media. The time needed for removing cultures from the smoking machine, changing media, and returning the cultures to the CO2 incubator varied from 1 to 2 hours, depending on the number of cultures utilized in each experiment. Rapid removal of media was done mainly to prevent later influence of other constituents than those contained in puffs of fresh smoke. Factors such as altered smoke constituents dissolved in media or cellular breakdown products due to pycnosis and necrosis of cultures after exposure to unfiltered cigarette smoke may have secondary “unspecific” effects on the cultures. In addition to this immediate change, media were changed again in exposed and control cultures 2—3 times a week in intervals from 24 to 72 hours. It should be mentioned that cultures exposed once to several puffs of cigarette smoke did not show any contamination, despite the fact that some cultures had to remain open for nearly 5 minutes.

For the assessment of the sequential alterations evoked by the smoke from the 3 types of cigarettes, the unfixed tissue cultures were examined under the phase microscope at intervals from 1 hour to 4 weeks. For cytologic and cytochemical studies of RNA and DNA, coverslips were harvested from 6 hours to 4 weeks after exposure. For the relative comparison of the RNA content, acidine orange staining was utilized. Fixation, staining, and analysis by Feulgen microspectrography for DNA content were done as previously described (7). For the determination of the mitotic index, H & E and Feulgen-stained preparations were utilized. Serial sections of organ cultures were stained with H & E, Feulgen, and periodic acid-Schiff according to Hotchkiss (6).

For the histologic, cytologic, and cytochemical studies of the organ cultures, the lung pieces were fixed for 1 hour in Lawdowsky’s fixative (25 ml of 95% alcohol, 20 ml of distilled water, 5 ml of formaldehyde C.P., 1 ml of glacial acetic acid), embedded in paraffin without the lens paper, cut into serial sections, and stained as above.

RESULTS

Effects of Fresh Smoke from Various Types of Cigarettes on Primary Kidney Tissue Cultures. No significant differences were observed between effects of whole fresh smoke and its
gas phase. However, there were marked differences between the 
effects of unfiltered cigarettes, those of charcoal-filtered 
cigarettes, and those of cigarettes containing cigar tobacco 
(Table 1). Furthermore, puffs of smoke from the second half 
(XE, UE) of unfiltered cigarettes and of cigarettes made up of 
cigar tobacco affected the cultures to a greater extent, and at a 
much faster rate than from the first half (XB, UB) (Table 1).

One exposure to 10 puffs from the first half or to 4 puffs 
from the second half of the unfiltered cigarette resulted in 
marked loss of RNA from cytoplasm and nucleoli, rounding 
up of cells, and pycnosis (Figs. 3b, 3e). Disruption of cell 
boundaries and extensive detachment of cultures from cover 
slips were observed as early as 6 hours after exposure to 10 
puffs. In most instances complete destruction and loss of 
monolayers were seen 24—48 hours later, particularly after the 
cultures were exposed to 4 puffs of the second half of the 
unfiltered cigarettes. All these changes were more widespread 
and occurred more rapidly if after exposure the medium was 
not removed directly, but allowed to be in contact with the 
culture for 6 hours or longer. The appearance of cultures 
exposed to puffs from charcoal-filtered cigarettes containing 
the same tobacco as the unfiltered cigarettes was strikingly differ-
ent. Even after exposure to 10 puffs from either the first or 
the second half of the charcoal-filtered cigarettes, there was no 
loss of RNA, nor was there cell degeneration for up to 4 
weeks. The cultures (Figs. 3c, 3f) resembled closely those of 
untreated controls of the same age (3a), with the exception 
that an increase in mitotic index over that of controls was 
observed 1—2 weeks after exposure to charcoal-filtered ciga-
rettes (Table 2). Some of these cultures also displayed a slight 
tendency towards proliferation. In cultures exposed to ciga-
rettes made of cigar tobacco, the damage was similar to that 
seen after exposure to unfiltered cigarettes. However, using 
the same number of puffs for U and X cigarettes, it was evident 
that degeneration of cells and of monolayers occurred more 
slowly and was not as marked as with unfiltered cigarettes. 
Furthermore, early stages preceding cell destruction and 
later stages of cultures differed somewhat from those 
exposed to unfiltered cigarettes. Rounding up of cells, loss of 
RNA, and pycnosis were less frequent. Instead, cells and nuclei 
often increased in size (Figs. 3d, 3g) and became balloon-
shaped and necrotic before cell death was observed. Further-
more, cultures in which only parts of the monolayers were 
destroyed displayed in the remaining parts some slight prolifer-
ation and slight increase in DNA synthesis (Chart 1). Early and 
later alterations evoked by unfiltered cigarettes, charcoal-
filtered cigarettes, and cigarettes containing cigar tobacco were 
reproducible in all 500 exposed cultures from 16 experiments 
and were not found in 200 corresponding control cultures of 
the same age.

Effects of Fresh Smoke from Various Types of Cigarettes on 
Lung Organ Cultures. Lung organ cultures showed early and 
later alterations (24 hours to 10 days) to whole smoke or its 
gas phase, which were essentially the same as those of mono-
layers of primary kidney tissue cultures. As can be seen from 
Fig. 4, one exposure to 8 puffs of the unfiltered cigarette (XE) 
led to nearly complete destruction (Fig. 4d) at 24 hours, while 
one exposure to 12 puffs of the charcoal-filtered cigarette 
(ZE) did not alter significantly the organ culture (Fig. 4b). As 
in kidney tissue cultures, exposure to cigarettes containing 
cigar tobacco (UE) also evoked considerable destruction of the 
lung organ culture (Fig. 4c), but again to a lesser degree than 
seen in cultures exposed to the unfiltered cigarettes (XE) (Fig. 
4d). Differences between the effects of unfiltered cigarettes, 
charcoal-filtered cigarettes, and cigar tobacco-containing ciga-
rettes are especially impressive when compared from 4 to 10 
days after exposure. While at this time the lung organ culture 
exposed to cigarette X consists mainly of eosinophilic necrotic 
material and nuclear debris (Fig. 5e), lung cultures exposed to 
cigarettes Z do not only resemble control cultures (Fig. 5a), 
but exhibit a slight tendency towards proliferation of bron-
chial epithelium (Figs. 5b, 5c). While lung organ cultures 
showed more areas of destruction (Fig. 4c) than mouse kidney 
tissue cultures (Figs. 3d, 3g) after exposure to smoke from 
cigarettes made of cigar tobacco, nevertheless such lung cul-
tures also displayed a tendency towards proliferation in re-
maining foci of preserved lung tissue (Fig. 5d). Early and later 
alterations evoked by unfiltered cigarettes and cigarettes con-

Table 1

<table>
<thead>
<tr>
<th>Type of cigarette</th>
<th>Number of puffs and part of cigarette</th>
<th>Time after exposure</th>
<th>Cells</th>
<th>Damage</th>
<th>Monolayer destruction</th>
<th>Stimulation</th>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>Decrease in RNA</td>
<td>Pycnosis</td>
<td>Necrosis</td>
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<tr>
<td>Unfiltered</td>
<td>6, 10; B</td>
<td>6—24 hr</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>++++</td>
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<tr>
<td>3, 4; E</td>
<td>48 hr—1 week</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Charcoal filter</td>
<td>10; B</td>
<td>6 hr—1 week</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10; E</td>
<td>1—3 weeks</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td>3—4 weeks</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Cigar tobacco</td>
<td>6, 10; B</td>
<td>6 hr—1 week</td>
<td>++</td>
<td>(±)</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1—4 weeks</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
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<tr>
<td>1—2 weeks</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Cytologic and cytochemical effects on growth of primary Snell's mouse kidney tissue culture after one exposure to puffs of fresh whole cigarette smoke or its gas phase from different types of cigarettes. R, remaining cells of monolayer; ±, doubtful; --, negative; + to ++++, significant alterations of varying degree; B, first half of cigarette; E, second half of cigarette.

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Table 2

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Time after exposure</th>
<th>Mitotic index (%) (n = 48)</th>
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</thead>
<tbody>
<tr>
<td>Control (5 days)</td>
<td>24 hours</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Control (19 days)</td>
<td>15 days</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>Z</td>
<td>0.15 ± 0.02</td>
<td></td>
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</tbody>
</table>

Mitotic index in primary kidney tissue cultures exposed once to 10 puffs of charcoal filtered fresh cigarette smoke (Z) (whole smoke or gas phase). n, number of cultures examined.

Effects of Cigarette Smoke on Tissue and Organ Cultures

Mitotic index in primary kidney tissue cultures exposed once to 10 puffs of charcoal filtered fresh cigarette smoke (Z) (whole smoke or gas phase). n, number of cultures examined.

DISCUSSION

The finding that in tissue and organ cultures the gas phase alone evokes essentially the same cell damage as whole cigarette smoke indicates that factors contributing to cell destruction are present mainly in the gas phase of cigarette smoke. That the gas phase of cigarette smoke also contains factors contributing to cell proliferation is suggested by our in vivo studies, which disclosed that incidence and spectrum of tumors in mice were increased over controls not only after inhalation of whole smoke but also (although to a lesser extent) after inhalation of the gas phase alone (manuscript in preparation). It thus appears that the gas phase of cigarette smoke has a pronounced biologic activity on cells and tissues in vitro and in vivo. Further experimental work, both in vitro and in vivo, concerned with comparison of biologic effects and of mechanism of action of whole, fresh cigarette smoke and of its gas phase on cell metabolism is urgently needed. This approach should be considerably assisted by the rapid development in recent years of new technics for analysis of gas phases,

Chart 1. Amount of DNA in nuclei (Feulgen microspec.) of primary kidney cultures (TB121 #5194) exposed to the gas phase of fresh cigarette smoke. (N = 544).
which have provided much information and new knowledge on
gas and vapor phase constituents of cigarette smoke (3, 4, 17).

The observation that the gas phase from the second half of
cigarettes has a stronger cell-damaging effect than that of the
first half is compatible with the findings of Newsome and Keith (16) who reported that the gas phase composition is
markedly altered when passing through a burning cigarette.
They observed that the gas mixture close to the burning cone
of the cigarette contains little or no oxygen, but relatively
large concentrations of reducing gases such as carbon monox-
oxide and methane; after passing through the burning cigarette
and in accordance with the distance from the burning cone,
the concentration of oxygen increases considerably, while car-on monoxide and other reducing gases decrease. Therefore, it
is not unexpected that cell-damaging effects of cigarette smoke
may be enhanced when cultures are under conditions of rela-
tively low oxygen and high carbon monoxide (puffs from the
ends of cigarettes, with short distance between burning cone
and exit of cigarette). An experimental exploration of the pos-
sible roles of reducing gases, particularly of carbon monoxide
present in the gas phase of cigarette smoke, for alterations of
cell metabolism appears to be of great interest, the more so since
humans as well as animals inhaling cigarette smoke show a
marked increase of carbon monoxide levels in their blood (11, 13).

The observation that charcoal filters absorb demonstrable
cell-damaging activity from whole, fresh cigarette smoke or its
gas phase is of interest in the light of the findings of Thayer
and Kensler (21), These workers reported that charcoal filters
reduce water-soluble components contained in gas and particu-
late phase of cigarette smoke condensates, which inhibit
growth of human cultured cells. This finding of reduction of

cell damage by use of a charcoal filter is in accordance with
our observation. One may be tempted to regard this result as
distinct progress in attempts to eliminate factors from ciga-
rette smoke which may interfere with cell metabolism. How-
ever, other observations of the present study indicate the need
for caution. Tissue cultures exposed once to puffs from char-
coal-filtered cigarettes, although not showing a striking cell
proliferation or DNA synthesis (Table 1, Chart 1), did reveal a
significant increase in the mitotic index about two weeks after
exposure (Table 2). That this increase was not observed at
later periods may simply be due to the fact that these cultures
were exposed only once to puffs of the charcoal-filtered ciga-
rettes. The possibility must be considered that repeated ex-
posure of cultures to these charcoal-filtered cigarettes over a
longer period of time—studies which we have just started—
may lead not only to persistence of cell mitosis, but may even
result in atypical proliferation similar to what has been found
in animals inhaling cigarette smoke repeatedly for longer
periods of time (10-12, 14, 18).

The observation that whole fresh smoke or the gas phase
from cigar tobacco evokes less and somewhat different cellular
alterations than that of cigarette tobacco would suggest that
constituents responsible for cell damage are not the same in
both types of tobacco. Further work in this direction is under
way in our laboratory.

While the model system described in this report has been
used by us so far only on a limited scale, the results already
obtained indicate its suitability as a tool for detecting differ-
ences between biologic effects of smoke from various types of

cigarettes. In view of the fact that damage to cells and cultures
can be detected within a short time after exposure, the
method appears especially useful for rapid screening of cell-
damaging factors in different types of cigarettes. Also, utiliza-
tion of this model system for long-range studies should help in
the assessment of other suspected factors in cigarette smoke,
such as those which may possibly contribute to normal and
abnormal cell proliferation, and in elucidation of their mecha-
nism of action. Of course, it is realized that this system can be
complementary to, but cannot replace, long-term chronic
inhalation studies in animals.

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vice and help in teaching us her technic for the preparation of lung
organ cultures. We are indebted to Mr. Otto Jenni, a technical engineer,
formerly with our department, for his competent construction of the
smoking machine.

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Fig. 1. Photograph of smoking machine for exposure of tissue and organ cultures to cigarette smoke. a, holder for Cambridge filter and cigarette; b, electric switch to turn on mechanism for aspiration of cigarette smoke; c, plastic box for placing cultures in a compartment; d, one of the 4 separate compartments; e, automatic counter for number of puffs; f, vacuum pump for evacuation of smoke; g, ashtray.

Fig. 2. Schematic drawing of essential parts of smoking machine. 1, holder for Cambridge filter and cigarette; 2, valve regulating admission and prevention of air flow into compartment; 3, one of the 4 separate compartments; 4, plastic box for placing cultures in a compartment; 5, chamber for equilibration of pressure; 6, connection to vacuum pump; 7, valves for control of aspiration and evacuation of cigarette smoke; 8, electromagnetic coil (solenoid) for control of valves; 9, box containing power supply, transformer, rectifier, and other electrical parts; 10, microswitch for control of 7 and 8; 11, rotating control cam (exchangeable) permitting selection of duration of cigarette puffs and intervals between puffs; 12, ashtray.

Fig. 3. Photomicrographs of 6-day-old primary mouse kidney culture. Feulgen, × 670.

Fig. 3a. Control culture not exposed to cigarette smoke.

Fig. 3b. Culture 24 hours after exposure to 10 puffs of the gas phase from first half of unfiltered cigarette (XB). Note pycnosis of nuclei.

Fig. 3c. Culture 24 hours after exposure to 10 puffs of the gas phase from first half of a charcoal-filtered cigarette (ZB). Note similarity to control culture.

Fig. 3d. Culture 24 hours after exposure to 10 puffs of the gas phase from first half of a cigar tobacco-containing cigarette (UB). Note increase in nuclear size.

Fig. 3e. Culture 24 hours after exposure to 4 puffs of the gas phase from second half of an unfiltered cigarette (XE). Note cell destruction and a few remaining pyknotic nuclei.

Fig. 3f. Culture 24 hours after exposure to 10 puffs of the gas phase from second half of a charcoal-filtered cigarette (ZE). Note similarity to control culture.

Fig. 3g. Culture 24 hours after exposure to 4 puffs of the gas phase from the second half of a cigar tobacco-containing cigarette (UE). Note increase in nuclear size.

Fig. 4. Photomicrographs of 5-day-old organ cultures of embryonic mouse lung. H & E, × 335.

Fig. 4a. Control culture not exposed to cigarette smoke. Note well-defined regular bronchial epithelium.

Fig. 4b. Culture 24 hours after exposure to 12 puffs of the gas phase from the second half of a charcoal-filtered cigarette (ZE). Note well-defined regular bronchial epithelium similar to that of control.

Fig. 4c. Culture 24 hours after exposure to 10 puffs of the gas phase from second half of cigar-containing cigarette (UE). Note marked destruction of culture, except for a few remaining foci of bronchial epithelium.

Fig. 4d. Culture 24 hours after exposure to 8 puffs of the gas phase from the second half of an unfiltered cigarette (XE). Note pyknosis and marked destruction of culture.

Fig. 5. Photomicrographs of 8-day-old organ cultures of embryonic mouse lung. H & E, × 335.

Fig. 5a. Control culture not exposed to cigarette smoke. Note well-defined regular bronchial epithelium and lung tissue.

Fig. 5b, 5c. Cultures 4 days after exposure to 12 puffs of the gas phase from second half of charcoal-filtered cigarette (ZE). Note well-preserved cultures with ciliated well-defined bronchial epithelium and well-preserved lung tissue.

Fig. 5d. Culture 4 days after exposure to 10 puffs of the gas phase from second half of cigar tobacco-containing cigarette (UE). Note considerable destruction of culture and remaining foci of bronchial epithelium and lung tissue.

Fig. 5e. Culture 4 days after exposure to 8 puffs of the gas phase from second half of unfiltered cigarette (XE). Note striking destruction of culture consisting of acellular material and nuclear debris.
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