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

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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8 December 1965

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

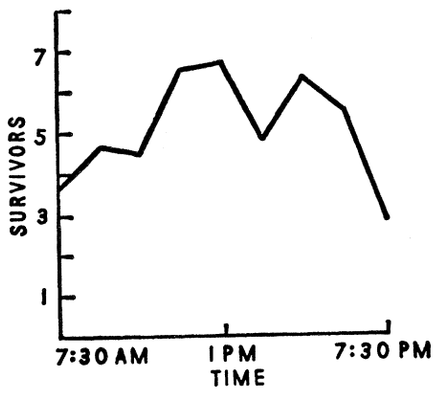

Fig. 1. Survival from pentobarbital sodium as a function of time of day. The curve represents the mean of six replications on widely separated days. Each point represents the mean survival rate of six groups of 18 animals given a dose of 130 mg of pentobarbital sodium per kilogram of body weight, intraperitoneally.

Data were subjected to the arcsin transformation and analyzed by the method of orthogonal polynomials (*3*). Theoretical variances were used to make chi-square tests of significance. Figure 1 shows the mean survival rate derived from the six experiments and Table 1 contains the analysis of variance of the data.

The analysis of variance shows in its upper portion evidence for or against the existence of various trends (linear, quadratic, and others); the lower portion relates to whether a given trend is similar from day to day. In detail, the table indicates first that a linear trend has not been demonstrated, but that this may be due to the heterogeneity between days. The quadratic term has been shown to exist and is similar from day to day. There is very little evidence for the cubic term, and even less for the quartic and quintic; all of these are similar on various days. The sextic term does not exist on the average but shows a high degree of inconsistency; therefore it may on occasion be real. The septic term exists on the average and is reasonably consistent. The octic term does not exist on the average, and there is little evidence of its being inconsistent between days.

Under the conditions of this experiment the LD_{50} dose (*4*) killed substantially more than 50 percent of the animals. This trend, which was so extreme on one day as to allow only one survivor in several groups, may have contributed greatly to inconsistency in the form of the curve between days.

Our data indicate the existence of short-term fluctuations in the toxicity of pentobarbital sodium in a mammalian species. The 4-hour periods at which such observations are usually made, and the even longer period used by Emlen and Kem (*5*) in studying this same drug in the mouse, are too long to reveal the full complexity of the toxicity curve.

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10 November 1965

Ozone: Nonlinear Relation of Dose and Injury in Plants

Abstract. *Ozone produces a sigmoidal dose-injury response in sensitive tobacco and pinto bean. A definite threshold concentration and presentation time are required before injury is initiated.*

Ozone was first recognized as a phytotoxic air pollutant of serious concern to agriculture when it was identified as the agent responsible for stipple in grape (*1*) and weather fleck in tobacco (*2*). Visual symptoms and pathological histology of ozone in a number of agronomic plants have been reported (*3*).

Recently, MacDowall *et al.* (*4*) correlated tobacco fleck in the field with amount of oxidant (which they term ozone) by multiplying the dose (time × concentration) by an empirical exposure factor, which they related to evapotranspiration. This exposure factor brought their dose-injury response into a linear relation, as suggested by Middleton (*5*). Using this relation, they found that, for a given total dose, conditions favoring high evapotranspiration would produce greater injury to tobacco than would conditions of high humidity and low wind (days which favor stagnation and large amounts of oxidant). The use of this empirical exposure factor, which does make their data fit Middleton's linear relation of dose to injury, ignores the extensive work that suggests that plants are more sensitive to a given phytotoxic air pollutant when moisture stress is low. Their conditions may favor the production of different types of phytotoxicants and may not necessarily be conditions that sensitize plants to one specific phytotoxicant such as ozone. Work in several laboratories suggests that values for ambient oxidant cannot be interchanged with ozone values.

Menser *et al.* (*6*), in view of Middleton's work (*5*), have also suggested, for four varieties of tobacco, that the relation between ozone concentration and injury is linear after a sensitivity threshold has been reached. They do not have sufficient data, however, to correctly interpret their injury curve; from their graphs, three of the varieties show a definite log-shaped curve for the few concentrations used. Their doses do not go high enough to show the shape of the upper end of the curve.

Middleton *et al.* (*7*) reported data on 4-hour exposures of pinto bean to from 10 to 50 pphm (parts per hundred million) of ozone per hour for a total dose of 40 to 200 pphm. They plotted injury against dose, fitted a linear regression line to their data, and found a significant positive correlation coefficient. Neither the linear regression nor the positive correlation coefficient necessarily demonstrated that a true linear dose-injury relation existed. The work reported by Middleton (*5*) restated the early work (*7*), but gave no additional experimental data on the dose-injury relation. Thus, the concept that plant injury is linearly related to ozone dose has been perpetuated even though it is based on preliminary data and an incomplete experimental design.

A more detailed study of the dose-injury relation of ozone in tobacco (*Nicotiana tabacum* L., var. Bel-W_3) and bean (*Phaseolus vulgaris* L., var. Pinto) was initiated in this laboratory to help explain some of the variability in environmental effects on plant sensitivity to ozone. Plants were grown in growth chambers (with a 14-hour, 27°C day and a 10-hour, 21°C night) and exposed to ozone in greenhouse exposure chambers for periods starting after 5 hours of light. Temperature, light intensity, and humidity were fairly constant during all exposure times. Four concentrations (10, 20, 35, and 55