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.

PHILIP S. THAYER
C. J. KENSLER
Life Sciences Division, Arthur D. Little, Inc., Cambridge, Massachusetts

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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-

eters, convection can be ignored (*3*). Heat conduction to the droplet along the wires is

$$\left(\frac{dQ}{dt}\right)_{wires} = C_w r_c \theta \quad (3)$$

where C_w is the heat conductance of both wires per unit length, and θ is the wet bulb depression. Heat conduction to the drop through the air is given by McAdams (*4*):

$$\left(\frac{dQ}{dt}\right)_{air} = \frac{4 \pi K_a \theta}{\frac{1}{r_d} - \frac{1}{r_c}} \quad (4)$$

where K_a is the heat conductivity of air. For $\theta < 1$, net radiation to the drop is approximately

$$\left(\frac{dQ}{dt}\right)_{rad} = 16 \pi \sigma r_d^2 T^3 \theta \quad (5)$$

where σ is the Boltzmann constant (emissivity of drop and leaf tissue is assumed to be 1), and T is the Kelvin temperature of the leaf tissue, which is assumed to be the same as that of the bath in which the chamber is immersed. The total heat input to the drop, the sum of Eqs. 3, 4, and 5, is substituted into Eq. 2:

$$\left(\frac{dm}{dt}\right)_{drop} = \frac{\theta}{L}\left(C_w r_c + \frac{4 \pi K_a}{\frac{1}{r_d} - \frac{1}{r_c}} + 16 \pi \sigma r_d^2 T^3\right) \quad (6)$$

At steady state the mass of vapor in the chamber is constant, so that $(dm/dt)_{drop} + (dm/dt)_{leaf} = 0$, or from Eqs. 1 and 6

$$\frac{\theta}{L}\left(C_w r_c + \frac{4 \pi K_a}{\frac{1}{r_d} - \frac{1}{r_c}} + 16 \pi \sigma r_d^2 T^3\right) + a k (e_{leaf} - e_{air}) = 0 \quad (7)$$

For the narrow range of θ normally encountered, θ is observed to be a linear function of Ψ_{air}, the water potential of the chamber air. θ and Ψ_{air} are both zero at saturation, so that

$$\theta = \alpha \Psi_{air} \quad (8)$$

where α is an empirically determined constant. If water vapor is an ideal gas, the e's in Eq. 7 may also be written as functions of Ψ.

$$e = e_o \exp \frac{V \Psi}{R T} \quad (9)$$

where e_o is the vapor pressure at saturation, R is the ideal gas constant, T is the Kelvin temperature, and V is the molar volume of water. (Ψ is expressed in bars where 1 bar = 10^6 erg cm^{-3}.)

With these substitutions and with the terms rearranged, Eq. 7 can be written

$$\frac{\exp \frac{V \Psi_{leaf}}{RT} - \exp \frac{V \Psi_{air}}{RT}}{\Psi_{air}} = \frac{\alpha}{a k L e_o}\left(C_w r_c + \frac{4 \pi K_a}{\frac{1}{r_d} - \frac{1}{r_c}} + 16 \pi \sigma r_d^2 T^3\right) \quad (10)$$

For $\Psi < 200$ bars, the exponentials in Eq. 10 may be replaced by the first two terms of the exponential series, with a truncation error of < 1 percent. Then Eq. 10 can be written

$$\Psi_{leaf}/\Psi_{air} = 1 + \beta, \text{ where } \beta = -\frac{R T \alpha}{a k L e_o V}\left(C_w r_c + \frac{4 \pi K_a}{\frac{1}{r_d} - \frac{1}{r_c}} + 16 \pi \sigma r_d^2 T^3\right) \quad (11)$$

The relative error of measurement is

$$\epsilon = \frac{\Psi_{leaf} - \Psi_{air}}{\Psi_{leaf}} = \frac{\beta}{1 + \beta} \quad (12)$$

Figure 1 shows ϵ versus k for $r_d = 0.1$ cm, $r_c = 1$ cm, $T = 298°$K, $C_w = 0.24 \times 10^6$ cal °C^{-1} cm, and $\alpha = -9 \times 10^{-5}$ °C bar^{-1}. As expected, if the product ka is large enough, in this case $> 10^{-4}$ g sec^{-1} mm-Hg^{-1}, the relation between θ and the potential is practically the same for calibrating solution and leaf. When $ka = 10^{-7}$, however, the relative error is more than 0.5; when $ka < 10^{-9}$, it is practically 1.0.

To determine leaf permeability under conditions similar to those probably existing in psychrometer chambers, leaves (three tobacco, two laurel, one philodendron) were placed in a dark wind tunnel (*5*) for 79,000 seconds, and weighed at intervals of 5000 to 56,000 sec. Carbon dioxide concentration was kept at 2 percent to simulate the high concentration resulting from respiration in the sealed psychrometer chambers. Relative humidity was controlled to within 0.2 percent. Leaf permeability was calculated from Eq. 1, assuming that e_{leaf} was the saturation vapor pressure at air temperature and that e_{air} was the same as that measured in the wind tunnel. The validity of these assumptions depends on low atmospheric resistance to diffusion of heat and vapor near the leaves; to ensure low atmospheric resistance, the leaves were placed between two perforated baffles. Air was forced through these perforations at 70 m sec^{-1} and moved the leaves noticeably. Thus, without lights and at these low transpiration rates the temperature difference between air and leaf would not likely invalidate the first assumption. Neither would the lowering of vapor pressure inside the leaf (by desiccation) be significant. For the k meas-

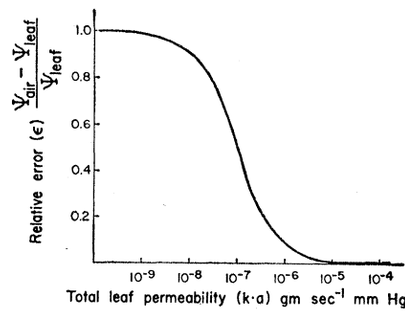

Fig. 1. Theoretical values for the relative error in water potential (Ψ) introduced by the presence of a water droplet in a thermocouple psychrometer as a function of the total permeability of the leaf tissue within the chamber. (See Eq. 11 for the psychrometer dimensions and boundary conditions given in text.)

Table 1. Water permeability of excised tobacco, laurel, and philodendron leaves at four different times during drying in a wind tunnel.

Leaf No.	0–7500 sec (Δe = 4.3 mm-Hg)*		7500–18,300 sec (Δe = 3.9 mm-Hg)		18,30–23,100 sec (Δe = 4.3 mm-Hg)		23,000–79,000 sec (Δe = 8.1 mm-Hg)	
	Final wt. (%)†	$k \times 10^8$‡	Final wt. (%)	$k \times 10^8$	Final wt. (%)	$k \times 10^8$	Final wt. (%)	$k \times 10^8$
				Tobacco				
1	94.8	3.38	90.4	2.22	89.3	1.06	59.3	1.39
2	94.8	3.25	88.1	3.24	85.1	2.96	57.0	1.25
3	95.3	3.31	88.3	3.76	86.1	2.43	53.1	1.65
				Laurel				
1	99.0	0.59	97.5	0.67	97.0	0.46	90.2	0.29
2	99.0	0.61	97.6	0.60	97.1	0.43	94.3	0.29
				Philodendron				
1	99.8	0.06	99.6	0.06	99.5	0.06	97.2	0.06

* $\Delta e = e_{leaf} - e_{air}$, if one assumes that e_{leaf} is at saturation at air temperature. † Leaf weight at the end of each drying period as a percentage of weight at 0 sec. ‡ k is grams of water vapor that move from a unit area of leaf per second when a difference of 1 mm-Hg exists in the water vapor pressure between the leaf and the air adjacent to it.

Table 2. The water potential, measured with a thermocouple psychrometer, of five leaves of a pepper plant and of the nutrient solution in which the plant was growing.

Sample	Water potential (bars)
Leaf 1	−4.6
Leaf 2	−4.0
Leaf 3	−4.3
Leaf 4	−4.3
Leaf 5	−4.0
Nutrient solution	−7.2

ured here the gradient in vapor pressure away from the leaves would not affect e_{air} significantly if the length of the equivalent diffusion path caused by atmospheric resistance to vapor diffusion was < 1 cm; undoubtedly, atmospheric resistance in the wind tunnel was not so great. The observed values for k are given in Table 1.

The k for tobacco ranged from an average high of about 3.4×10^{-8}, when 95 percent of the initial leaf weight remained, to about 1.4×10^{-8} when 56 percent of the initial weight remained. The k for laurel also decreased with water loss. So little water was lost from philodendron that no decrease in permeability was detected. In all three species, leaf permeability was sufficiently small to cause considerable error in measurement of water potential in leaves with this psychrometer. For example, if 20 cm^2 of leaf at the greatest hydration shown in Table 1 were placed into the psychrometer for which Fig. 1 is drawn, the relative error of measurement for tobacco would be about 0.14; for laurel, about 0.36; and for philodendron, about 0.85. At the least hydration, the error for tobacco would be about 0.24 and for laurel about 0.66.

To determine how closely Eq. 6 predicts the evaporation rate from a drop in a real psychrometer, a drop was measured onto the wet bulb, θ was measured as time passed, and finally the total loss from the drop was measured. As is seen by integrating Eq. 6, the area under the curve relating observations of θ to t equals the loss in mass from the drop divided by a term which is constant for any psychrometer at constant temperature. An idealized psychrometer with a spherical sample chamber is assumed in Eq. 12 and Fig. 1; different geometry would be expected to change the evaporation rate from the drop. For a cylindrical chamber 1.5 cm in diameter and 5.5 cm long, with other variables the same as for Fig. 1, loss from the drop was within 10 percent of that predicted by Eq. 6 for a spherical chamber with $r_c = 1.0$ cm; the errors predicted above are realistic.

The magnitude of error that can be introduced by the presence of the water drop in the chamber is illustrated in Table 2. The pepper plant that yielded these data grew in nutrient solution, with polyethylene glycol added to decrease the water potential in the root. Prior to sampling, the plant was kept in the dark overnight to slow transpiration. The water potentials of five leaves and of the nutrient solution were determined in psychrometers similar to the one described in the preceding paragraph. The data show the water potential of the leaves to be about 3 bars higher than that of the nutrient solution. Since the water potential gradient must be in the opposite direction for water to move passively from the roots to the leaves, these data indicate that, without a correction for leaf permeability, large errors may be introduced into measurements made with this thermocouple psychrometer of water potential in leaves.

STEPHEN L. RAWLINS*
Department of Soils and Climatology, Connecticut Agricultural Experiment Station, New Haven

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* Present address: U.S. Salinity Laboratory, Riverside, California.

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Biological Rhythms: A New Type in Strains of a Mutant of Neurospora crassa

Abstract. *The formation of growth bands in* Neurospora, *with periods ranging from 15 to 90 hours, depends strongly on temperature and on composition of the growth medium, but not upon cycles of light and dark. The rhythm is endogenous but not "circadian."*

Insight into cellular processes may be gained by observation of biological rhythms. Goodwin (*1*) explains in his recent book how the observed period of these rhythms can be determined by a statistical treatment of biochemical reactions. Endogenous daily rhythms with a period of approximately 24 hours, which have been termed "circadian" rhythms (*2*), are prominent examples of systems of this kind. However, these rhythms exhibit a number of additional special properties which relate to their inferred role in a biological clock mechanism. First, they may be synchronized, within limits, by appropriate light-dark cycles simulating day-night conditions of illumination. Second, in the absence of light-dark cycling (under constant conditions of light and temperature), the rhythms persist with a well-defined period close to 24 hours. Their deviations from 24 hours are useful diagnostic features since we thereby deduce that no environmental factor is synchronizing the rhythm. Third, the period, when observed under constant conditions, is relatively independent of the physical and chemical aspects of the environment; the so-called temperature-independence of biological clocks is well known.

We have examined mutants of *Neurospora crassa* that possess an endogenous rhythm which is not circadian in character. The role of this rhythm may only be related to a series of repetitive biochemical events rather than serve as a special adaptation to the day-night cycle. The linear growth rate of the several "clock" mutants (*3*) is constant, but their hyphae form a regular series of periodic bands by increasingly dense terminal branching toward the end of a period (Fig. 1). A new growth band is initiated by the relatively small number of surface hyphae advancing beyond the terminal growth edge at random locations. This process is repeated at regular intervals depending on temperature and medium composition. In contrast to previously reported rhythmic *Neurospora* isolates (*4*) Sussman's strains form very distinct bands with no conidia between 16° and 35°C. Formation of bands continues as long as adjacent fresh medium is available for growth. Since there is no overgrowth from one band to another, a permanent record of their growth pattern is left. No bands form on a liquid medium, but they do form on Millipore filters floating on liquid medium.

Although some of the mutants have a period of about 24 hours at room temperatures, the rhythm bears no special relation to the light-dark cycle and it is not possible to demonstrate synchronization. When strain A is main-