

and the total radioactivity of amino acids in nascent protein bound to polyosomes at different times after infection are shown in Fig. 3. The pattern of labeled amino acid incorporation into acid-insoluble peptides of whole-cell homogenates is also shown in Fig. 3. The striking feature of these data is the remarkable correlation between the specific activity of peptides from homogenates of pulse-labeled cells (1), the specific activity of peptides extracted from the cytoplasm of pulse-labeled cells (Fig. 1), the amounts of cytoplasmic polyosomes recovered, and the amounts of pulse-labeled peptides bound to these polyosomes at different times after infection. These data indicate that inhibition of protein synthesis early and late after infection is due to a corresponding breakdown of cytoplasmic polyosomes, that the increase in protein synthesis between 4 and 8 hours after infection is due to stimulation of cytoplasmic polyosome formation, and that the bulk of viral proteins is probably made in the cytoplasm. In this connection it is noteworthy that the polyosomes formed after 4 hours of infection sediment more uniformly and slightly faster (Fig. 2) than those of uninfected cells.

Our results bear on two aspects of virus multiplication in animal cells. First, the inhibition of host protein synthesis with herpes simplex, a DNA virus, is the immediate result of breakdown of cytoplasmic polyosomes. The mechanisms of inhibition of protein synthesis in cells infected with DNA virus shown here and with RNA virus (5) appear similar. However, in both instances the cause of breakdown of host polyosomes is unknown.

Second, the data suggest a discrepancy in the time and site of synthesis of viral proteins and their utilization. Our data indicate that viral proteins are mostly made in the cytoplasm between 4 and 12 hours after infection. On the other hand, more than 95 percent of the final yield of virus is made between 12 and 17 hours after infection (6). The discrepancy in the time of synthesis of virus constituents and their utilization may be due to a physical separation of the sites of viral DNA and protein synthesis. Although mature virus accumulates in the cytoplasm, viral DNA is made and acquires at least one coat in the nucleus (7). The time required to transport protein subunits into the nucleus may account for the in-

terval between the time of synthesis and utilization of viral components.

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References and Notes

1. B. Roizman, G. S. Borman, M. Kamali-Rousta, *Nature* **206**, (1965); B. Roizman and P. R. Roane, Jr., *Virology* **22**, 262 (1964).
2. B. Roizman, and P. R. Roane, Jr., *Virology* **15**, 75 (1961).
3. ———, *ibid.* **19**, 198 (1963).
4. J. R. Warner, P. Knopf, A. Rich, *Proc. Nat. Acad. Sci. U.S.* **49**, 122 (1963).
5. S. Penman, K. Scherrer, Y. Becker, J. E. Darnell, *ibid.*, p. 654; E. F. Zimmerman, M. Heeter, J. E. Darnell, *Virology* **19**, 500 (1963).
6. B. Roizman, L. Aurelian, P. R. Roane, Jr., *Virology* **21**, 482 (1963).
7. C. Morgan, J. Rose, M. Holden, E. Jones, *J. Exp. Med.* **110**, 643 (1959).
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Calibration of β Gauges for Determining Leaf Water Status

Abstract. The recent development of β gauges for the indirect determination of the water status of plants has had a widespread impact on plant ecological-physiological research. Calibration of these instruments has, however, been tedious and cumbersome, involving repeated simultaneous measurements of β absorption and leaf water content. This procedure can be much simplified, as long as the β absorption at a single known relative water content is measured, together with the turgid and dry weights of a sample of leaf discs.

Beta-particle gauging is being used increasingly to provide continuous, non-destructive measurement of the water status of plant leaves (1). A source of β particles is arranged on one side of a leaf and a radiation detector on the other. The absorption of particles by the leaf depends on the energy of the β particles and on the mass per unit area of the leaf. In experiments lasting only a few hours, changes in the mass per unit area, the "effective thickness," are largely the result of changes in the water content of the leaf, since changes in dry-matter content cause only very small changes in effective thickness. Changes in actual leaf thickness closely parallel changes in effective thickness, but direct measurements of the former quantity lack the accuracy and sensitivity of a β gauge and disturb

the microenvironment around the leaf.

Leaves differ one from another in effective thickness according to species, age, position on the plant, and environmental conditions during development. Even if the area of the lamina occupied by main veins is excluded, differences in effective thickness also occur from point to point on the leaf surface. Hence, every time a β gauge is used, it should be calibrated for that area of the leaf between source and detector, and, strictly speaking, the results obtained are valid only for that area. Clearly, if this method is to be used extensively, a simple and reliable method of calibration is required which involves as few measurements as possible.

Calibration in terms of leaf water potential (2) is most desirable, but is cumbersome because of the methods at present available for determining water potential. Relative water content (also called relative turgidity) (3) can be interpreted as water potential with an acceptable degree of accuracy by means of sorption curves relating the two (4). Relative water content also provides a suitable basis for calibration because it can be related to effective thickness, in the following manner.

If R is the relative water content (percent) defined as

$$R = \frac{100 (W_f - W_d)}{W_s - W_d} \quad (1)$$

and D is the effective thickness (mg cm⁻²) given by

$$D = \frac{W_f}{A} \quad (2)$$

Eq. 2 can be substituted in Eq. 1 to give

$$R = 100 \left(\frac{AD - W_d}{W_s - W_d} \right) \quad (3)$$

where W_f , W_s , and W_d are the weights (mg) of a tissue sample of area A (cm²), when sampled, fully turgid ($R = 100$), and oven dried, respectively. (In fact $W_s \cong W_f \cong W_d$.) The slope of the relationship between relative water content and effective thickness, dR/dD , is then obtained by differentiation, giving

$$\frac{dR}{dD} = \frac{100 A}{W_s - W_d} \quad (4)$$

Thus the slope is determined by the water content at $R = 100$, and, knowing the slope, the intercept can be obtained from one estimate of D at a

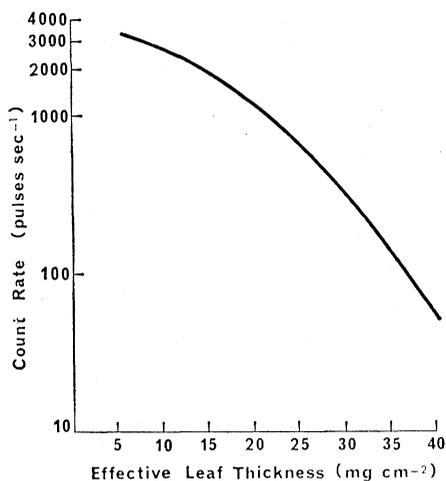


Fig. 1. Relationship between count rate and effective thickness, with the same source-detector characteristics and geometry as for experimental measurements.

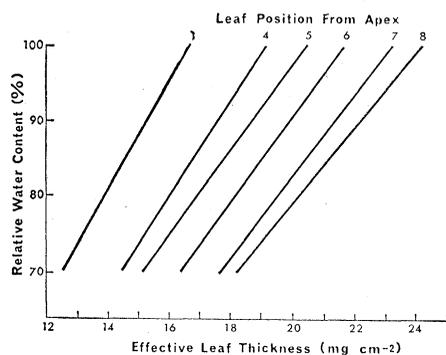


Fig. 2. β -Gauge calibration curves, for a series of cotton leaves numbered from the apex, obtained with Eqs. 2 and 4.

known value of R . Since, for the particular source-detector geometry and characteristics used in a β gauge, D can be uniquely related to count rate, calibration can therefore be simply achieved by determining only: (i) the count rate-effective thickness relationship for the system being used; (ii) the weight (W_s) of a disc sample of known area at $R = 100$, and when oven dried (W_d).

The calibration procedure we adopted was to use the β gauge as set up for experimental measurements. The relationship between count rate and effective thickness, which is independent of absorber characteristics (5), was first established with the use of leaf discs of varying water content or aluminium foil of varying thickness. For this purpose, discs punched out of foil were mounted in small plexiglass holders with Mylar windows, each holder being arranged, in turn, in the β gauge so that the foil discs were in

the same position as a leaf used for experiments. The relationship is shown in Fig. 1. A series of measurements could then be made with a leaf in position, no further count rate-effective thickness calibrations being required as long as source-detector geometry and characteristics remained unchanged.

At the end of each experimental run, a disc sample of known area was punched out of the leaf being monitored and was then rehydrated to the stage $R = 100$ by use of the procedure outlined by Barrs and Weatherley (3). Each disc was then placed in one of the standard plexiglass holders and a count rate was obtained. The sample was then weighed to obtain W_s and oven-dried to obtain W_d . The slope dR/dD was then obtained from Eq. 4 and the intercept from Eq. 2, W_s being substituted for W_f . A range of typical calibration curves for a series of leaves of different age on a single cotton plant is shown in Fig. 2.

The accuracy of the calibration curves was checked by measuring the count rate of samples of leaf discs having a range of relative water contents. The results obtained for two leaves of another cotton plant are given in Fig. 3. The points plotted as open circles relate relative water content to count rate and reflect the curvilinear relationship characteristic of most calibrations at present in the literature. The points plotted as filled circles relate relative water content to effective thickness, as in the present calibration procedure.

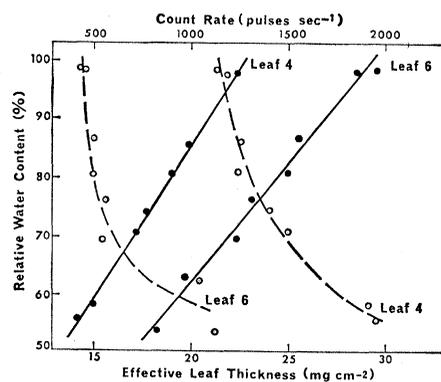


Fig. 3. The relationship between relative water content, effective leaf thickness (solid lines, filled circles), and count rate (dashed lines, open circles) for a young, thin cotton leaf at position 4 from the apex and an older, thicker leaf at position 6. Each curve is based on separate estimates from two samples of ten discs, 1 cm in diameter, which were allowed to lose water slowly over several hours.

The standard error of the estimate of R for leaf 4 and leaf 6 did not exceed 1.19 percent and 1.25 percent relative water content, respectively.

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References

1. W. R. Gardner and R. H. Nieman, *Science* **143**, 1460 (1964); H. J. Mederski, *Soil Sci.* **92**, 143 (1961); F. S. Nakayama and W. L. Ehrler, *Plant Physiol.* **39**, 95 (1964); P. C. Whiteman and G. L. Wilson, *Australian J. Biol. Sci.* **16**, 140 (1963).
2. R. O. Slatyer and S. A. Taylor, *Nature* **187**, 922 (1960).
3. H. D. Barrs and P. E. Weatherley, *Australian J. Biol. Sci.* **15**, 413 (1962).
4. R. O. Slatyer, *Bull. Res. Council Israel Sect. D* **8**, 159 (1960).
5. R. A. Faires and B. H. Parkes, *Radioisotope Laboratory Techniques* (George Newnes, London, 1958), pp. 16-24.

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Influence of the Lethal Yellow (A^y) Gene on Estrous Synchrony in Mice

Abstract. *Introduction of an adult male induces partially synchronous estrus in female laboratory mice that have been caged in groups. In the inbred YS/ChWf strain, this effect was observed only when the male was non-yellow (aa), while males heterozygous for the lethal yellow allele ($A^y a$) failed to induce synchrony.*

Estrous cycles are prolonged and irregular in female laboratory mice housed in groups. Introduction of a male shortens the cycle and elicits heat. Because of partial synchrony of this process more females mate on the third night after being placed with the male than on any other night (1). Yellow ($A^y a$) and nonyellow (aa) males of the inbred YS/ChWf strain were compared for effectiveness in this respect. The Whitten effect (estrous synchrony) (2) was observed only when the male was nonyellow.

All mice used were from the YS/ChWf strain maintained by sib matings with forced heterozygosity for the lethal yellow (A^y) and nonagouti (a) alleles at the *agouti* locus; they were housed and fed in the manner described (3). Females were weaned when 3 to 4 weeks old and housed three to ten per cage. Several weeks later, matings of two females (mean age, 8.6 weeks) and one young adult male were set up in clean cages. During the next 6 to