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9. Research supported by NSF grant GA 36092 and the Graduate School, University of Minnesota. I thank P. Hudleston for his penetrative review and numerous colleagues whose continued interest and curiosity stimulated this work.

2 April 1974

Long-Lived Chemiluminescence in Cigarette Smoke

Abstract. *Cigarette smoke contains high concentrations of unstable molecules that react with oxygen to produce chemiluminescence. The chemiluminescent activity is concentrated in the aerosol phase that can be absorbed on glass-fiber filters and extracted into organic solvents. Cigarette smoke in* N,N*-dimethylformamide produces a long-lasting luminescence visible to the dark-adapted eye. We have demonstrated the oxygen dependence and have measured the kinetics, activation energies, emission spectra, and absolute photon intensities of this chemiluminescence. The total light emission from a single puff (35 cubic centimeters) of cigarette smoke is greater than* 10^{12} *photons. There was a significant correlation between smoke chemiluminescence and tar content. It is suggested that the chemical production of electronically excited states of aromatic hydrocarbons is equivalent to photoexcitation in the promotion of the carcinogenicity of these agents.*

If one has a phototube with good sensitivity and very low dark noise, it is possible to measure light emission from most chemical reactions and to a degree from most biological oxidations (*1*, *2*). It was therefore of great interest to verify that a low-level luminescence issued from cigarette smoke exhaled into a glass vial (*3*).

We now report that this light emission is not a trivial process. Cigarette smoke contains high concentrations of unstable molecules that react with molecular oxygen to produce electronically excited states. Under proper conditions the photon yield of this chemiluminescence can be increased many orders of magnitude. The aerosol fraction of a single puff (35 cm^3) of cigarette smoke contains sufficient chemiluminescent precursors so that when they are extracted into organic solvents at 37°C a persistent chemiluminescence becomes visible to the dark-adapted eye.

Cigarettes were puffed (35 cm^3 in 2 seconds) through Whatman GF/C or GF/F glass-fiber filters mounted in Millipore Swinex-25 filter holders. The GF/C and GF/F glass-fiber filters and Millipore filters (pore size, 0.1 μm) were of essentially equal efficiency in collecting the portion of aerosol giving rise to the observed chemiluminescence. The filter was immediately removed from the holder, placed in 5 ml of organic solvent [*N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or dioxane] in a glass vial, and agitated for 30 seconds on a Vortex mixer. Chemiluminescent intensities were measured in calibrated photometer geometries with the use of d-c amplifier techniques and single photon counting. Absolute photon intensities were measured by direct comparison with a ^{14}C-activated luminous reference source previously calibrated for the absolute photon emission of dinoflagellate bioluminescence (*4*). Measurements were also made in the liquid scintillation counter. The phototube gain was set for single photon counting and the counter was operated in the "coincidence off" mode (*5*). All operations were performed in the dark or in dim red light. The smoke extracts are subject to light-induced chemiluminescence. This is probably a pathway that could account for the reported photo-induced degradation of cigarette smoke extracts (*6*). Glassware, subject to considerable photo-excited phosphorescence, was also kept in darkness prior to use.

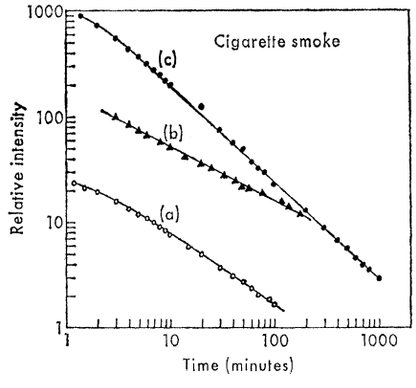

Fig. 1. Log-log plot of relative intensities of chemiluminescence versus time of (a) cigarette smoke aerosol deposited on a glass-fiber filter, (b) a 5-ml DMF extract of cigarette smoke, and (c) a 1 : 100 dilution of an initial extract. The relative scales apply to each curve separately and are not intended to compare the absolute photon intensities measured for (a), (b), and (c).

There is a requirement for molecular oxygen. Degassed smoke extracts in DMF and DMSO solvents were prepared under freezing and thawing conditions in a vacuum sufficient to observe a substantial photoreduction (bleaching) of aqueous solutions of flavin mononucleotide at 436 nm. Upon readmission of oxygen, chemiluminescent intensities of smoke extracts increased by factors of 20 to 50. A direct interaction with molecular oxygen would be consistent with the large free-energy requirements for chemical luminescence (*7*).

The apparent Arrhenius activation energies for smoke chemiluminescence in DMF, DMSO, and dioxane solvents were between 12 and 15 kcal $mole^{-1}$ in the temperature range from 10° to 60°C. It is also possible to collect smoke samples on glass-fiber filters, freeze them on Dry Ice, and reactivate the chemiluminescence a day or two later by inserting the filter into DMF at room temperature.

The reactions giving rise to the chemiluminescence are complex, as shown in Fig. 1, in which we have plotted the logarithm of chemiluminescent intensity as a function of logarithmic time for smoke aerosol on a glass-fiber filter and for smoke extracts in DMF solvent. For relative measurements of the chemiluminescence of smoke extracts we arbitrarily measured the intensity at 90 seconds after the delivery of the smoke sample to the glass-fiber filter. While the order of the chemiluminescent reaction was higher than first order with respect to time, the variation in chemiluminescent intensity with dilution was approximately linear below concentrations in which self-absorption in the

colored initial extract was important.

A significant number of reactive vapor phase molecules pass through both Millipore and glass-fiber filters. When 35 cm^3 of filter-passing vapor from a freshly puffed cigarette was bubbled through 1- to 2-day-old smoke extracts, an additional chemiluminescence was produced which was not seen when the vapor was bubbling through DMF alone, but the intensity was less than 10 percent of that observed for the original smoke extract. As a check, we condensed this filter-passing vapor in a cold "finger" in a mixture of Dry Ice and acetone, extracted it into DMF, and added this to old smoke extracts. The increase in chemiluminescence intensity was no greater than that observed when the vapor was bubbling into DMF alone. The fact that no significant chemiluminescence was observed upon delivery of the vapor to DMF alone implies that, while the filter-passing molecules are not themselves chemiluminescent, they are reactive enough to stimulate chemiluminescence in smoke extracts. We conclude that the major source of smoke chemiluminescence is contained in the aerosol deposited on the glass-fiber filter.

The observed chemiluminescent intensities of smoke extracts in DMF were increased by factors of 3 to 10 by the additions of fluorescein to final concentrations of $10^{-4}M$. Since these additions also changed the order of the decay kinetics with respect to time, the reaction is probably more complex than a sensitized chemiluminescence. Conversely, the addition of small amounts of ethylene glycol ($0.4 \times 10^{-3}M$) to solvent extracts of smoke quenched the chemiluminescence by approximately a factor of 10. This would be consistent with quenching of a radical precursor to chemiluminescence. A similar effect was observed after addition of small amounts of water.

The chemiluminescent emission spectra of cigarette smoke in its aerosol form and in DMF solution are shown in Fig. 2, a and b, respectively (*8*). These were measured by means of Corning glass color filters and narrow-band interference filters for the aerosol and with a 1-m focal length f/3 spectrometer (*9*) in combination with a low-noise phototube (EMR 541N-01-14) for the smoke extracts. The data of Fig. 2b were corrected for the spectral response of the phototube. The extremely broad chemiluminescent emission spectrum in DMF is typical of the fluorescence observed from smoke extracts. The distortion evident in the aerosol emission spectrum is most likely due to self-absorption of blue light within the highly concentrated droplets of the disperse phase. The loss of the blue region of the spectrum would be consistent with the broad excitation spectra for fluorescence of these extracts, which extends from the blue down to the long-wavelength ultraviolet (*8*). The smoke chemiluminescence emission spectra extend to longer wavelengths than the spectra of the ^{14}C-activated luminous standard or of the dinoflagellates. Since the spectral efficiencies of the phototubes (EMI 9789QA) in the chemiluminescent intensity measurements fall off rapidly for wavelengths above 500 nm (S-11 photocathode response), the absolute values of photon intensities and total emissions reported may be underestimated by as much as a factor of 2.

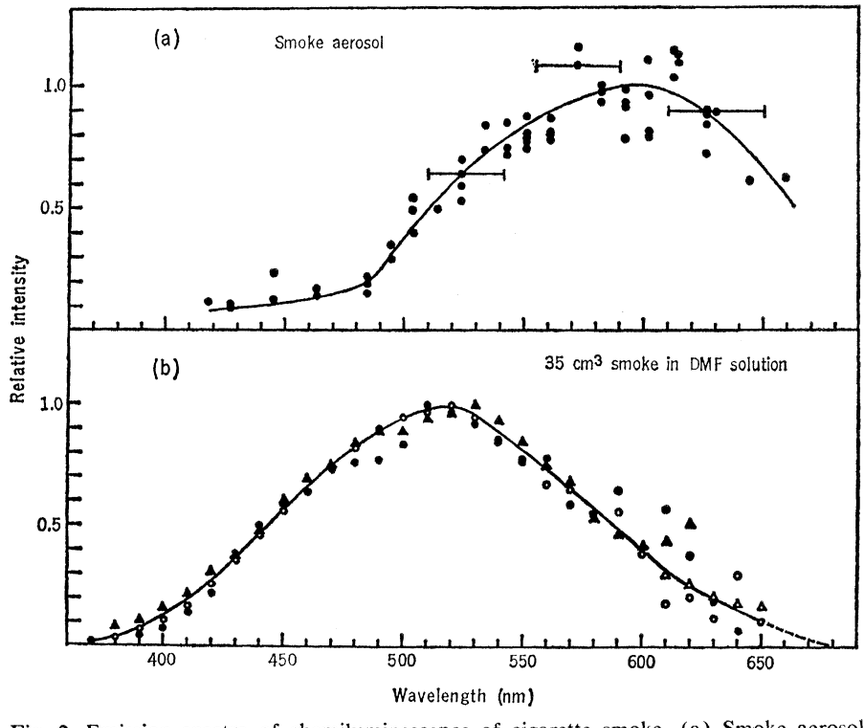

Fig. 2. Emission spectra of chemiluminescence of cigarette smoke. (a) Smoke aerosol in air, contained within a glass vial. The horizontal bars are representative of the range of wavelengths about a central wavelength transmitted by the interference filters; (b) a single puff of smoke extracted into 5 ml of DMF (●, △, and ▲), further diluted by one third (○).

We measured the chemiluminescent intensities of DMF extracts of smoke from 17 brands of cigarettes having a tar content ranging from 1 to 30 mg per cigarette (*10*). The intensity at $t = 90$ seconds (where t is time) for a 35-cm^3 puff of smoke from a fresh cigarette, within the initial 15 mm of length, could be measured for the cigarettes from a single pack with coefficients of variation between 30 and 40 percent. For any individual cigarette, puffs of 35 cm^3 of smoke assayed at the beginning, middle, and end of the burning length were indistinguishable from one another within the relative precision given. Of these there were 12 brands of filter cigarettes that were also assayed without their filters, making a total of 29 different cigarette types. Those brands with filtered tips were labeled f^+; in each case, when the smoke chemiluminescence was measured with the filter cut off, the symbol f^- was used. There was a significant correlation between the chemiluminescent intensity at $t = 90$ seconds and the tar content of the cigarette brand ($r = .606$; $N = 17$). Student's t-test of the paired statistic $f^- - f^+$ was extremely significant (that is, $t = 3.96$; $N = 12$), which confirmed that the removal of tars by the filters also removes chemiluminescent activity.

At high concentrations of reactants in the condensed aerosol droplets there can be considerable radical recombination and limiting oxygen concentrations. Both of these, as well as self-absorption of the emitted chemiluminescence, could be responsible for the low

intensity of chemiluminescence observed from cigarette smoke directly. There is considerable self-absorption even in the initial DMF extracts. Therefore, absolute measurements of intensities and integrated light emissions were made with 1 : 100 dilutions of the initial extract. The mean initial intensity of chemiluminescence (at time 0) from a 35-cm^3 puff of cigarette smoke was 8×10^9 photons per second. As can be inferred from Fig. 1, light emission from cigarette smoke is measurable for days. At room temperature, the intensity decays by roughly a factor of 1000 over a period of 1000 minutes. For the determination of total light emission we arbitrarily integrated intensity over a 24-hour period. On this basis, a single puff of cigarette smoke emits more than 10^{12} photons. An interesting mnemonic is that this is approximately the number of photons emitted by a firefly in a single flash (*11*) (see also Fig. 3).

Cigarette smoke is not alone in exhibiting spontaneous chemiluminescence. We have observed a significant chemiluminescence in the side-stream smoke of cigarettes (*12*), in cigar smoke, pipe tobacco smoke, and the smoke of dried oak, maple, dogwood, and tea leaves. Cellulose smoke from cigarette paper or from wood shavings produced much less chemiluminescence, but a significant chemiluminescence can be measured from particulates captured on GF/F filters from air samples taken in areas where smoking has occurred.

The aerosol portion of cigarette smoke was captured on filters in order to eliminate the possibility that luminescence was caused by phosphorescence of the glass vial, produced by surface recombination or neutralization of smoke radicals. Enhancement of the luminescence intensity upon extraction into organic solvents eliminated the phosphorescence of pyrolysis-excited aggregates or particulates as another possible source of luminescence. The relatively low apparent energies of activation measured in the three solvents are characteristic of intermediate hydroperoxides or endoperoxides which decompose to produce excited state product molecules. The enhancement by oxygen indicates that chemiluminescent precursors produced in the burning tip of the cigarette do not combine with oxygen immediately upon being formed. They are most likely unstable radicals, inferred by the observation that fresh cigarette smoke contains more free radicals than aged smoke does (*13*). There is no statistically significant correlation between total free radicals in cigarette smoke condensates and carcinogenesis (*12*). However, if the excited electronic states given in this report are in any way correlatable to the promotion of carcinogenesis, only the unstable radicals that give rise to chemiluminescence would be of significance, and a correlation between the concentration of total free radicals and carcinogenesis might not be expected.

The addition of *t*-butoxide ions (*14*) to DMF and DMSO extracts of cigarette smoke further enhances the intensity of chemiluminescence. The strong organic base accelerates the oxygen attack on the smoke radicals and the oxygenation of the polynuclear aromatic hydrocarbons present in the extract. There is no necessity to invoke a singlet oxygen intermediate in the spontaneous chemiluminescence of smoke extracts. The products of pyrolysis contain sufficient concentrations of unstable radicals that can react with ground state oxygen directly or that can produce radicals of other polynuclear aromatic hydrocarbons. The apparent kinetic order of the luminescence is consistent with a radical chain reaction mechanism.

The potential chemiluminescence of polynuclear aromatic hydrocarbons (PAH's), including the carcinogens dibenzanthracene, dimethylbenzanthracene, and benzopyrene (which are also found in cigarette smoke), was demonstrated by Anderson (*15*) many years ago. In a brilliant paper, he predicted with great insight that there should be a chemiluminescence accompanying the metabolic hydroxylation of PAH's and that this chemiluminescence might be the causal agent of malignant transformation. This was the original concept that "dark" chemical reactions, and particularly biochemical reactions, could result in excited states and products identical to those produced by direct photoexcitation; the latter has been shown to promote the mutagenicity and carcinogenicity of aromatic hydrocarbons (*16*). Anderson's concept has been restated and demonstrated by a number

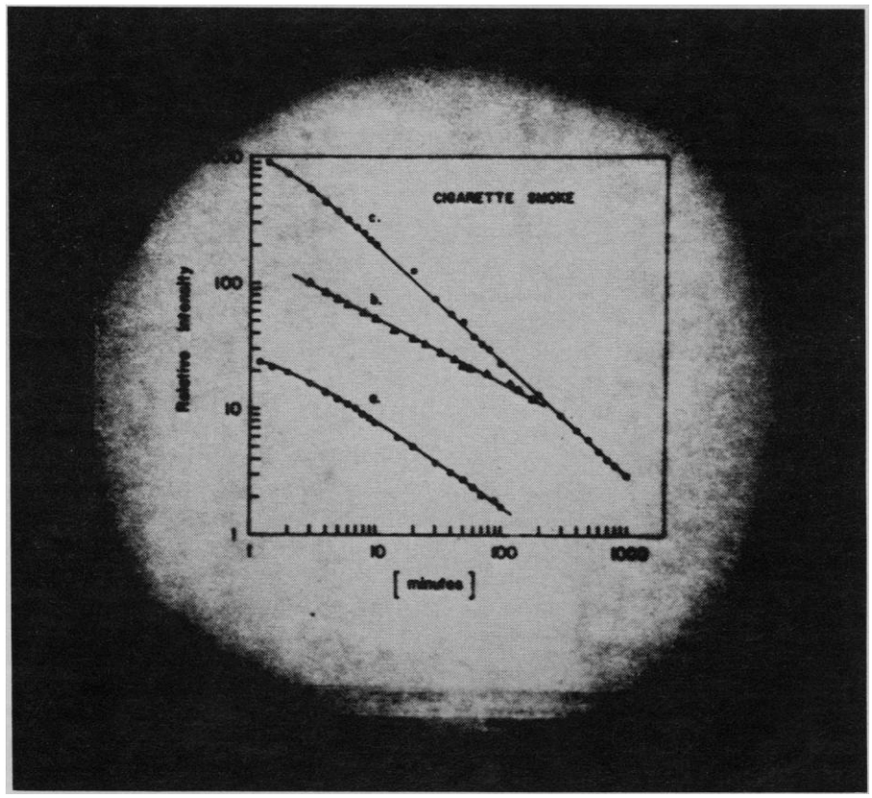

Fig. 3. A contact photograph illustrating the kinetics of the spontaneous chemiluminescence of cigarette smoke. The light for this exposure came solely from chemiluminescent emission of the smoke from a single cigarette. Smoke was collected on a glass-fiber filter and extracted in total darkness into 60 ml of *N,N*-dimethylformamide. The extract, initially at 75°C, was positioned above a Polaroid slide, type 146L, the latter resting on a Polaroid type 410 high-speed emulsion. Exposure time, approximately 10 minutes.

of workers (*17*) and has recently been proposed as a nonradiative model for aromatic hydrocarbon–induced carcinogenesis (*18*).

There is no experimental evidence relating chemiluminescence to any mechanism for carcinogenesis. However, cigarette smoke contains relatively stable carcinogens that may be metabolically activated. The spontaneous chemiluminescence observed indicates the potential for the production of electronically excited states within the lung over and above those metabolically produced excited states proposed by Anderson. The photon emission of cigarette smoke provides a minimum number for the excited state product molecules formed chemically.

Except for bioluminescence, in which an evolutionary selection has been made for chemiluminescent substrates that give high photon yields, most chemiluminescent reactions have very low photon yields. The detection of photon emission produced by these highly exergonic chemical reactions is in one sense fortuitous; nonradiative pathways and fluorescence quenching might have been so efficient as to make the luminescence, and therefore the presence of these reactions, undetectable. A possible interference in experiments on the promotion of carcinogenesis of tars by photoexcitation can be inferred from the self-absorption effect shown in Fig. 2a. A method for painting tars on the skins of mice and rabbits calls for a 50 percent solution (weight to volume) in acetone (*12*). This slurry produces a thick layer of tar so that incident light is absorbed by the outer layers of tar molecules—those not in contact with or absorbed by the skin. In view of the transient nature of the unstable radicals in cigarette smoke and their possible involvement in the promotion of carcinogenesis by activation of carcinogens already present, or as activated carcinogens themselves, it would appear that experiments attempting to relate cigarette smoke to carcinogenesis should also mimic the true physiological time exposures of the test organisms to the observed chemiluminescence [see also (*3*)].

Carcinogenic aromatic hydrocarbons are present in the air we breathe as the result of the burning of organic material, exclusive of tobacco. When tars and other latent carcinogenic molecules are already present in the lungs, the inhalation of chemiluminescent precursors in smoke from any source, as well as from tobacco, could result in a chemically mediated electronic excitation of these molecules, that is, a promotion of carcinogenesis. The long-lived nature of the chemiluminescence from smoke implies that, while smokers who inhale subject their lungs to relatively high intensities of chemiluminescence because of particulate retention, the chemiluminescent emission that occurs from exhaled smoke and the side-stream smoke subjects smokers and nonsmokers alike to a significant chemiluminescent dose.

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29 March 1974

Microwave Hearing: Evidence for Thermoacoustic Auditory Stimulation by Pulsed Microwaves

Abstract. *Acoustic transients can be thermally generated in water by pulsed microwave energy. The peak pressure level of these transients, measured within the audible frequency band as a function of the microwave pulse parameters, is adequate to explain the "clicks" heard by people exposed to microwave radiation.*

When a person's head is illuminated with pulsed microwave energy, he can perceive "clicks" in synchrony with the individual microwave pulses (*1–3*). The pulses must be moderately intense (typically 0.5 to 5 watt/cm^2 at the surface of the head). However, they can be sufficiently brief (50 μsec or less) that the maximum increase in tissue temperature after each pulse is very small ($< 10^{-5}$ °C). This is the only unequivocal biological effect of microwave radiation that is not accompanied by or produced by observable tissue heating. Because of the current debate over possible effects on the central nervous system of low-power, radio-frequency radiation (*2*), it appears important to understand the underlying mechanisms for this phenomenon.

Electrophysiological experiments in cats have demonstrated the presence of