the organism whatever limiting factor falls to a low level at hour 0 (see Fig. 1). Since the primary function of light for these autotrophic cells is to provide energy, this result may indicate a depletion of the energy pools of the cells during the night phase. Further experiments along these lines are necessary to distinguish these alternatives. Further experiments will also be necessary to determine what relationship, if any, this oscillation has to the retardation of the clock by cycloheximide and hence to the mechanism of the circadian clock.

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Cigarettes: Chemical Effects of Sodium Nitrate Content

Abstract. *Although addition of sodium nitrate to cigarettes is reported to reduce the components and properties of cigarette smoke that are associated with tumorigenicity, we find that the additive nevertheless significantly increases the levels of certain vapor-phase constituents of smoke that are known to inhibit ciliary movement; and also it produces other effects of questionable value.*

Cigarettes with $NaNO_3$ added produce smoke that is less tumorigenic and toxic (in mice) and contains less particulate matter, benzo[*a*]pyrene, nicotine, and phenol than does smoke from standard cigarettes (*1*). These effects are attributed partly to the thermal decomposition of the nitrate into oxygen and nitrogen oxides, the former enhancing combustion of tobacco and the latter inhibiting free-radical reactions leading to formation of benzo[*a*]pyrene and other polynuclear aromatic hydrocarbons.

Table 1. Yields of the components of the vapor phase of smoke (fifth puff, 35 ml) from cigarettes without or with $NaNO_3$ added; averages of ten or more determinations. The method did not distinguish between NO and NO_2; the lower limit of detection of N_2O was 1.0 μg.

Component	Without $NaNO_3$	With $NaNO_3$
	Mole percentages	
H_2	1.38	1.64
O_2	13.46	12.46
CO	3.00	3.55
CO_2	7.43	7.86
	Micrograms	
$NO+NO_2$	22.9	48.7
N_2O	<1.0	11.0
HCN	23.0	23.3
H_2S	4.5	0.3
CH_4	107.5	90.0
C_2H_6	37.8	36.4
CH_3CHO	74.0	146.3
CH_3COCH_3	40.6	55.3
CH_3CN	14.3	29.3
$CH_2=CHCHO$	9.2	18.4
CH_2O	5.3	5.6

Compounds (*2*) other than those cited above, however, are believed to contribute to the reported biological activity (*3*) of cigarette smoke. Several of these, including ciliostatic agents (*2*) and irritants (*4*), are present in the vapor phase of cigarette smoke, and their concentrations may be affected adversely by the addition of $NaNO_3$ to cigarettes. Moreover, thermal decomposition of the additive may increase the concentrations of nitrogen oxides in smoke and lead to formation of other compounds (*5*) of questionable activity such as nitroalkanes.

We have studied the effect of $NaNO_3$ on the qualitative and quantitative composition of the vapor phase of tobacco smoke. Table 1 compares the concentrations of several vapor-phase constituents of smoke from cigarettes with and without addition of 8.3 percent $NaNO_3$. The data show that addition of $NaNO_3$ leads to significantly (at least twice) greater quantities of undesirable vapor-phase constituents such as nitrogen oxides, acetaldehyde, acrolein, and acetonitrile. Acetaldehyde and acrolein strongly inhibit ciliary movement (*2*). Certain nitrogen oxides (*6*) have been implicated in ciliostasis. From the nature of the additive one expects higher concentrations of nitrogen oxides; of them, however, N_2O apparently occurs only in the smoke of treated cigarettes (11.0 μg per 35-ml puff); if it is produced by normal cigarettes, it is below the level of detection (< 1 μg per 35-ml puff). Our data also show that concentrations of CO, CO_2, HCN, and HCHO remain essentially unchanged, while that of H_2S is significantly reduced. Although not indicated in Table 1, levels of low-boiling olefinic hydrocarbons are increased in proportion to decrease in levels of paraffins.

Our findings lead one to conclude that, although $NaNO_3$ reduces properties of cigarette smoke that are reportedly associated with tumorigenicity and toxicity, it increases the concentrations of certain vapor-phase constituents that play important roles in inhibition of ciliary movement; it may also lead to formation of new compounds of questionable activity.

We used commercial cigarettes (without filters and 85 mm long) that were smoked by machine. Each puff passed through a Cambridge filter before being collected in a bulb for subsequent analyses. All analyses were conducted on fifth puffs (volume, 35 ml; duration, 2 seconds; interval between puffs, 60 seconds). Reported colorimetric methods (*7*) were used for determination of HCN, H_2S, HCHO, and nitrogen oxides. Analyses by gas chromatography, with appropriate molecular-sieve or polyaromatic-resin columns, were used to determine the other components. During the smoking, the temperatures of burning were monitored with a thermocouple. Cigarettes containing $NaNO_3$ burned at 788°C; normal cigarettes, at 850°C.

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