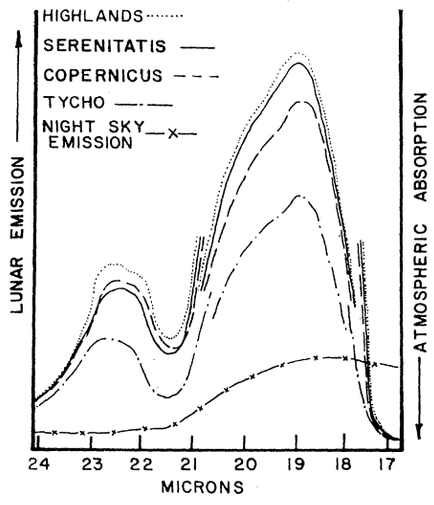

Fig. 3. Emission spectra of four lunar surface features through the 16- to 24-μ window. The amplifier gain was 1.5 between 17 and 24 μ and 2.5 between 21 and 24 μ.

42-inch (107-cm) telescope at Lowell Observatory, Flagstaff, Arizona, on 26 February 1964. Unfortunately, the humidity was unusually high (38 percent in late evening to 33 percent early morning at −5°C and −7°C, respectively) and hence the windows were not as clear as would be expected. A Perkin-Elmer model 98 single-beam spectrometer with KBr prism was modified by constructing an enlarged slit wedge, which allowed the slits to be opened to a maximum of 6 mm, and by using an Eppley Laboratories Golay detector equipped with a KBr window which was fed directly from the exit slit with a 4:1 reducing torroidal mirror. Conversion of the $f/33$ beam from the telescope was effected by using fore optics, constructed by Sinton (*6*), in which a KBr lens was used and the oscillating chopper mirror was replaced by a fixed 2.54-cm, 114-mm mirror.

The spectra recorded through the 16- to 24-μ region are shown in Fig. 3. The curves shown are direct traces, without smoothing, of the continuously recorded emission spectra from four lunar-surface features modified by the atmospheric absorption where the sample size on the moon was approximately 80 × 480 km. The noise level was less than 1 percent and the resolution better than 0.4 μ. Between 17 and 21 μ the spectra were recorded with a gain setting of 1.5, while for those shown between 21 and 24 μ the dynamic range was increased by increasing the gain to 2.5.

If the usual assumption is made that the lunar surface is emitting as a black body, then recorded spectra from various lunar regions should appear as a family of curves representing transmission through the atmosphere from a source in which the temperature had been varied. Subtraction of the atmospheric absorption or one curve from another then yields a set of smooth, almost parallel curves. This is not the case for the present observations (Fig. 3), and the discrepancy between the emissions from Copernicus and Serenitatis is the most apparent. At 19 μ the emission from Serenitatis is stronger than from Copernicus, while at 23.5 μ the relative intensities of emission are reversed. The curves do in fact actually cross.

Such an effect cannot be explained as being due to differences in surface roughness, changes in atmospheric absorption during the observations, or to some peculiar distribution of emitted energy resulting from temperature differentials within one sample area. The surface temperature in one region would have to be predominantly something less than 140°K to produce the type of difference in spectra we observe here. Black-body emission at this temperature would not be detected by our Golay detector, which was operating at about 300°K. Also the Copernicus and Serenitatis spectra were recorded consecutively and repeatedly, so that neither changes in the transmission through the atmosphere nor in instrument performance would be expected to have caused the observed anomalies.

We are, therefore, led to the conclusion that the comparative differences in intensities of the recorded spectra are due to the superimposition of differences in spectral emission from the lunar surface materials upon the absorption of the atmosphere and result from compositional differences between the lunar features examined. What these anomalies are remains to be determined.

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Cigarette Smoke: Charcoal Filters Reduce Components That Inhibit Growth of Cultured Human Cells

Abstract. *Water-soluble components of total cigarette smoke inhibit cell growth and protein synthesis by the KB line of human cells. The cytotoxic components were in both the gas phase and the particulate phase of smoke. Conventional filters of cellulose acetate reduced cytotoxicity of the particulate phase in proportion to the weight of particles trapped, that is, they did not alter the specific activity of the particulate phase. Appropriately designed filters containing activated charcoal granules selectively reduced cytotoxic components in cigarette smoke which would have appeared in both phases, although the reduction, as anticipated, occurred to a greater extent in the gas phase.*

A filter containing activated charcoal granules designed to remove gas phase components selectively from cigarette smoke has been used to study the inhibitory effects of cigarette smoke on mammalian ciliary transport activity (*1*). Our present results, another phase of this work, show that the water-soluble components of cigarette smoke condensate inhibit the growth of mammalian cells in culture; this activity is found in both phases (gas and particulate); and the charcoal granule filter reduces activity, particularly by reducing the components in the gas phase. The toxic effects of cigarette smoke, smoke condensates, or components thereof on mammalian cells in culture have been previously investigated by several workers (*2–4*). In one investigation, bubbles of "gases" from cigarettes and other combustion products were passed directly over the surface of cells with resultant "blebbing" and cell death (*5*). Studies of *Paramecium* (*6*) showed some morphological effects as well as lethality due to mainstream smoke.

Three types of 85-mm cigarettes were used to evaluate the cytotoxicity of cigarette smoke and the effects of a charcoal filter on it: type A, with a three-part filter, consisting of two sections of cellulose acetate on either side of a compartment containing charcoal granules; type B, identical to type A except that the center compartment is empty; and type C, with no filter but containing the same blend of tobacco as used in the first two. All cigarettes were smoked to a butt-length of 28 to 32 mm, requiring seven to nine 40-ml puffs per cigarette (averaging 8.1 to 8.3 puffs for all three types of cigarette).

Cigarettes were smoked by a method similar to that of Keith and Newsome (*7*), with smoke condensate collecting on glass beads in a glass trap immersed in liquid air. For separation into gas and aerosol phases, a Millipore glass-fiber absolute filter (*8*) was interposed between the cigarette and the smoke trap. Condensates were removed from the trap with water, added before thawing, the volume being adjusted to 10 ml per cigarette smoked. The thawed solution was kept overnight at room temperature. Aerosol-phase materials from the finely dispersed filters on which they were trapped were extracted in water. Further dilutions were done in tissue culture medium. The weights of condensate were determined by the increase in the weight of the trap (or filter), with a correction for condensation of atmospheric water. These values are approximate in that they represent small increases over large tare values.

The KB tumor cells were cultured by the method of Eagle and Foley (*9*) as modified by Smith *et al.* (*10*). Serial twofold dilutions of the water-soluble materials were incorporated in the medium (Eagle's) before inoculation. After 72 hours, growth was measured by increase of cell protein. Control tubes show approximately 8- to 12-fold growth over the original inoculum in this system. The concentration of cigarette material which would produce 50 percent inhibition of growth (ID_{50}) was calculated on the basis of "cigarettes per milliliter." The number of milliliters of solution of this concentration which can be prepared from one cigarette is reported in Table 1 as ID_{50}'s per cigarette and is equivalent to the reciprocal of the ID_{50}. "Specific activity" is calculated as the number of such ID_{50} units per milligram of solids.

Several statements may be made about the data summarized in Table 1:

1) The unfiltered cigarette (type C) had the highest activity, approximately 60 percent of which was trapped as aerosol phase.

2) Filtration with cellulose-acetate filters (type B) reduced the total activity with no effect on that in the gas phase, but there was a significant decrease ($p < .005$) in the activity of the particulate (aerosol) phase. The reduction in specific activity of the aerosol (ID_{50} per milligram of condensate) was not significant, the decrease in activity being roughly parallel to the decrease in total solids.

3) The addition of charcoal granules to the filter (type A) reduced the activity in the total smoke by about 60 percent compared with the nonfilter cigarette, or about 50 percent compared with the cigarette with the cellulose-acetate filter, both of these differences being highly significant.

4) As might be expected for a material which adsorbs gaseous substances, the charcoal significantly reduced the activity by approximately 60 percent in the gas phase (when compared with either of the noncharcoal cigarettes) and significantly lowered the specific activity. Although there are reservations regarding the validity of the weight of the gas phase, this result would indicate the removal of relatively small amounts of quite active material.

5) The charcoal significantly reduced the total activity in the aerosol phase (a less expected result), and the specific activity was also significantly reduced. The overall reduction, compared with the nonfilter cigarette, was also approximately 60 percent, of which a 35-percent portion is accounted for by the cellulose-acetate filter, so that the remaining 25 percent may be attributed to effects of the charcoal.

The sum of the separate activities of the gas and aerosol phases explains the activity of the total smoke very well, with recoveries of 99 to 107 percent for the averages. When individual corresponding preparations are com-

Table 1. Inhibition of growth of KB cells by gas phase, aerosol phase, and total condensate of cigarette smoke. The results are the means of six sets prepared for each factor compared. The numbers in parentheses indicate the range.

Factor compared	Type			Results of *t*-test (*p*-values)		
	A	B	C	A vs. B	A vs. C	B vs. C
			Total smoke			
ID_{50} per cigarette	318 (295–370)	654 (590–715)	768 (665–910)	$< .001$	$< .001$	.01–.025
Condensate per cigarette (mg)	39.8 (25.3–47.1)	43.3 (36.8–49.6)	53.8 (51.7–57.9)	.2–.4	.001–.005	$< .001$
ID_{50}/mg condensate	8.3 (6.7–12.1)	15.2 (14.3–16.0)	14.3 (11.9–17.4)	$< .001$	$< .001$	.2–.4
			Gas phase			
ID_{50} per cigarette	143 (100–205)	351 (300–400)	349 (310–385)	$< .001$	$< .001$	>0.50
Condensate per cigarette (mg)	14.3 (10.7–16.7)	13.5 (11.0–16.7)	14.3 (9.4–16.0)	> 0.50	>0.50	>0.50
ID_{50}/mg condensate	9.9 (8.9–12.3)	26.2 (23.0–30.4)	25.0 (22.2–33.0)	$< .001$	$< .001$	>0.50
			Aerosol phase			
ID_{50} per cigarette	189 (145–270)	297 (250–335)	463 (345–625)	$\sim .001$	$< .001$	.001–.005
Condensate per cigarette (mg)	24.4 (18.7–28.6)	26.8 (22.1–32.0)	34.7 (30.8–41.1)	.2–.4	$\sim .001$	$\sim .005$
ID_{50}/mg condensate	7.7 (6.5–9.5)	11.1 (10.5–11.6)	13.5 (8.4–17.6)	$< .001$	.001–.005	$\sim .10$

pared there is a somewhat larger spread, as follows: charcoal filter cigarette, 80 to 128 percent; plain filter cigarette, 87 to 108 percent; and unfiltered cigarette, 86 to 137 percent.

The reduction of activity in the aerosol phase by the cellulose-acetate filter is explained by its property of reducing total smoke solids, but the additional effect of the charcoal requires further consideration. This reduction is due to removal from the original gas phase of materials which would otherwise appear in the smoke-collecting system on the absolute filter. Their presence in the aerosol sample, when there is no charcoal filter, might be due to absorption by aerosol material either before or after deposition on the absolute filter, or possibly to adsorption (or absorption or condensation) on that filter without interaction with the aerosol material.

Since the gas phase of cigarette smoke contains a large number of materials, many of unknown biological activity, and since many of them are adsorbed by activated charcoal, it is not obvious what the growth inhibitor would be. We have found that the growth inhibitory potencies of some components (hydrogen cyanide, acetaldehyde, acrolein) and their concentration in smoke are not sufficient of themselves to explain the observed activity, although these components may be contributory. Neither nicotine nor phenol inhibits growth appreciably in measure consistent with their concentration in smoke.

When cells were cultured directly on cover-slips in the presence of smoke preparations and stained after growth, there were no pronounced morphological changes in the surviving cells, even at inhibitory concentrations of total condensate, aerosol phase, or gas phase.

As with all laboratory studies of the effects of tobacco products on nonhuman or biological systems in vitro, the significance of the aforesaid results for the human smoker cannot be estimated with any degree of certainty. However, the basic biochemical similarity of animal cells of diverse origin gives some ground for comparison. In reviewing the experience of many workers with cell lines of diverse origins, Foley states that reports on the differential response of cell lines to inhibitory agents indicate "little in the way of unequivocal evidence for significant and predictable differences among the response of cells to inhibitors *in vitro*" (*11*). In work specifically related to cigarette smoke, Pace and Elliott (*3*) showed that the growth-inhibiting effects of acetaldehyde on HeLa cells (a human tumor cell similar to the KB line) were essentially similar to those on strain L mouse fibroblasts and mouse liver epithelial cells. For these reasons, the KB cell may be a valid presumptive means of detecting general growth-inhibiting activity in cigarette smoke and evaluating methods for its removal.

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Systematic Error in Leaf Water Potential Measurements with a Thermocouple Psychrometer

Abstract. *To allow for the error in measurement of water potentials in leaves, introduced by the presence of a water droplet in the chamber of the psychrometer, a correction must be made for the permeability of the leaf.*

In 1958 Richards and Ogata (*1*) described a thermocouple psychrometer which they used mainly to measure the water potentials of soils. Later Ehlig (*2*) used it to measure the water potential of leaf tissue. The psychrometer consists of a pair of thermocouple junctions in a closed chamber. One junction is kept wet by a droplet of water held in a small ring; the other is kept dry and at chamber temperature. The instrument is calibrated with salt solutions, of known water potential, carried by filter paper lining the wall of the chamber. Use of this calibration technique for measuring the water potential of leaves implies that the temperature depression of the wet bulb is related to the potential within the leaf exactly as it is related to the potential in the calibrating solution. Vapor continually diffuses from the wet junction to the leaf, if the leaf is not saturated. If the resistance of the leaf to the diffusion of water vapor is not negligible, the vapor pressure at the external leaf surface will be significantly greater than that within the leaf. In this case the temperature depression of the wet bulb will depend on the permeability of the leaf to water vapor, as well as on the potential within the leaf. When leaf resistance is not negligible, extraneous sinks for water vapor in the chamber, such as salt contaminating either the leaf or the chamber, could also change the relation between depression of the wet bulb and the potential.

If we consider only the error resulting from the presence of the water drop on the wet junction, an expression can be derived for the difference between the water potential inside the leaf and that indicated by the psychrometer when a spherical psychrometer chamber is lined by leaf tissues with different permeabilities to vapor. Let the radius of the chamber be r_c and the radius of the drop at the wet junction be r_a. The rate of mass transfer (dm/dt) of vapor from the leaf into the chamber is

$$\left(\frac{dm}{dt}\right)_{leaf} = a\,k\,(e_{leaf} - e_{air}), \qquad (1)$$

where k is leaf permeability per unit area, a is area of the the leaf, and the e's are the vapor pressures in the leaf and in the air adjacent to the leaf. Evaporation from the drop is also controlled by the gradient in vapor pressure away from it, but at steady state this gradient is determined by the rate of heat flow, dQ/dt, to the droplet:

$$\left(\frac{dm}{dt}\right)_{drop} = \frac{1}{L} \cdot \frac{dQ}{dt} \qquad (2)$$

where L is the latent heat of vaporization.

Heat can flow to the drop by conduction along the thermocouple wires and through the air in the chamber, as well as by radiation. For chambers of the dimensions used in these psychrom-