DEPTH CONTROLLED DEUTERON IRRADIATION OF LACTUCA SATIVA SEEDS. II. ENERGY LOSS IN THE OUTER SEED LAYERS

JOHN W. PREISS and S. KLEIN

DEPARTMENT OF BIOPHYSICS AND DEPARTMENT OF BOTANY, JOSEPH H. HENRY LABORATORIES, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

In a previous paper (2) the germination and subsequent growth of deuteron-irradiated lettuce seeds was described, with special emphasis on the influence of penetration depth. It was shown that depth of penetration is the predominant factor in eliciting the effects described, i.e., germination percentage and root length are affected at different depth levels. The general phenomena observed in our experiments do not differ essentially from effects of radiation described by other workers. It was possible, however, to pin down the radiation effects locally, and to make a more detailed analysis of the radiation data by following the energy loss through the various layers. The results obtained suggest that this application of techniques of deuteron bombardment to a biological problem is of value in the study of multicellular organisms where a localization of certain physiological activities is required.

Since range accuracy and dosimetry are of utmost importance in work of this kind these aspects will be discussed in this article. A method by which the energy lost in the outer seed layers was determined with the deuterons themselves will be described. It will be shown that germination induced by ionizing radiation can be ascribed solely to effects produced on the endosperm.

Determination of Radiation Energy Loss in the Outer Seed Layers: Determination of the relative stopping power of the outer seed layers with respect to air (N.T.P.): In order to obtain the mean ranges of deuterons in an average protein of density 1.3, the rate of energy loss per unit distance was calculated with the Bethe-Bloch formula, and a numerical integration of the reciprocal of this rate was performed. The average excitation potentials used for carbon, hydrogen, nitrogen, and oxygen were those listed by Pollard (3). It was found that the mean range of deuterons in proteinaceous material, measured in microns, is five times their mean range in aluminum, expressed as mgm/cm². In stopping power, 1.53 mgm/cm² of aluminum is equivalent to 1 cm of air at N.T.P. (4).

Values for protein were used when calculating depth of penetration into the embryo. They could not, however, be applied directly to the layers surrounding the embryo. The problem arose not so much from the different chemical constitution of these layers but from their inhomogeneous structure. The fruit coat, the external part of the seed, has a very uneven surface and contains large air spaces. The endosperm, the layer closest to the embryo, is much thinner but more dense. Therefore, direct measurement of the thickness of these structures would not give an indication of their ability to retard deuterons. A method had to be found whereby their stopping powers relative to air or aluminum could be measured. With this information their thicknesses could be expressed in equivalents on a calculated average protein scale.

A range cell containing the usual variable air pressure chamber and continuously evacuated beam pick-up chamber was constructed. The variable pressure chamber, which is 6.9 cm long, was isolated from the cyclotron vacuum and the beam pick-up vacuum by 5.5 mgm/cm² and 11.0 mgm/cm² aluminum foils respectively (equivalent in stopping power to 3.6 cm and 7.2 cm of air). Since a 4-Mev deuteron is stopped by about 15 cm of air at N.T.P., these foils, together with the air column in the range chamber at somewhat less than atmospheric pressure, stopped the beam. A fixture containing five no. 68 drill holes was placed in the chamber between the foils, either with the holes open, or with fruit coats or combined seed coats and endosperms clamped in place so as to

---

1 Received January 20, 1958.
2 Participation of J. W. Preiss was made possible by a grant from the John A. Hartford Foundation to the Yale Biophysics Department.
3 Present address: Hebrew University, Jerusalem, Israel. Participation of S. Klein was made possible by grants from the Anna Fuller Fund and from the Yale University Atypical Growth Committee (American Cancer Society) to A. W. Galston.

---

Fig. 1. "Number-distance" curves used to measure the stopping power of the outer seed layers relative to that of air. O—Air, corrected to N.T.P. +—Air plus the fruit coat. △—Air plus the endosperm and seed coat. The mean range of 15 cm (air curve) corresponds to a kinetic energy of 4.1 Mev for the bombarding deuterons.
block them. Although the amount of beam current coming through the five holes was small it could be read on a sensitive galvanometer which was not quite critically damped. The three range curves in figure 1 were obtained by measurement of the change in detected current as the air pressure was varied. The horizontal separations were measured at several ordinate values. The average thickness of the seed coat—endosperm was found to be equivalent to $11.5 \pm 1 \mu$ of 1.3 density protein; that of the seed coat alone was found to be $5 \pm 1 \mu$ of protein. This latter is a mean value, for the range curve of the fruit coat tends to approach the range curve of air near the tail. This is an indication that a small number of deuterons traverse sections equivalent to less than $5 \mu$ of protein. Furthermore, when cotyledon damage was observed, the damaged area was (for the least energetic deuterons which reached this region) traversed by narrow strips of undamaged tissue. Therefore, since these followed fruit coat ridges, it must be concluded that the ridges represent much more than $5 \mu$ of protein. However, the only effect of taking the average value for Bragg curve calculations is the prediction of too high an energy loss in the dense inner regions of the seed. Since our basic aim is to show that deuteron irradiation of the embryo is not required to overcome the dark dormancy of the lettuce seed, our argument will therefore be more conservative than necessary.

Construction of Bragg curves for a protein absorber: If a monoenergetic beam of ionizing particles passes through a layer of matter, the particles emerge with a certain distribution of energies about some mean value, since all of them do not produce exactly the same number of ionizations and excitations. Therefore, the distances they travel before being stopped in an absorber will likewise be distributed about a mean value. The width of this distribution is a measure of the "straggling" of these particles.

If the aim of a radiation experiment is, as in this study, to find out if or to what extent a physiological function is located in a certain layer of an organism, the straggling phenomenon may place a serious limitation on the depth resolution. This is especially true when the thickness of the layer to be investigated is comparable to the straggling distance. Since the protein-equivalent thickness of the endosperm is only about $10 \mu$, special consideration had to be given to this problem.

In figure 1, the distance along the range axis, from the point where the detected current just begins to fall to the point where it is just detectable was measured. This distance represents about 1.3 cm of air at N.T.P. or about $10 \mu$ of average protein (density 1.3). A deuteron which can traverse 1.3 cm of air has a kinetic energy equal to about 0.8 Mev. Since the sloping portion of a "number-distance" curve can be approximated by a normal distribution function about the mean range, R microns of protein (1), it follows that a plane, normal to the deuteron beam at $(R - 5)$ microns, can be considered as a source of deuterons with energies varying from 0.0 to 0.8 Mev. From this it follows that a normal density function, the mean of which is at R microns, can be used as an energy weighting function. This procedure was carried out in constructing Bragg curves for a protein absorber, some of which are shown in figure 2. The wide energy distribution undoubtedly allows for possible cyclotron beam energy inhomogeneity. The assumption was made that straggling was produced to the same degree among the original 4.1-Mev deuterons traversing a thin aluminum foil and penetrating deep into the lettuce seed, as among those traversing a thick aluminum foil and penetrating a shorter distance into the seed. This permits a single straggling correction for all mean ranges, since only atoms of low atomic number are involved, both in the foil and in the organism.

The Bragg curves show that the energy loss rate varies from about $7 \times 10^{-9}$ n ergs/cm$^2$/micron in the 5th micron, beyond the mean range to $1.25 \times 10^{-7}$ n ergs/cm$^2$/micron in the 5th micron before this point. (n = the applied dose in deuterons/cm$^2$.) At the mean range, it is about $8 \times 10^{-8}$ n ergs/cm$^2$/micron of protein. The total integrated energy loss beyond the calculated mean range is about $10^{-7}$ n ergs/cm$^2$. The peak of the Bragg curve for undamaged cotyledon is at 86%, for partially damaged at 69%, and for totally damaged at 53%.
As an illustration of the deuterons with a mean penetration of 7.5 \( \mu \) in protein material, \( 2 \times 10^{12} \) deuterons lose \( 2 \times 10^6 \) ergs/cm\(^2\) in the seed, \( 2 \times 10^2 \) ergs/cm\(^2\) of which appear beyond the mean range. These data are taken from the 53% germination curve of figure 2. Only \( 1.4 \times 10^4 \) ergs/cm\(^2\) are lost in the 5th micron beyond the mean range. No visible embryo damage was seen with the highest doses applied. If \( 2 \times 10^{12} \) deuterons/cm\(^2\) penetrate to a mean depth of 15 \( \mu \), about \( 2 \times 10^5 \) ergs/cm\(^2\) out of a total of \( 4 \times 10^6 \) ergs/cm\(^2\) appear beyond this point. This treatment causes a very slight but visible embryo damage, which is due to a small amount of energy straggling past the 16.5 \( \mu \) depth. (See fig 2.)

**Localization of the Seed Region Involved in Deuteron-Induced Germination:** It was shown (2) that when deuterons were stopped in the endosperm a minimum dose of about \( 10^{13} \) deuterons/cm\(^2\) completely overcame the light requirement for lettuce seed germination. When the deuterons penetrated part of the embryo itself, a dose about an order of magnitude lower produced the same effect. The former case establishes the fact that ionizations do not have to occur in the embryo to initiate germination, i.e., it is sufficient to irradiate only the endosperm. However, the latter case in which both endosperm and embryo are irradiated suggests that it may not be necessary to irradiate the endosperm. A more critical analysis will show that irradiation of the endosperm is both necessary and sufficient to obtain an increase in dark germination.

In figure 2 various Bragg curves have been plotted for a protein absorber. The boundaries between the lettuce seed layers, as previously established, have been included. The curves represent the energy loss rates for the five deuteron irradiations, each resulting in a different germination percentage, which are listed in table I.

For the mean penetration ranges here represented, germination can always be raised to 100% by increasing the number of deuterons per square centimeter (dose). The 53% and 86% curves, for which the mean range is 7.5 \( \mu \), illustrate this. No counter effect, even with a dose as high as \( 10^{14} \) deuterons/cm\(^2\), was ever observed. A low germination due to a lethal effect occurred when penetration depths were of the order of 100 \( \mu \).

### Table I

**Irradiations for Which the Bragg Curves in Figure 2 Were Constructed**

<table>
<thead>
<tr>
<th>Deuterons/cm(^2)</th>
<th>Mean Penetration</th>
<th>Dark Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1.8 \times 10^{12} )</td>
<td>7.5 microns</td>
<td>53%</td>
</tr>
<tr>
<td>( 6.0 \times 10^{11} )</td>
<td>20.0 microns</td>
<td>60%</td>
</tr>
<tr>
<td>( 7.2 \times 10^{11} )</td>
<td>7.5 microns</td>
<td>86%</td>
</tr>
<tr>
<td>( 1.8 \times 10^{10} )</td>
<td>36.0 microns</td>
<td>100% (A)</td>
</tr>
<tr>
<td>( 1.8 \times 10^{2} )</td>
<td>15.0 microns</td>
<td>100% (B)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>30%</td>
</tr>
</tbody>
</table>

**Fig. 3.** Energy loss in the endosperm as a function of germination percentage. (Integrals of the five sample Bragg curves of figure 2 from 7 to 16.5 \( \mu \).) Such a single-valued function would not be obtained for integrals over the fruit coat or embryo regions.

That the endosperm is the seed region responsible for deuteron induced germination is deduced as follows. The sensitive seed region must lie partly or wholly within the regions bounded by the origin and the range axis intercepts of the Bragg curves (fig 2) since deuteron induced germination occurs in each case. Since germination is a function of dose, it follows that it must also be a function of the energy lost throughout this sensitive region. Furthermore, it is a non-decreasing function of the energy lost in the sensitive region, because no dose was ever high enough to decrease germination even when the mean range of the bombarding deuterons was 36 \( \mu \). Both dose (number of deuterons/cm\(^2\)) and deuteron energy determine the energy loss per \( \mu \) in the sensitive layer and therefore the energy loss throughout that layer. Therefore, no Bragg curve associated with a certain germination percentage should ever lie above another with a higher percentage in the region where deuterons induce germination. In other words, whenever germination percentage cannot be represented by a non-decreasing function of the energy loss in a particular layer of the seed, this layer must be discounted as far as germination effects produced by ionizations are concerned.

In the region of the fruit coat total energy losses (integrals) for the 53% and 86% curves are higher.
than those for 100 \% curves. Since there exists no non-decreasing functional relationship between germination percentage and energy loss in this region, ionizations in the fruit coat cannot be responsible for the induced germination. Likewise, in the region of the embryo there is a 69 \% germination curve which lies above a 100 \% curve. (In both of these cases visible embryo damage could be seen.) Therefore, germination increase cannot be ascribed to ionization effects in the embryo. However, the required functional relationship between germination percentage and energy loss is satisfied in the endosperm region (fig 3). The points on this curve were obtained by integrating the five rate curves of figure 2 from 7 to 16.5 \mu.

It can be concluded that a sub-surface shell-like region of the seed exists where ionizations induce germination. The measured values of the stopping powers of the outer seed layers relative to a standard protein absorber allow us to identify this region as the endosperm.

**Summary**

Low energy deuterons were used as probes to establish which morphological entities of the lettuce seed are responsible for ionization-induced germination. For this purpose, the deuteron retarding ability of the fruit coat and seed coat-endosperm was measured with a range cell. Bragg curves for irradiations at various deuteron energies were constructed. It was found that it is necessary and sufficient to irradiate the endosperm to overcome the dark dormancy of lettuce seeds.

The authors would like to express their appreciation to Professors E. C. Pollard and A. W. Galston and Drs. Harry Rappaport and H. J. Morowitz for their criticism and interest in this work.

**Literature Cited**


**GIBBERELLIC ACID, PART VI. THE BIOLOGICAL ACTIVITY OF ALLOGIBBERIC ACID AND ITS IDENTITY WITH GIBBERELLIN B\(^1\)**

P. W. BRIAN, JOHN FREDERICK GROVE, H. G. HEMMING,
T. P. C. MULHOLLAND AND MARGARET RADLEY

AKERS RESEARCH LABORATORIES, IMPERIAL CHEMICAL INDUSTRIES, LTD.,
WELWYN, GREAT BRITAIN

Mild acid hydrolysis of gibberellic acid (I) yields allogibberic acid (II) (5, 6) with aromatization of ring A and evolution of carbon dioxide. Cross (5) has presented evidence that allogibberic acid is identical with gibberellin B, a substance obtained from culture filtrates of Gibberella fujikuroi by Yabuta et al (10) along with gibberellin A, and also obtained (11) by treatment of gibberellin A with mineral acid at low temperature.

\[
\begin{align*}
\text{II} & \quad \text{OH} \\
\text{I} & \quad \text{CO} - \\
\text{CH}_3 & \quad \text{CO} - \\
& \quad \text{OH}
\end{align*}
\]

Gibberellin B was reported by Yabuta et al (10) to produce elongation of rice seedlings (i.e., the "bakanee" effect) in the same way as gibberellin A, but that it was markedly less active. Cross (5), on the basis of data on growth of dwarf peas supplied by one of us, reported that allogibberic acid was biologically inactive. Stowe and Yamaki (8) have drawn attention to this apparent contradiction, and have suggested that the presumed identity of allogibberic acid and gibberellin B should be reconsidered. As they rightly point out, it is of some importance to know whether or not ring A of gibberellic acid is necessary for biological activity.

In this paper we present in greater detail the evidence for the identity of allogibberic acid and gibberellin B and report further evidence that allogibberic acid is inactive in inducing shoot extension. In addition, since Stowe and Yamaki (8) have also stated that gibberellin B may stimulate root growth, whereas gibberellic acid and other gibberellins usually do not, we have examined the effects of allogibberic acid on root growth.

**Methods and Materials**

**Dwarf Pea Test:** The materials to be examined were applied to 14-day-old dwarf pea seedlings, var. Meteor, in doses of 0.01 to 10.0 \mu g in 2 \mu ethanol solution. The height of the plants was measured at the beginning of the experiment and 3, 7 and 10 days afterwards. Sixteen replicates of each treatment were used. The method has been described in greater detail.

\(^1\) Received February 27, 1958.