antipodal distribution of continents and oceans could easily have been caused by a random process, whereas the results described in this paper show that there is less than 1 chance in 14 that the present distribution has been caused by a random process. Thus there is some disagreement between the two methods.

One factor which Evison and Whittle may not have accounted for fully is the possibility of the continents overlapping each other. They attempted to allow for this, but it is not clear that the method they used was adequate. Their method seems to give a value for the mode (the most likely value) which is too high. For instance, in the case where two circular continents were used, of area 0.25 and 0.2 of the earth's surface, Evison and Whittle's method gives a mode of 75 percent, which is impossible for circular continents (Fig. 2). Even with triangular continents of this size, the *maximum* amount of continent opposite ocean is scarcely greater than 75 percent. Thus Evison and Whittle's method gives a mode which is much too high. In order to check that Evison and Whittle's method gives consistently high readings, a set of Monte Carlo calculations was done for six circular continents of equal size. Each continent was made 0.007, 0.014, 0.028, and 0.056 of the earth's area, in turn. In each of the four calculations, the continents were placed on the sphere 2000 times. The means, medians, and modes were calculated for each. The results are shown in Fig. 3. As can be seen, the most likely value calculated with Evison and Whittle's method becomes progressively larger than the values of the mean, median, and mode from the Monte Carlo method as the size of the continents is increased. It may be concluded that Evison and Whittle's method gives a good estimate of the mean percentage when the total continental area is small, but values which are too large when the continental area is an appreciable fraction of the earth's area.

The conclusion which can be drawn from this work is that there is less than 1 chance in 14 that the present antipodal distribution of continents and oceans is the result of a random process. Thus it appears probable that a nonrandom process, such as the presence of large-scale convection currents in the earth's mantle, has caused the present distribution of continents over the earth's surface.

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Ciliastatic Components in the Gas Phase of Cigarette Smoke

Abstract. *The gas phase of cigarette smoke, separated into its components by gas chromatography, was passed across a ciliated specimen. The acetaldehyde, acrolein, and hydrogen cyanide produced strong inhibition of the ciliary beat. A filter which removed most of the acetaldehyde and acrolein from smoke did not reduce the inhibitory effect of the gas phase of that smoke, whereas a filter that removed most of the hydrogen cyanide did reduce inhibition.*

The inhibitory effect of cigarette smoke on cilia movement was first demonstrated more than two decades ago. In recent years attempts have been made to identify and eliminate the components of smoke which cause ciliastasis. A number of compounds known to be present in cigarette smoke have been reported to affect the cilia of such animals as rabbits, cows, clams, frogs, and cats. These compounds include the acids, formic, acetic, propionic (*1*), and hydrocyanic (*2*), and the aldehydes, formaldehyde, acetaldehyde, propionaldehyde, acrolein, and crotonaldehyde (*3*).

The activity of a compound when pure is not necessarily its activity in the presence of smoke, as Bernfeld and co-workers (*4*) showed with phenol. They found that while pure phenol vapor showed little or no ciliastatic activity, it was markedly ciliastatic in the presence of smoke. Ideally then, one would like to remove each component of smoke selectively to determine its part in ciliastasis.

The work presented here deals only with the gas phase of cigarette smoke which is separated from the particulate phase by a glass-fiber filter. This work was planned to obtain more information about the ciliastatic components of the gas phase and to determine the changes in activity of the gas phase when some of the ciliastatic agents are selectively removed. (*Ciliastasis* herein means inhibition of the ciliary beat, rather than complete loss of motion.)

The Lamellibranch, *Anodonta cataracta*, which is a freshwater mussel, was chosen as test specimen. For exposure to smoke, 4-mm by 20-mm sections of the reflected lamellae of the inner gill plates were placed in temperature-controlled physiological solutions (*5, 6*). The method for measuring the ciliary beat is well known and is used by many investigators (*5, 7*). A stroboscope was synchronized with the metachronic wave of in vitro mussel cilia permitting quantitative measurement of any change in ciliary frequency. The frequency was measured with an error of less than 1 percent.

The puffing machine, consisting of a stainless-steel piston attached to a motor, drew a 35-ml puff in 2 seconds at a frequency of one puff per minute. It automatically diluted the puff with air to 100 ml and pushed it across the specimen. Eight puffs were taken on each cigarette. The glass-fiber filter used to separate the gas phase from the particulate phase was about 2 mm thick and 45 mm in diameter. Touey has reported that a filter of this type removes 99 percent of the liquid/solid phase of cigarette smoke and that the low-boiling aldehydes and ketones are not retained by the filter (*8*). Since the ciliary beat is sensitive to changes in temperature, the specimen temperature was controlled at 30°C to 31°C by passing water from a constant-temperature tank through coils in the base of the exposure chamber which was mounted on a microscope.

The gas phase was separated into two fractions, condensable gases and permanent gases, distinguished by whether they condense at dry ice/alcohol temperature. To determine the activity of the permanent gases as re-

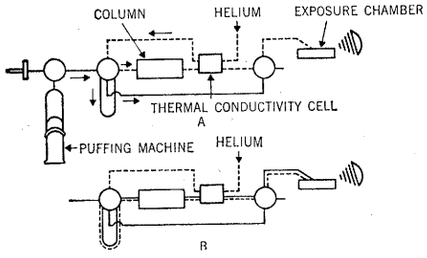

Fig. 1. Apparatus for testing condensable gases.

gards cilia, a cold trap was placed between the smoking machine and the specimen to remove the condensable gases. The cold trap was a ¼-inch by 12-inch (⅔-cm by 30½-cm) copper U-tube containing 0.8 g of Chromosorb P coated with 10 percent (by weight) Ucon Oil 280-X. A dry ice/isopropyl alcohol bath was used for cooling. A tube maintained at 26°C was included to insure that the permanent gases returned to room temperature before passing across the specimen.

The permanent gases could be passed across the specimen in the same way as the total gas phase, that is, eight puffs from each cigarette at one puff per minute, and the frequency of the cilia could be measured after each puff. By this method the permanent gases showed little or no ciliastatic activity. By the fourth puff there was no reduction at all in the ciliary beat and there was less than 5 percent reduction by the eighth puff. In contrast, the total gas phase had reduced ciliary beat by 40 percent by the fourth puff and 55 percent by the eighth. This would appear to eliminate such compounds as 1,3-butadiene, NO, and CO as major contributors to ciliastasis, since they appear in the permanent gases.

A method was not available for determing the activity of the condensable gas phase on a puff-by-puff basis. For analysis of this fraction, we devised a gas chromatographic technique which employed dual detectors—a thermal conductivity cell and a clam specimen (Fig. 1*A*). The smoke was puffed through the dry ice–cooled trap, where the condensable gases remained. The permanent gases were either vented to the atmosphere or passed across the specimen. When smoking was complete the condensable gases were flushed by a boiling water bath onto the chromatographic column (Fig. 1*B*). After passing through the column they were carried through the thermal conductivity detector and across the ciliated specimen. The chromatographic column consisted of a 3/16-inch by 20-inch copper tube containing 60 to 80 mesh Chromosorb W coated with 10 percent Ucon Oil 280-X.

Several regions of the condensable gases thus fractionated were found to be active. The dotted line in Fig. 2 represents a ciliastasis curve plotted on a gas chromatogram. The two curves were recorded simultaneously from the same gases. By superimposing the curves in this way, the compounds which were passing across the specimens when inhibition occurred were observed. There were four regions of interest, a small peak (*A*), and a larger peak (*B*) in the acetaldehyde/isoprene region with rapid and total recovery, sudden permanent paralysis (*C*) in the acetone/acrolein region, and strong inhibition (*F*) in the HCN/benzene region with no significant recovery.

A complete ciliastasis curve could not, of course, be run on a single specimen, owing to paralysis at *C*. To measure the activity of the gases which eluted after this region, we made runs in which we vented to the air everything through region 2. The initial rapid stimulation and leveling at *D* was due to the introduction of a helium atmosphere where there had been air. In the acetonitrile area (region 3), the metachronicity of the wave was usually disrupted but was quickly recovered.

Care must be taken, however, however, in attributing the cause of a ciliastasis peak to the compounds which were eluting at that time: delayed reactions are quite possible. To eliminate this problem, runs were made in which the gases representing a single chromatographic peak were vented before they reached the clam. If ciliastasis were due to the vented compounds, a peak would be eliminated from the ciliastasis curve. When acetaldehyde and HCN were vented, the ciliastasis curves shown in Fig. 3 were obtained. As expected, the second ciliastasis peak in Fig. 2 disappeared and paralysis still occurred at *C*. The acetaldehyde peak has been shown by mass spectrometry to be 95 percent pure. When acrolein was vented, there was no paralysis or inhibition at C. The peak representing acrolein is about 75 percent pure. The venting of acetone and other nearby compounds had no noticeable effect. The venting of air eliminated the small peak at *A*. The air peak contains more than just air, but separate experiments

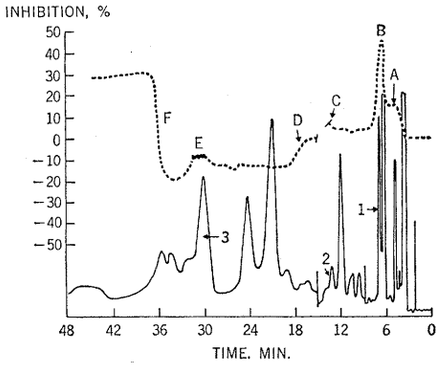

Fig. 2. Chromatogram of condensable gases with ciliastasis curve. (1) Acetaldehyde/isoprene. (2) Acrolein. (3) Acetonitrile.

showed that air alone can account for the peak. The ciliary beat is quite stable in air; it merely beats at a lower frequency than in helium. When HCN was vented (Fig. 3) inhibition did not occur in the usual place but was found when the gas stream was turned back across the clam. This effect may possibly be due to extensive trailing of HCN. The chromatographic method, then, pointed mainly to acrolein and HCN. Due to the rapid recovery of the clam from acetaldehyde, it is doubtful that this compound would contribute significantly to total ciliastasis in these short-term tests.

Filters were prepared which removed the major part of the compounds of interest. One contained a polymeric hydrazide; another contained a common base. These are shown in Table 1 along with a cellulose acetate filter and two carbon filters. All of the compounds in the filters were bonded to cellulose acetate tow. The filters were 20 mm long except for the 100-mg carbon filter, which was 10 mm long. The resistance to draw of the

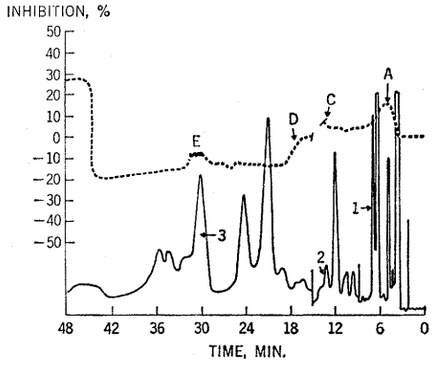

Fig. 3. Chromatogram of condensable gases with acetaldehyde and HCN vented. (1) Acetaldehyde/isoprene. (2) Acrolein. (3) Acetonitrile.

200-mg carbon filter was somewhat greater than that of the others.

Also shown in Table 1 are the amounts of ciliastatic materials in the condensable phase that the filters removed. Formaldehyde is included here because it has been reported to be ciliastatic, but its activity cannot be correlated by vapor phase chromatography (VPC) with that of the other compounds because it does not elute from the chromatographic column. The HCN (*9*, *10*) and NO, NO_2 (*11*) determinations were done by colorimetric methods described in the literature. Formaldehyde was determined by gas chromatographic analysis of its 2,4-dinitrophenylhydrazone (*12*). Acetaldehyde and acrolein were determined by a VPC method (*13*).

The effects of the five filters on ciliastasis are shown in Fig. 4. The data was analyzed statistically by the incomplete block method. There is no significant difference between points within the same circle. Points that fall in overlapping areas are not significantly different from points in either of the overlapping circles. At the 95-percent confidence level there was little significant difference in the early puffs. By the time the eighth puff was reached, however, differences could be seen. The smoke vapors through the basic filter were less ciliastatic than any of the others shown in the figure. The smoke through the plain cellulose acetate filters and the 100-mg carbon filters was less ciliastatic than the control, but no significant difference could be seen between the hydrazide and the control. The smoke through the 200-mg carbon filter was less ciliastatic than any of the others.

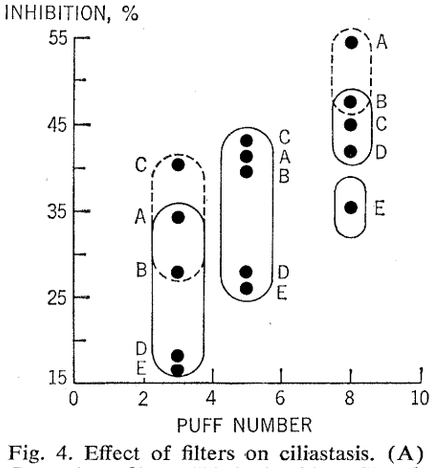

Fig. 4. Effect of filters on ciliastasis. (A) Control-nonfilter; (B) hydrazide; (C) cellulose acetate; (D) carbon; (E) base.

In conclusion, hydrogen cyanide appears to be an important contributor to the activity of smoke. This was shown partly by the chromatographic technique when the HCN strongly inhibited the cilia and partly by the sharp reduction in ciliastasis when most of the HCN was filtered from the smoke by the filter which contained base. Acetaldehyde seems to contribute little to the activity of smoke. Removal of most of it by the hydrazide filter showed no change in ciliastasis. This is not surprising, since it was noted in the chromatography of smoke that the cilia rapidly recovered from the effects of acetaldehyde. When the whole gas phase is smoked over the clam, the cilia would have time to recover from acetaldehyde before ciliastasis is recorded. And in fact some rapid recovery is seen immediately after a puff is taken.

Acrolein presents an interesting problem. The chromatographic method pointed to acrolein as a powerful ciliastat—at least as powerful as HCN; yet removal of most of the acrolein by the hydrazide filter had no effect on ciliastasis while removal of most of the HCN markedly reduced ciliastasis. One possible explanation is that while pure acrolein is a strong ciliastat, it is deactivated in the presence of smoke. Another explanation is that the acrolein passing across the specimen was more concentrated in the VPC method than in the puffing method, and the VPC method therefore did not give a true picture of its importance. Another possibility is that perhaps it is necessary to remove more than 60 to 70 percent of the acrolein to produce a reduction in ciliastasis; that is, the 25 μg acrolein in the filtered smoke may be about as effective as the 70 μg present in unfiltered smoke.

Table 1. Removal of gas phase components from cigarette smoke by filters.

Filter additive	Component removed (%)				
	NO, NO_2	HCHO	CH_3CHO	CH_2=CHCHO	HCN
None	< 10	16	0	0	0
Base	< 10	48	0	0	68
Hydrazide	< 10	61	82	66	0
Carbon, 100 mg	< 10	79	86	95	56
Carbon, 200 mg	< 10	92	> 95	> 95	80

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1 August 1966

North Atlantic Deep-Sea Fertility

Abstract. *Observations have been made on two cruises in the North Atlantic in which large numbers of microscopic, unicellular flagellates have been found throughout the aphotic zone below 1000 meters. Preliminary measurements also indicate the uptake of dissolved organic substances, suggestive of an apparently viable, actively metabolizing community at these depths.*

The aphotic zone of the oceanic water column is generally considered to be sparsely populated with phytoplankton. Phytoplanktologists agree that organisms exist throughout the depths, but that both their biomass and rate of production are negligible relative to that in the euphotic layers. The view that aphotic microorganisms are insignificant can be attributed to three major reasons: first, those deep-sea expeditions concerned with this problem found few cells in abyssal samples (*1*); second, heterotrophy, so necessary for existence at these depths, has not been demonstrated to be a widespread nutritional mode in phytoplankton; third, the quantity of both dissolved and particulate organic ma-