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PIGMENT CONVERSION IN THE FORMATIVE RESPONSES OF PLANTS TO RADIATION

BY S. B. HENDRICKS, H. A. BORTHWICK, AND R. J. DOWNS

AGRICULTURAL RESEARCH SERVICE, PLANT INDUSTRY STATION, BELTSVILLE, MARYLAND

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Many aspects of growth and maturation of plants are known from the close similarity of their action spectra to be controlled by a reversible photoreaction.[1] Among these responses are germination of seeds of some higher plants[2] and of fern spores,[3] photoperiodic control of flowering,[4] coloration of some fruits,[5] and etiolation.[6] The photoreaction can be written as follows:

$$\text{Pigment}_1 + (\text{A reactant}) \underset{\text{Far-red radiation (Max. near 7350 A) or Darkness}}{\overset{\text{Red radiation (Max. near 6600 A)}}{\rightleftharpoons}} \text{Pigment}_2 + (\text{Changed reactant})$$

(red-absorbing) (far-red absorbing)

The essential contribution herein is the expression of the physiological response as a function of the fractional conversion of the pigment into the effective form,

whichever that may be, and the finding of minimum values for the products of the respective absorption coefficients and the quantum efficiencies for conversion of the two pigment forms in vivo. These ends are attained without knowledge of the chemical nature of the pigment or of its concentration. The method depends upon physiological information and upon the fact that the photoreaction is reversible and follows first-order kinetics with respect to energy in both directions. That the reaction rate is not limited by molecular collision in either direction is shown by temperature coefficients of unity for the photoreactions in both directions for germination of lettuce seed between 6° and 26° C.[7]

The method of analysis is similar to the one used by Warburg and Negelein[8] to find the products of the absorption coefficients and quantum efficiencies for dissociation of cytochrome oxidase–carbon monoxide by photoreversal in vivo of the poisoning with carbon monoxide of the respiration function of the oxidase.

After irradiation with energy E in an absorption region $\lambda_1 \pm \Delta\lambda_1$, an unknown fraction F of the pigment in one form is converted to the other according to the differential equation $dF/dE = k(1 - F)$. The solution of this equation is $kE = \log [1/(1 - F)]$. If the energies required to give two physiological responses, 1 and 2, have the relationship $\alpha = E_2/E_1$, then $\alpha \log [1/(1 - F_1)] = \log [1/(1 - F_2)]$. The same responses in the reverse direction, obtained with the energies of the reversing radiation in an absorption region $\lambda_2 \pm \Delta\lambda_2$, will have a different ratio β, with $\beta \log [1/F_2] = \log [1/F_1]$. Thus, if α and β are measured for any two arbitrary degrees of physiological expression, values of F_1 and F_2 can be calculated. The only limitation for the equations as set up is that the dark reaction be slow and the radiant energy used for one response be relatively free of that inducing the other, but $\Delta\lambda$ need not be small.

The products of the absorption coefficients, ω (cm^2/gram molecular weight), and the quantum efficiencies, ϕ, for both the red and the far-red form of the pigment can be determined from the fraction, F, of the pigment converted in a given spectral region by a given incident energy E (in einsteins/cm^2). Thus $\omega\phi X$ = number of effective quanta = F/E, where X is the fraction of the incident energy reaching the region of absorption. The molar extinction coefficient, in American usage, is $[\omega/1{,}000] \log_e 10$.

Internode elongation of the bush Pinto bean is the first example considered. If these beans are grown with daily light periods of several hours under light sources such as "white" fluorescent lamps having low intensities in regions beyond 7000 A, the developing internodes are short. But if they are exposed after the end of each light period on four successive days to moderate energies, which can be delivered in a few minutes or less, in the region beyond 7000 A the internodes increase in length several fold; essentially the beans are changed from "bush" to "pole" at this stage of growth. If the plants are irradiated with red light following exposure to high energy in the far-red region of the spectrum at the end of the light period, the lengthening stimulus of the far red is nullified. The internodes may attain lengths, depending on the red energies, no greater than those of control plants. This is the reversal. Essentially the length of the second internode of the bean is a function of the fraction of the pigment converted by radiation, and it can be used as an index of the conversion irrespective of the nature of the function.

Results of an experiment are given in Table 1. The mean internode lengths are the averages for lots of 24 plants. If two responses are taken as the internode

lengths 40.3 and 65.2 mm., respectively, the value of β for far-red radiation is 8.00/4.00 = 2.00. From interpolation on a plot of the third columns against the first columns, the reverse times to give the two internode lengths with the arbitrary red

TABLE 1

MEAN LENGTHS OF SECOND INTERNODES OF PINTO BEANS RESULTING FROM IRRADIATIONS FOR VARIOUS PERIODS WITH FAR-RED RADIATION AND WITH RED RADIATION FOLLOWING SATURATING FAR-RED EXPOSURES

PERIODS OF IRRADIATION (MIN.)*	MEAN INTERNODE LENGTHS FOLLOWING IRRADIATION (MM.) Far-red	Red	PERIODS OF IRRADIATION (MIN.)*	MEAN INTERNODE LENGTHS FOLLOWING IRRADIATION (MM.) Far-red	Red
0.00	26.3	88.0	2.00	31.7	59.6
0.25	27.0	86.6	4.00	40.3	50.3
0.50	28.7	81.3	8.00	65.2	35.0
1.00	29.6	77.5	16.00	96.0	30.7

* Each radiation source had a power equivalent to about 0.25 milliwatt/cm^2 at the wave length of maximum action integrated over the region of action.

radiation source are 5.95 and 1.63 minutes, respectively, giving α = 5.95/1.63 = 3.65. The corresponding solutions of F_1 and F_2 are 0.10 and 0.316. A measure of precision is indicated by calculation for β = 2.00 and α = 3.30 or 4.00. The respective values of F_1 and F_2 are 0.12 and 0.348 and 0.08 and 0.284. The value of k for the far-red irradiance is accordingly 0.35 per minute of irradiation. The complete curve of physiological response as a function of pigment conversion by far-red radiation as calculated for this value of k is shown in Figure 1. The fact that

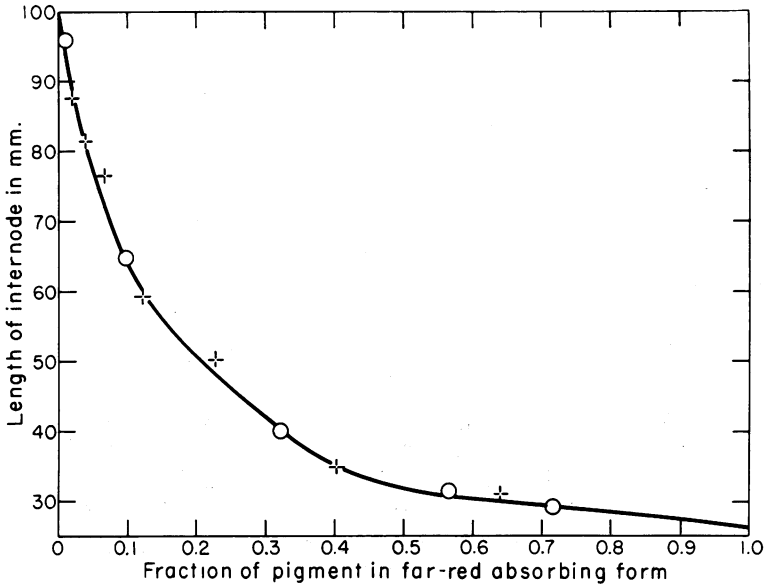

FIG. 1.—Internode lengths of Pinto beans as a function of pigment conversion: circles are for irradiation in the red part of the spectrum of beans with the pigment initially in the red-absorbing form, and crosses are for irradiation in the far red with the pigment initially in the far-red-absorbing form.

the points for both red and far-red radiation fall along the same curve validates the first-order kinetics.

Values of $\omega\phi X$ are next determined for the pigments in the spectral regions of

maximum action. These values are based upon internode lengths for beans from which one of the two primary leaves was removed so that the plants might be irradiated in a spectrum on four successive nights following their growth periods under fluorescent lights. One group of 18 plants was irradiated in the region 7100–7600 A and another group in the region 6300–6700 A after they had received a saturating exposure to far-red radiation. The first group received an incident energy of 9.1×10^{-7} einstein/cm^2 in each daily irradiation and the second 1.84×10^{-7} einstein/cm^2. The fractions of the pigment converted for the two groups were 0.81 and 0.21, respectively, as based on the variation of internode lengths of similar beans, with a single leaf, as a function of incident energy. The values of $\omega\phi X$ are 0.09×10^7 and 0.11×10^7 for the far-red and the red-absorbing forms of the pigment, respectively.

Before this procedure is extended to other examples, attention is again directed to the fact that the "mean" physiological response of bean internodes serves as an index of the pigment conversion. Each internode value listed in Table 1, for example, is a mean obtained from 24 individuals that varied in internode lengths. Reasons for the variations were many, including variation in the fraction of the pigment converted by a given incident energy.

The procedure used for analysis of internode growth may be applied with equal validity to germination of seeds, despite differences in the character of results obtained in the two cases. The length of an internode following a light treatment may have any value between that for the untreated and that for saturated control plants, but the response of a seed following light treatment is either germination or lack of germination. After red irradiation, some imbibed seeds germinate upon being held in darkness at a favorable temperature while others remain dormant. At a slightly higher red energy the germination requirements of these same seeds and those of an increment are met. The seeds of this increment are different from all others in that their energy requirements for germination are greater than the first energy given but less than the second. The crucial point is that the analysis is based only on such an increment, and this increment is the same whether reached by promotion of germination with red or inhibition of germination with far-red radiant energy.

The germination responses of lettuce, *Lactuca sativa*,[7] and of pepper grass, *Lepidium virginicum*,[9] seeds to an increasing series of red and far-red energies have been measured, as illustrated for *L. virginicum* in Figure 2. Action spectra giving the incident energies required for given percentage germinations are also known.[7, 9] All the information thus is available for calculations of the germination response as a function of the fractional conversion of the pigment and for finding the product of the absorption coefficient, the quantum efficiency, and X, that is, $\omega\phi X$. The germination functions for the two kinds of seed as calculated are shown in Figure 3.

Lepidium seed imbibed in 0.2 per cent KNO_3 solution requires about 4.5×10^5 ergs/cm^2 at the action maximum of 6400–6600 A for 50 per cent germination at a constant temperature of 20° C. This per cent germination requires 0.17 conversion of the pigment. An energy of 7.0×10^4 ergs/cm^2 at the action maximum of 7200–7500 A is required for 50 per cent inhibition of fully promoted seed. The values of $\omega\phi X$ calculated from these quantities are 0.067×10^7 for the red-absorbing form of the pigment and 1.9×10^7 for the far-red-absorbing form. For lettuce seeds

these values are 5.0×10^7 and 0.11×10^7, respectively, with 50 per cent germination corresponding to 50 per cent pigment conversion.

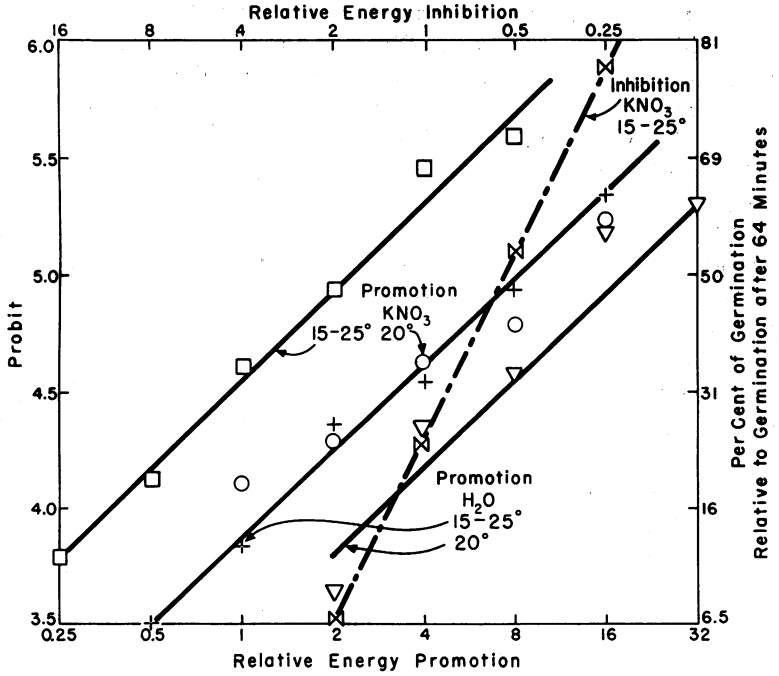

FIG. 2.—Variation in germination of *Lepidium virginicum* seeds with times of irradiation for promotion in the red part of the spectrum and with inhibition in the far-red after full promotion. Conditions under which the seeds were held are indicated.

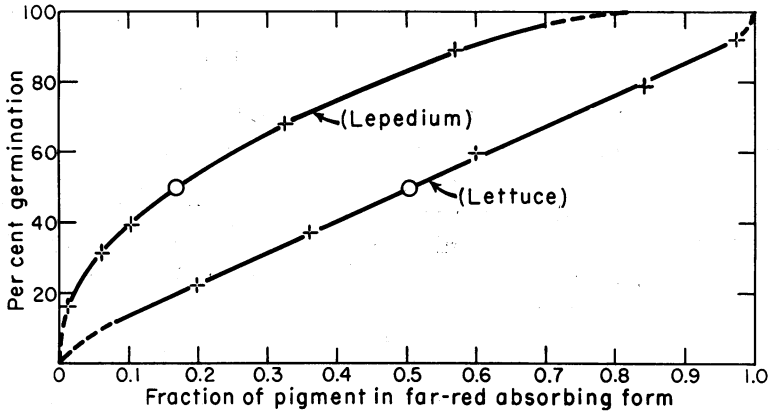

FIG. 3.—Variation in germination of *Lepidium virginicum* seeds and of Grand Rapids lettuce seeds with pigment conversion. The circles represent the interpolated values for 50 per cent germination.

An immediately evident fact is that the values of $\omega\phi X$ vary for the several objects, but the question whether the variation is in ω, ϕ, or X involves further considerations. These considerations can be based on results for germination of *L.*

virginicum seed as reproduced in Figure 2. As conditions affecting germination are varied, the several lines expressing promotion of germination as a function of incident energy are displaced without change of slope. This means that the fraction of pigment converted for a given germination is constant but that the incident energy required for this conversion varies. Since the seed is not changed in its light-absorbing or light-scattering characteristics by the several conditions, X is approximately constant. The conclusion is that ϕ, the quantum efficiency for pigment conversion, varies with conditions of seed germination. A deduction to account for variation in ϕ is that a reactant other than the pigment is involved in the photoreaction in both directions.

A probable maximum value of the quantum efficiency for pigment conversion per chromophoric group is 1.0. The value of X also cannot be greater than 1.0, and an order of magnitude for seeds and leaves would be in the range of 0.1–0.5 or less. The maximum values found for $\omega\phi X$ are 5.0×10^7 for red absorption in lettuce seed and 1.9×10^7 for far-red absorption in *Lepidium* seed. The minimum value of the molecular absorption coefficients of the pigment, $\omega/1{,}000$, in both forms at the action maxima, then, are of the order of 10^5. The oscillator strengths, accordingly, are of the order of 1.0, the intrinsic time constant of the excited states being of the order of 10^{-9} second.

The values of absorption coefficients of the two pigments in the region between 4000 and 5200 A are of the order of 0.01 times those at the action maxima in the red and far-red portions of the spectrum. This was shown[7] by the rate of approach with increasing radiant energy to an equilibrium physiological response in the region 4000–5200 A of objects in which the pigment systems were displaced to the two extremes by absorption in the red or far red. The method is independent of possible overlapping absorptions. The significance here is that cyclic tetrapyrolle systems, such as are present in porphyrins and might naturally be suspected here, are eliminated as possibilities for the pigment structures on account of their strong absorptions in the region in question. Instead, the pigments are probably open-chain conjugate systems, which include the phycocyanins of the blue-green algae as possibilities. The molecular extinction coefficient of c-phycocyanin at the absorption maximum of 6150 A is 2.76×10^5 as measured by Svedberg and Katsurai.[10] The corresponding probable value of the molecular absorption coefficient referred to a single chromophoric group is 1.59×10^5, based on four groups in each molecule. Allophycocyanin has an absorption maximum at 6500 A[11] and its relative absorption coefficients at other wave lengths correspond closely to those of the red-absorbing form of the pigment. Polyenes, of types as yet unknown, are not excluded as possible structures of the pigments.

The details of the photochemical reaction are of interest. With the reactant's presence now demonstrated, its general nature can be anticipated. The photochemical reaction probably involves the radiationless transition of the molecule from an excited singlet- to a triplet-state. The triplet-state free radical probably accepts hydrogen from the reactant in one form and donates hydrogen to the oxidized reactant in the other form. The variations in quantum efficiency arise from the variations in the ratio of the oxidized and reduced forms of the reactant in the biological object depending upon ambient conditions. For the photoreaction to be first order with respect to energy in the presence of the reactant implies con-

tinuous association of either the oxidized or the reduced reactant and the pigment, as is characteristic of condensed systems.

The pigment in its far-red-absorbing form is evidently biologically effective. This deduction rests on three arguments, none of which is rigorous. The first argument is that, in two of the three objects considered, half-maximum physiological response was obtained with conversion of the order of 0.1 of the pigment from the red- to the far-red-absorbing form, and conversion of 0.75 approached physiological saturation. This type of dependence upon the fraction of the active far-red-absorbing form corresponds to that usually found in enzymatic control of a reaction. The second argument rests upon the fact that some seeds with the pigment in the red-absorbing form will lie dormant for about a century but will germinate in a few days when exposed to light, with consequent appearance of the far-red-absorbing pigment form. Inhibitors of germination present in seeds usually disappear after a year or more, so that discharge of the final dormancy would seem to depend upon the far-red-absorbing pigment form acting as an activator rather than the red-absorbing form acting as a suppressor of an inhibitor. The third argument is that photoreversibility in photoperiodism is lost, an indication of biological change, shortly after the pigment is converted to the far-red-absorbing form.

The pigment is not sensitive to destruction by photo-oxidation in either of the two forms. It is freely available for photoconversion. This follows from the fact that the pigment system was fully effective in the green bean leaf after an 8-hour light period at high energy, as shown by the results given in Table 1. During the day the fraction of the pigment in the far-red-absorbing form in plants growing in the open is determined by the ratio of the effective red and far-red radiation in the radiation from the sun and by the ratio of the oxidized to the reduced form of the reactant in the leaf. With darkness the far-red form of the pigment changes slowly by a thermal reaction toward an equilibrium with the red-absorbing form.[7]

Summary.—Variations of physiological response with the fraction of pigment conversion as obtained from the reversibility and the first-order character of the conversion with respect to energy in the controlling reversible photoreaction were measured for internode lengthening and seed germination. Knowledge of this fraction permitted calculation of absorption coefficients and quantum efficiencies for pigment conversion. The method is a general one applicable to photoperiodism and other responses controlled by the reversible photoreaction.

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*PROGRESSIVE CONFIGURATIONAL CHANGES, DURING CELL DIVISION, OF CELLS WITHIN THE APICAL MERISTEM**

By Edwin B. Matzke

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY

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During the past several decades the three-dimensional shapes of cells which are essentially undifferentiated morphologically have been studied by a number of investigators. Many different organisms have been used. Some research has also been done on configurational changes, during cell division, in epidermal layers. Thus far, however, no analysis has been made anywhere of the shape changes, step by step, which take place during the course of cell division. Furthermore, no study whatever, from this standpoint, has heretofore been carried out on cells deep within a tissue. In view of the increased emphasis, both morphological and physiological, placed upon terminal and lateral plant meristems, the internal cells, within the stem apex, were chosen for this study of the progressive shape changes of dividing cells.

Previously comparisons have been drawn between the shapes of interphase cells and those of cells in division in the epidermis of the apical meristem of *Anacharis densa* (Matzke[1]); Mozingo[2] has traced the configurational alterations, during division, of epidermal cells of living roots of *Phleum pratense*. Various aspects of cell division have also been considered in a series of publications by Lewis.[3]

It is now known as a result of the research of numerous workers that cells which are essentially undifferentiated morphologically, in tissues, have an average of approximately 14 faces and that no one type is achieved. Much of the literature dealing with this has already been summarized (Matzke[4]). Cells with 8 hexagonal and 6 quadrilateral faces, as in the tetrakaidecahedra of Kelvin,[5] occasionally occur, but they are infrequent. In analyzing the factors responsible for shape determination, experiments have been performed on compression of lead shot by Marvin[6] and on bubbles in foam by Matzke.[7] Comparisons have been made between the shapes of cells in tissues and grains in a metal alloy by Williams and Smith[8] and by Smith.[9] Detailed analyses of similarities and differences in the data presented below and the results of previous investigators will be published elsewhere.

Materials and Methods.—Vigorously growing vegetative stem apices of *A. densa* were fixed in Craf, sectioned lengthwise at 40 μ, and stained in Feulgen and in ruthenium red. Only the median section of a tip was used, and it was examined under oil immersion with a Leitz Ortholux microscope. The cells were selected from the region of the apex above the visible origin of lateral primordia, and they were at least 4 cells from the periphery in any direction. This region is shown by the brace in Figure 1.