TECHNICAL PAPERS

Chromosome Numbers for Two Species of Mexican Commelinaceae

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Although the Commelinaceae have provided classic material for cytological and cytotaxonomic studies (*1–3*), sufficient material has never been available for thorough treatment of the Central American representatives of the family. The authors report the following collections from Mexico:

Tradescantia iridescens Booth ex Lindl. $n = 6$. Borders of oak woods near Real del Monte, State of Hidalgo, Mexico. Altitude, 8500–8700 ft. Aug. 1, 1948. *H. E. Moore, Jr., C. E. Wood, Jr., E. Atchison, 4247.*

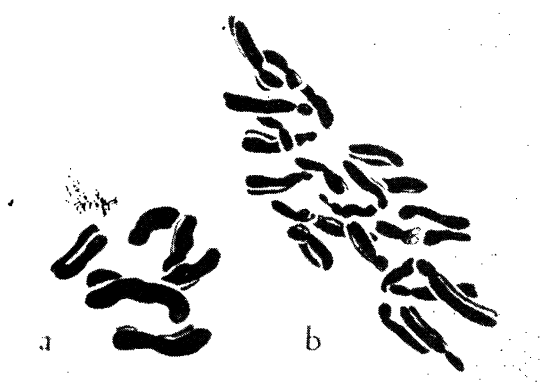

FIG. 1. (a) *T. iridescens,* pollen mitosis, $n = 6$. (b) *W. candida,* root-tip mitosis, $2n = 24$.

Weldenia candida Schult. f. $2n = 24$. Wet pockets on sides of granitic rock masses above Pueblo Nuevo, road from Real del Monte to El Chico, State of Hidalgo, Mexico. Altitude, 9000–9500 ft. July 25, 1948. *H. E. Moore, Jr.,* and *C. E. Wood, Jr., 4082.*

T. iridescens, $n = 6$ (Fig. 1a), is of particular cytotaxonomic interest since it is the first diploid species to be reported among the Mexican tradescantias. The species is apparently endemic to the area around Real del Monte; it is known only from the type collection, *H. E. Moore, Jr., 3111,* and the collection cited above. *W. candida,* $2n = 24$ (Fig. 1b) is found in the alpine regions of central Mexico and Guatemala.

Specimens will be deposited in the Bailey Hortorium, Gray Herbarium, and Museum of the Department of Agriculture, San Jacinto, D.F., Mexico.

References

1. ANDERSON, E., and SAX, K. *Bot. Gaz.,* 1936, **97**, 433.
2. ANDERSON, E., and WOODSON, R. E. *Contr. Arn. Arb.,* 1935, **9**, 1.
3. DARLINGTON, C. D. *J. Genet.,* 1929, **21**, 207.

The Application of the Beer-Lambert Law to Optically Anisotropic Systems

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The determination of the concentrations of various substances in cell structures by the measurement of their absorption spectra in the cell is becoming a widely used experimental procedure (*3, 4, 6, 11*). Caspersson and his co-workers (*3*), for example, have made quantitative estimates of the relative, and sometimes absolute, concentrations of nucleic acids, proteins, and other substances in cells by the use of this technique.

The quantitative evalution of such microspectrophotometric measurements is dependent on the validity of the assumption that the decrease in intensity of a beam of monochromatic light on passing through a cell structure is quantitatively related to the number of molecules of absorbing material in the path of the light beam. Recognizing this limitation, Caspersson has shown (*1*) that in the passage of a light beam through microheterogeneous structures such as cytoplasm, the losses due to nonabsorption processes are relatively small and calculable. This consideration of nonspecific light losses, however, does not accomplish the complete validation of intracellular extinction measurements as an index of concentration. Even though the difference in intensities of the incident and transmitted light beams may actually be due to absorption of photons by a given cell constituent, it is still necessary to know the quantitative relationship between the amount of light absorbed and the number of molecules in the path of the light beam. Usually it is assumed that this relationship is described by the Beer-Lambert law, $E = \log I_0/I = \varepsilon cd$.[1]

This law may be derived using the following assumptions: (a) that a single molecular species is absorbing the light, and (b) that variations in concentration do not affect the interactions either between solute molecules or between solute and solvent in such a way that the prob-

[1] E = extinction, I_0 = intensity of the incident light beam, I = intensity of the transmitted beam, c = concentration of absorbing material in moles/liter, d = thickness of the sample in cm, and ε = molar extinction coefficient.

ability of absorption of a light quantum by a single solute molecule is changed. No published data are available on the applicability of the Beer-Lambert law to light absorption by molecules in the complicated states of aggregation characteristic of cell structures. Since interpretations of absorption measurements (e.g., by Caspersson and co-workers) are based on the above proportionality between c and E, and because the theoretical conclusions derived from them have been of considerable interest to biologists, it is necessary to consider to what degree the Beer-Lambert law is valid for the actual conditions of intracellular measurements. It is the purpose of this paper to examine one of these conditions—the orientation of the absorbing molecules—for its effect on the validity of the Beer-Lambert law.

It has been suggested (*9*) that the absorption by a molecule of a light quantum of appropriate energy is due to the excitation of electronic oscillations in the molecule. Except in the case of an optically symmetrical molecule, the probability of absorption of a given quantum depends on the spatial relationship between the molecule and the electric vector of the incident light. Specifically, this probability is proportional to $\cos^2 \theta$, where θ is the angle between the direction of the electronic oscillation (an optical axis of the molecule) and the electric vector of the light beam. Consequently, the probability of absorption is zero when the electric vector is perpendicular to the optical axis and increases to a maximum as the angle becomes zero. The effect of orientation on the absorption spectra of optically anisotropic molecules has been studied by Weigert (*16*), Taylor (*15*), Scheibe (*12*), Lewis and co-workers (*10*), Jelley (*7*), and Sheppard (*14*). If such molecules are examined with unpolarized light in dilute solution they show no unusual optical properties. In this case the electric vectors of the light beam are randomly distributed in a plane at right angles to the beam and the dissolved molecules are oriented at random. On the other hand, if the optically anisotropic molecules are oriented and examined with plane polarized light, then they exhibit dichroism: the extinction varies with the orientation of the plane of polarization. Caspersson (*2*) has shown that partially oriented films of thymonucleic acid exhibit this behavior at 265 mμ. Furthermore, the work of Schmidt (*13*), Frey-Wyssling (*5*), and others indicates that the nucleic acids in cell structures may be oriented to a greater or lesser degree. The same is true of other optically anisotropic substances occurring in cell structures.

Since practically all cellular extinction values are obtained with unpolarized light, one might expect that the above factor would not interfere with the measurements. As we shall show below, such an expectation is unwarranted. A consideration of the quantitative relationship between the number of molecules in the light beam and the extinction (E) shows that this function is dependent on the degree of orientation of the optical axes of the molecules *even when the measurements are made with unpolarized light.*

We shall limit our considerations to a molecule in which there is possible a transition which corresponds to a distinctive axis. In other words, the molecule must have sufficiently low symmetry so that it has one optical axis which is different from all of the other possible optical axes. The cyanine dyes fall into this category; the optical axis corresponding to the main absorption band of longest wavelength coincides with the largest dimension of the molecule (*9*). Malachite green also falls into this group. It has three different optical axes; one corresponds to the absorption band at 625 mμ and another to the band at 423 mμ (*8*). It should be noted that crystal violet also has three optical axes, but that two of them are identical. The discussion which follows would pertain only to the one axis which is distinctive. The purines and pyrimidines would, like malachite green, have three different optical axes. The following comments apply here to absorption corresponding to any one axis.

The relationships between the extinction (E) for *unpolarized light* and the number of molecules of the type described above have been derived for three degrees of orientation of a given optical axis relative to the light beam, as follows:[2]

Case I. Completely random orientation of the optical axes. In this case the relationship is

$$\log_e \frac{I_0}{I} = \frac{\alpha N}{3}, \text{ or } E = \frac{\alpha N}{3 \times 2.303}.$$[3]

Case II. The optical axes all lie in a stack of planes with each plane perpendicular to the light beam, but the axes in each plane are randomly oriented. Here the relationship takes the form

$$\log_e \frac{I_0}{I} = \frac{\alpha N}{2}, \text{ or } E = \frac{\alpha N}{2 \times 2.303}.$$

Case III. The optical axes are all parallel and the light beam is in a plane perpendicular to these axes. The relationship now becomes $\log_e \frac{I_0}{2I - I_0} = \alpha N$, or $E = 0.3 - \log_e (e^{-\alpha N} + 1)$.

These three functions are plotted in Fig. 1. It can be seen that for the case of a completely oriented group of molecules (Curve 3, Case III) E is approximately proportional to the number of molecules only at extinction values less than 0.15. Above this value the slope of the curve changes until it approaches zero, so that as E approaches 0.3 the amount of light absorbed becomes independent of the number of molecules. In other words, if a completely oriented aggregate of optically anisotropic molecules such as we have described above is examined with monochromatic unpolarized light, then the amount of incident light absorbed can never be above 50%. This same type of behavior is exhibited by a sheet of Polaroid. If we consider a group of optically anisotropic molecules whose orientation corresponds to Case II (see Curve 2, Fig. 1), the relationship between E and N is a linear one, but not quantitatively the same as if the molecules were randomly oriented. For a given number of molecules, E for random orientation in a plane is 50% greater than

[2] We are indebted to Prof. S. I. Weissman for the derivation of these equations and for many helpful discussions of the problem.

[3] In these equations N = the number of molecules per unit area of light beam, and α = the absorption coefficient per molecule when the optical axis of the molecule and the electric vector of the light beam are parallel.

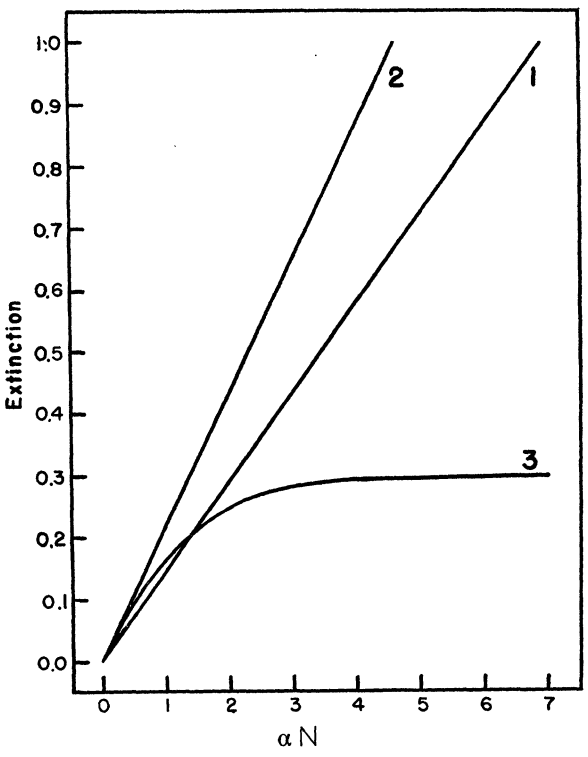

FIG. 1.

E for random orientation in three dimensions. If similar calculations are made for samples in which only a part of the absorbing molecules are fully oriented, one obtains curves lying between Curves 1 and 3 (Fig. 1). These considerations lead us to the conclusion that the Beer-Lambert law must be applied with caution to groups of optically anisotropic molecules which may be more or less oriented.

As previously noted, there is evidence for some degree of orientation in practically all cytological structures other than liquid vacuoles (*5*, *13*). Further, most biologically important molecules are optically anisotropic. Hence the assumption that the concentration of such an absorbing substance in a cell structure can be calculated from the ratio of its extinction value to the extinction of a given number of molecules of the substance in solution is not valid. Furthermore, unless the orientation of the optical axes in two cytological structures is identical, the assumption that the ratio of their extinctions per unit thickness is equal to the ratio of their content of such absorbing material is also invalid. Thus, in cytological absorption measurements made with unpolarized light, variations in extinction values may arise from differences in the degree of orientation rather than from differences in content of any specific substance. It seems clear that the entire problem of interpreting intracellular extinction measurements needs to be reexamined with the realization that one is dealing not with true solutions but with oriented aggregates of molecules.

References

1. CASPERSSON, T. *Skand. Arch. Physiol.*, 1936, **73**, Supp. 8.
2. ———. *Chromosoma*, 1940, **1**, 605.
3. ———. *Sympos. Soc. exp. Biol.*, 1947, **1**, 127.
4. COMMONER, BARRY. *Ann. Mo. bot. Gard.*, 1948, **35**, 239.
5. FREY-WYSSLING, A. *Submicroscopic morphology of protoplasm and its derivatives.* New York: Elsevier, 1948.
6. GERSH, I. and BODIAN, D. *J. cell. comp. Physiol.*, 1943, **21**, 253.
7. JELLEY, E. E. *Nature*, Lond., 1936, **138**, 1009; 1937, **139**, 631; *Ind. eng. Chem.*, 1941, **13**, 196.
8. LEWIS, G. N. and BIGELEISEN, J. *J. Amer. chem. Soc.*, 1943, **65**, 2102.
9. LEWIS, G. N. and CALVIN, M. *Chem. Rev.*, 1939, **25**, 273.
10. LEWIS, G. N., *et al. J. Amer. chem. Soc.*, 1941, **63**, 3005; 1942, **64**, 2801; 1943, **65**, 520.
11. POLLISTER, A. W. and RIS, H. *Cold Spr. Harb. Sympos. quant. Biol.*, 1947, **12**, 147.
12. SCHEIBE, G. *Kolloid Z.*, 1938, **82**, 1; Scheibe, G. and Kandler, L. *Naturwiss.*, 1938, **26**, 412.
13. SCHMIDT, W. J. *Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma.* Berlin: Borntraeger, 1937.
14. SHEPPARD, S. E. *Rev. mod. Phys.*, 1942, **14**, 303.
15. TAYLOR, W. H. *Z. Krist.*, 1935, **91**, 450.
16. WEIGERT, F. *Z. Physik*, 1921, **5**, 410.

Opalescence of Serum after Total Body X-Irradiation as a Prognostic Sign of Death[1]

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During an investigation of the clotting reaction of blood after irradiation with 200-kv X-rays, the author noted the appearance of a marked opalescence in the serum and plasma of rabbits, which subsequently died a few days after exposure to a single lethal dose of total body irradiation. This opalescence appeared within 24 hr following the exposure to radiation. In all cases, it disappeared completely 72 hr after exposure. A review of the literature has failed to reveal any mention of this phenomenon.

Rabbits of the New Zealand white strain were given over the total body single doses of 200 kv X-irradiation, calibrated in air by a Victoreen ionization chamber. Dosage ranged from 200 to 1000 roentgens (r). Blood samples were obtained by cardiac puncture before radiation and at various intervals after radiation (up to 30 days). Serum was obtained from the clotted blood and plasma was obtained by centrifugation of either citrated or oxalated blood. In all cases the opalescence, when present, was noted in both serum and plasma.

The opalescence was noticeable as a pearly white tint homogeneously distributed throughout the sample. Various degrees of intensity have occurred and can be classified as marked, moderate, and slight, as shown in Fig. 1. All animals showing marked opalescence died as a

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