different spindle fibers serves as a partial explanation of certain phenomena which were difficult to reconcile with previous models of spindle structure, for example: the interference with anaphase movement of one chromosome when the movement of its neighbor is inhibited by a bridge, and the relative interdependence of individual chromosome movements in anaphase as compared with prometaphase.

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10. Three European companies, to our knowledge, already manufacture this system, although in the United States it is little used. The Zeiss equipment that we used was a prototype instrument made available to one of us (R.D.A.) through the kindness of Dr. H. Piller of Carl Zeiss (Oberkochen). It has high-quality polarizing and beam-splitting elements and is designed for use with strain-free planachromatic objectives and condenser.
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14. This investigation was conducted at Princeton University in May 1965; supported by grants NSF GB 3335 to A. Bajer and NIH GM 08691 to R. D. Allen. We thank Professor W. Jackson of the Department of Biology, Dartmouth College, Hanover, New Hampshire, for supplying us with plant material in the proper stage. Our studies were made possible by Professor Jackson's work on the developmental cycle of *Haemanthus* (NSF GB 705). Finally, we are grateful to Dr. H. Piller of Carl Zeiss (Oberkochen) for making available to us the prototype of a superb research instrument (Nomarski system).

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Gaseous-Diffusion Porometer for Continuous Measurement of Diffusive Resistance of Leaves

Abstract. *We describe a porometer that enables continuous monitoring of the stomatal diffusive resistance of leaves. The flux is measured of a gas—such as nitrous oxide—diffusing through a leaf that divides an enclosing chamber into two compartments. Nitrous oxide is added in known concentration to the airstream passing through the compartment on one side of the leaf and is recovered from the airstream passing through the opposite compartment. From measurements of the difference in concentration across the leaf and of the flux, the diffusive resistance of the leaf to N_2O is calculated; this value, adjusted for resistance external to the leaf, gives a continuous record of internal diffusive resistance. This record can be made simultaneously with measurements of transpiration and photosynthesis.*

In studying the fluxes of carbon dioxide and water vapor into and from leaves, one frequently requires a continuous, independent measure of stomatal aperture. Most conventional porometers have disadvantages (*1*). Diffusion porometers have the following advantages over the more commonly used viscous-flow porometers: operation can be continuous without disturbance of the gas and vapor concentrations around the leaf or of their fluxes; flow through the leaf is by molecular diffusion and thus similar in principle to that of water vapor and carbon dioxide; no external pressure is applied to the guard cells. The use of diffusion porometers using unusual gases, such as hydrogen (*1*), has been limited by difficulty in selecting a suitable gas that does not affect the plant and that can be continuously and inexpensively analyzed. Thus the flux of water vapor itself is often used to give a measure of diffusive resistance of the stomata (*2*, *3*).

Such estimates are complicated by doubts as to whether the resistance so measured arises entirely in the stomatal pore or is partly caused by resistances to vapor flow in the interfibrillar spaces of the mesophyll cell walls [it is generally assumed that resistance in the intercellular space is negligible (*4*)]. This uncertainty becomes of particular importance when independent estimates of stomatal aperture (such as by direct examination or by stomatal-imprint techniques) seem to indicate that, under apparently constant environmental conditions, the transpiration rate has varied without detectable compensating change in stomatal aperture. In such situations it is sometimes assumed that a significant source of resistance to vapor flow has developed in the cell walls but, because of difficulty in estimating stomatal diffusive resistances from measurements of stomatal apertures or by viscous-flow porometry (*5*), the assumption is difficult to substantiate. Thus a measurement of stomatal diffusive resistance is required that is unaffected by changes elsewhere in the path length of the water vapor.

We have developed a procedure in which the flux of N_2O is measured diffusing through a cotton leaf mounted in a specially designed chamber (*6*). The chamber permits independent measurements of transpiration from upper and lower surfaces of the leaf and, simultaneously, measurements of gaseous-diffusive resistances to water vapor and CO_2.

For measurements of stomatal diffusive resistance a controlled flow of N_2O, at known concentration, is introduced into the airstream flowing through the lower chamber. The N_2O diffuses through the leaf by way of the lower stomata, intercellular air spaces, and upper stomata and is then collected from the airstream passing through the upper chamber; the differential value (or absolute value in the upper airstream) is detected in an infrared gas analyzer sensitive to N_2O.

Table 1. Effects of four concentrations of N_2O (means of determinations at beginning and end of runs) on photosynthesis and transpiration. Light intensity: 0.2 cal cm^{-2} min^{-1} in the visible. VP, vapor pressure.

Concn. N_2O (vol %)	Duration (hr)	Net uptake CO_2 (%)	Background		Transpiration (%)
			CO_2 (vol %)	VP (mm-Hg)	
0	2.0	100	0.032	6.0	100
0.1	2.0	100	.032	6.0	107
.5	1.25	93	.032	6.0	97
1.0	0.55	102	.031	5.7	104
10.0	1.20	104	.029	5.4	112

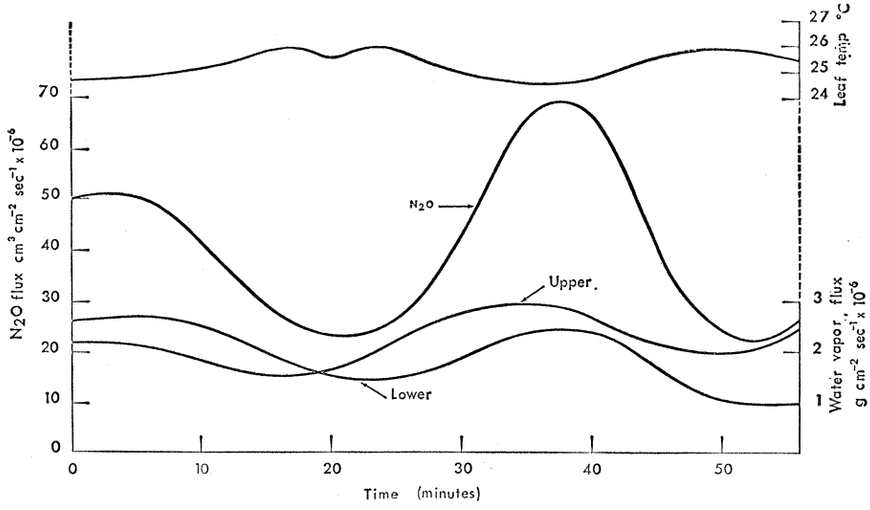

Fig. 1. Progressive changes in flux of N_2O through a cotton leaf, transpiration from upper and lower surfaces of the leaf, and leaf temperature.

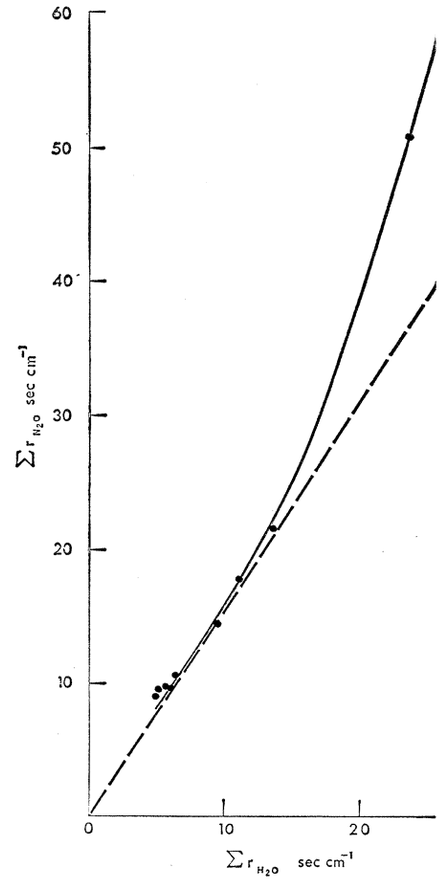

Fig. 2. Relation between total resistance to diffusion of N_2O through a cotton leaf, Σr_{N_2O}, and the series sum of the diffusive resistances to water-vapor transfer from both sides of the leaf, Σr_{H_2O}. The dashed line shows $\Sigma r_{N_2O} = \Sigma r_{H_2O}$ for $D_{H_2O}/D_{N_2O} = 1.54$.

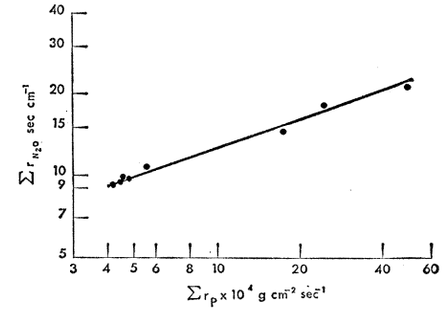

Fig. 3. Relation between log total resistance to diffusion of N_2O through a cotton leaf, Σr_{N_2O}, and log total resistance to viscous flow of air under a pressure difference, $\Sigma r_P : \Sigma r_{N_2O} = \Sigma r_P^{0.33}$.

Problems could arise if the gas being used was absorbed by, or had toxic effects on, the particular plant material. Under test for toxic effects of N_2O, a 6-week-old cotton plant was exposed to stepped increases in concentration from 0.02 to 10 percent (by volume) during two days, while net photosynthesis and transpiration were recorded at each concentration. Such concentrations had no apparent effects (Table 1). Another cotton plant was exposed to 0.5 percent N_2O throughout the photoperiod of a whole day without effect on net photosynthesis and transpiration, or on subesquent appearance of the plant. Since concentrations suitable for use in our porometer range from 0.1 to 0.5 percent, no deleterious effects of N_2O may be expected.

About 1.17×10^{-3} g N_2O is soluble in 1 ml water at 25°C; thus we thought it possible that a significant amount of N_2O might be absorbed in the leaf. However, when 0.1 percent N_2O in air was applied to the leaf for 2 hours, no uptake was detectable with an infrared gas analyzer sensitive to within less than 1 ppm.

The appropriate flow equation for N_2O transport is

$$q_{N_2O} = \frac{c_a{}^l - c_a{}^u}{r_a{}^l + r_i + r_a{}^u} \qquad (1)$$

where q_{N_2O} is the N_2O flux (cm^3 cm^{-2} sec^{-1}), c_a is the concentration of N_2O in air (cm^3 cm^{-3}), r_a is the external (boundary-layer) diffusive resistance, and r_i is the resistance to flow in the leaf (sec cm^{-1}); superscripts l and u refer to the lower and upper compartments of the leaf chamber, respectively.

For experimental measurements, $r_a{}^l$ and $r_a{}^u$ can be kept very low relative to r_i by using high stirring rates above and below the leaf surfaces to make the effective boundary layer as thin as possible. Alternatively, the external resistances can be determined separately by measuring the rate of evaporation from a surface-wetted leaf or from a piece of wet blotting paper, and by adjusting the r_a values, so obtained, by the ratio of the diffusion coefficients for water vapor and N_2O (*7*).

As far as we know, this porometer is the first to permit continuous monitoring of stomatal diffusion resistance simultaneously with uninterrupted measurements of transpiration, photosynthesis, and associated resistances to transfer of water vapor and CO_2. (If infrared gas analysis is used for measurements of both CO_2 and N_2O exchange, interference with one by the other must be prevented.) This has considerable significance for a wide variety of studies relating stomatal movement and function to plant-water relations and plant growth generally.

Figure 1 shows an example of the use of the porometer for a cotton leaf in which cyclic opening and closing of the stomata on the upper and lower surfaces is induced. The cycling is slightly out of phase on the two surfaces, as indicated by the separate transpiration curves. The diffusive flux of N_2O, however, provides a continuous record from which the sum of the diffusive resistances across the leaf can be calculated.

In Fig. 2 determinations of leaf diffusive resistance, obtained from N_2O flux measurements, are plotted against estimates from water-vapor diffusion from each side of the leaf, summed in series, for a range of stomatal apertures. There is a close, almost linear, relation between the two until the stomata are almost closed. At this stage

the relation curves because the contribution of cuticular transpiration appreciably influences the estimates of the water-vapor flux. The dashed line shows

$$\Sigma r_{N_2O} = \Sigma r_{H_2O} \text{ for } D_{H_2O} / D_{N_2O} = 1.54.$$

The close agreement between Σr_{N_2O} and Σr_{H_2O}, under conditions of open stomata, indicates that there were no detectable sources of resistance to movement of water vapor in the leaf, other than those associated with the stomata.

The method allows precise determination of the relation between the diffusive transfer of N_2O or water vapor and the viscous flow of air through the leaf (Fig. 3). This relation is particularly significant, since most published measurements of stomatal resistance have been made with viscous-flow porometery and difficulties are encountered in reconciling the two (*1, 5*).

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7. Unable to find in the literature a diffusion coefficient for N_2O in air (D_{N_2O}), we determined the ratio of the diffusion coefficients of N_2O and water vapor in air (D_{N_2O}/D_{H_2O}) experimentally. A thin metal membrane with pores, of about 45-μ diameter, regularly arranged at intervals of about 150 μ, and with an exposed area of about 2.25 cm^2, was mounted over a hole in a thin celluloid plate, which was placed in the leaf chamber in the leaf position. Air, containing 0.1 percent N_2O and water vapor at about 20×10^{-6} g cm^{-3}, was passed through the lower chamber, and the diffusive fluxes of N_2O and water vapor through the membrane into the upper chamber were estimated from the steady-state increases in concentration obtained there. The air supplied to the upper chamber contained no N_2O and water vapor at about 10×10^{-6} g cm^{-3}. Air flowed through both chambers at about 42 liter hour^{-1} and rapid stirring was maintained at a constant rate. The air lines leaving the chambers were open to atmospheric pressure, and a manometer connecting the chambers showed no difference in pressure. Concentrations and fluxes of N_2O were measured in the manner described; those of water vapor were determined with a differential psychrometer (*8*). The ratio of the sums of the resistances, Σr_{N_2O}: Σr_{H_2O}, was obtained from equations, of the form of Eq. 1, for N_2O and for water vapor:

$$\Sigma r_{N_2O} : \Sigma r_{H_2O} = [(c_a^l - c_a^u)(q_{H_2O})] / [(w_a^l - w_a^u)(q_{N_2O})] \quad (2)$$

where q_{H_2O} is the water-vapor flux (cm^3 cm^{-2} sec^{-1}), w indicates water-vapor concentration (cm^3 cm^{-3}), and the other symbols are as in Eq. 1. Since $r = h/D$ where h is the effective diffusive path length:

$$D_{H_2O} : D_{N_2O} = \Sigma r_{N_2O} : \Sigma r_{H_2O} = 1.54$$

If one assumes that D_{H_2O} is 0.28 cm^2 sec^{-1} at 28°C (*9*), D_{N_2O} is approximately 0.18 cm^2 sec^{-1}. (In view of the interest in the ratio D_{H_2O}:D_{CO_2} in studies of leaf resistances to CO_2 transfer (*3, 10*) and of the unsatisfactory knowledge of D_{CO_2}, this ratio was measured in the same way at approximately 1.68 at 28°C.)
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Pentobarbital Sodium: Variation in Toxicity

Abstract. *The survival rate of groups of female mice given a standard dose of pentobarbital sodium varied during a 12-hour period. When survival rate was plotted against time, a curve with several inflections was described.*

When degree of susceptibility of an insect to a toxic agent was plotted against time (*1*) the curve showed several inflections within a 12-hour period. We wished to learn whether the susceptibility of a mammal to pentobarbital sodium would vary during a similarly short time. Davis (*2*) had conducted a related experiment on the periodicity of barbiturate anesthesia but had worked with a longer (4-hour) interval between injections.

Groups of 18 mice were given intraperitoneally an LD_{50} (lethal dose, 50 percent effective) of the drug, 130 mg per kilogram of body weight, at intervals of 1½ hours during a 12-hour period. Each day's experiment was based on 162 mice; the series, on 1134. Final counts of survivors were made on the day after the injection. The mice were females, of the Swiss strain, weighing 15 to 20 g on arrival; at this time they were randomly assigned to cages on one side of a cage rack so that none were on the shaded side. Except in the first experiment nine mice were placed in each cage (30 by 23 by 18 cm). During the next 14 days the light was cycled automatically as follows: on at 7:30 a.m. and off at 7:30 p.m.; no daylight could reach the animal quarters.

Temperature was maintained at approximately 24.5°C. Water and Purina laboratory chow were allowed as desired. Two cages were taken in sequence for the experiment at each time period. Each mouse was weighed immediately before injection. No mice were used more than once. The experiment was repeated seven times over a 9-month period. The results of one day's experiment were excluded from the statistical analysis since a number of technical difficulties led to its not being done in the same manner as were the other six experiments.

Table 1. Analysis of variance of survival rate of mice given pentobarbital sodium intraperitoneally. Data were subjected to the transformation $\sin^{-1}(x/n)^{\frac{1}{2}}$ where n is the number (that is, 18) per group and x is the number of survivors per group; the theoretical variance for the transformed variable is $821/n$. χ^2, Sum of squares/theoretical variance; df, degrees of freedom; N.S., not significant.

Source	df	Sums of squares	χ^2	P
Days	5	5027.58	110.23	<.005
Time periods	8	1440.03	31.57	<.005
Linear	1	36.80	0.81	N.S.
Quadratic	1	945.38	20.73	<.005
Cubic	1	88.95	1.95	<.17
Quartic	1	20.56	0.45	N.S.
Quintic	1	48.57	1.06	N.S.
Sextic	1	81.88	1.80	<.18
Septic	1	210.22	4.61	<.04
Octic	1	7.66	0.16	N.S.
Day × time	40	2386.07	52.31	<.10
Day × linear	5	452.25	9.92	<.08
Day × quadratic	5	154.94	3.40	N.S.
Day × cubic	5	252.49	5.54	N.S.
Day × quartic	5	11.56	0.25	N.S.
Day × quintic	5	218.69	4.79	N.S.
Day × sextic	5	611.60	13.41	<.02
Day × septic	5	330.21	7.24	<.20
Day × octic	5	354.33	7.77	<.17