MECHANISM(S) OF FLUORIDE INDUCED CHLOROSIS¹ ²
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Chlorosis and necrosis have long been recognized as the first visible symptoms of fluoride injury to plants (5, 6, 8, 10, 11, 12, 17). These symptoms occur when extraneous soluble fluoride compounds are introduced into the environment of either the roots or the leaves. In some cases, the lethal threshold concentration of tissue fluoride is reached suddenly, as the cells appear quite normal up to the time of visible injury. Solberg et al (16) and Adams and Solberg (1) described the microscopic injury, concurrent with the appearance of macroscopic symptoms, as a disintegration of chloroplasts followed by a collapse of the cells.

While visible symptoms have been studied extensively, only a few investigations have been made to determine whether or not any changes occur prior to the appearance of chlorosis and necrosis, or to describe the mechanism of action. Bandurski (2) found that in vivo synthesis of carotenoids could be inhibited with fluorides. However, Bogorad (3) was unable to demonstrate any fluoride inhibition of the conversion of porphobilinogen to uroporphyrinogen or uroporphyrin, probable precursors to chlorophyll. Katz and Shore (7) suggested that fluoride compounds cause a degradation of chlorophyll by conversion to the corresponding pheophytins. The following investigation was initiated to elucidate further the mechanism of action of fluoride-induced chlorosis.

GENERAL EXPERIMENTAL PROCEDURES

Bush beans (Phaseolus vulgaris L. var. Burpee Tender Pod) or soy bean (Glycine max Merr. var. Bansei) were germinated and grown in vermiculite watered with 80% Hoagland's solution. All treatments were made under controlled temperature at 24° C, and when necessary, exposed to constant fluorescent light at 400 ft.-c. Chlorophyll determinations were made according to the method of Koski (9), measuring chlorophyll a, chlorophyll b, and protochlorophyll at 663, 644, and 624 mua.

Carotenoids were separated by paper chromatography and measured at 446 mua by the method described by Goodwin (4). Essentially, the procedure of Smith (14) was used to measure magnesium. A modification in this procedure consisted of dry ashing the chlorophyll samples. Absorption spectra were obtained using a Beckman DK2 ratio recording spectrophotometer.

FLUORIDE EFFECT ON PIGMENT SYNTHESIS. Bush beans were grown in the dark until the leaves were approximately one centimeter long. The stems were excised 2.5 cm below the cotyledonary node and placed in large petri dishes containing calcium-free, but otherwise complete, culture solution with and without fluoride (1.31 × 10⁻⁵ m NaF). The dishes were then exposed to light. At 24 hour intervals leaf samples were removed and the chlorophylls and protochlorophyll extracted and measured. The results (figs 1 & 2) are expressed in terms of milligrams pigment per ten plants. This was found to be as accurate for comparative purposes as dry weight measurements. The increase of both chlorophylls in the control plants followed a sigmoid curve (fig 1), but there was a definite reduction in the amount of both pigments when the plants were treated with fluoride. The effect of fluoride on protochlorophyll content is shown in figure 2. During the period of exposure to light the plants grew considerably, which would account for the apparent increase of protochlorophyll per plant in the control treatments. The plants exposed to fluorides did not show any increase in total protochlorophyll although they grew in a similar manner. These results suggest that fluoride may be inhibiting the synthesis of these pigments or causing their destruction.

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Fig. 1. The change in content of chlorophyll a and b in etiolated leaves treated with fluoride for 1, 2, and 3 days.

Fluoride Effect on Protochlorophyll to Chlorophyll Transformation. All procedures in this experiment were conducted in total darkness, with the exception of the 1 minute light exposure. Etiolated bush beans, 15 cm high, were excised at the base of the stem and partially immersed in culture solutions with and without fluoride (2.63 × 10⁻³ M NaF). After 61 hours treatment individual leaves were removed and placed in a spectrophotometer accessory kit as described by Smith, et al (15). This adapter is the same shape as the cell holder for the Beckman DK2 spectrophotometer, but holds a leaf between a piece of clear glass and a piece of opal glass. The opal glass window corrects for scattered light so that a consistent percentage is directed toward the photo-cell for each measurement.

The absorption spectra from 600 to 700 mμ were measured for the control and fluoride treated etiolated leaves. They were then exposed to 1 minute of light (incandescent, 1,000 ft-c) and the absorption spectra again measured.

Figure 3 shows a large decrease in protochlorophyll in both the treated and untreated leaves after exposure to 1 minute of light. At the same time there was an increase in the amount of chlorophyll present. After an additional 30 minutes in the dark there was very little change in protochlorophyll content, and the chlorophyll peak shifted only slightly. This shift has been described by Shibata (13). The changes in protochlorophyll and chlorophyll were almost identical in both the fluoride-treated and the untreated leaves. This indicates that fluoride does not affect the conversion of protochlorophyll to chlorophyll under the conditions of these experiments. If the reduction in chlorophyll content of fluoride treated leaves is due to an inhibition of chlorophyll synthesis, the inhibition must precede the protochlorophyll-chlorophyll transformation.

Fluoride Effect on Total Ether Soluble Magnesium. Etiolated bush beans, 15 cm high, were excised 2.5 cm below the cotyledonary node and placed in large petri dishes with and without fluoride (5.26 × 10⁻³ M NaF). The dishes were then placed in a constant temperature chamber (24° C) at three different light intensities (60, 120, 400 ft-c). After 84 hours the leaves were removed and chlorophyll a and b, and total ether soluble magnesium were measured.

It is apparent from table I that the quantities of ether-soluble magnesium present under the different treatments varied in the same directions and with the same magnitudes as did the chlorophylls. If there was an inhibition of some step between an ether sol-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Light intensity (ft-c)</th>
<th>Chlorophyll a mg/g dry wt</th>
<th>Chlorophyll b mg/g dry wt</th>
<th>Ether soluble Mg, mg/g dry wt</th>
<th>Total carotenes mg/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60</td>
<td>11.02</td>
<td>3.07</td>
<td>0.361</td>
<td>0.726</td>
</tr>
<tr>
<td>Fluoride</td>
<td>60</td>
<td>7.07</td>
<td>2.15</td>
<td>0.226</td>
<td>0.468</td>
</tr>
<tr>
<td>Control</td>
<td>120</td>
<td>11.24</td>
<td>3.26</td>
<td>0.361</td>
<td>0.684</td>
</tr>
<tr>
<td>Fluoride</td>
<td>120</td>
<td>8.26</td>
<td>2.42</td>
<td>0.328</td>
<td>0.516</td>
</tr>
<tr>
<td>Control</td>
<td>400</td>
<td>10.26</td>
<td>2.90</td>
<td>0.418</td>
<td>0.666</td>
</tr>
<tr>
<td>Fluoride</td>
<td>400</td>
<td>5.34</td>
<td>1.55</td>
<td>0.188</td>
<td>0.330</td>
</tr>
</tbody>
</table>

70% Confidence limits ±0.48 ±0.12 ±0.043 ±0.03
then removed, the pigments extracted, and the chlorophylls and carotenoids measured (fig 4). The decrease in concentration of all three pigments was nearly linear with increase in fluoride concentration. When the decrease was calculated as per cent of control, it was essentially the same for all pigments. As a result, the rate of change of chlorophyll a with respect to the rate of change of total carotenoids was a constant. These results show that fluoride reduces the concentration of the three pigments to a similar degree but do not indicate whether the reduction is due to a degradation of the pigments or to an inhibition of their synthesis.

**Fluoride Effect on Chloroplast Structure.** The leaves of fluoride-treated and control plants were sectioned and examined microscopically. As chlorosis developed, there was a simultaneous disintegration of the chloroplasts. This occurred prior to any apparent disruption of the cell. The breakdown of chloroplasts and simultaneous mixing of their contents with the cytoplasm could explain the results obtained in the previous experiments, that is, the decrease of all chloroplast pigments occurring simultaneously and to the same degree.

**Discussion & Summary**

Leaf tissue was cultured in a calcium-free mineral nutrient solution, with and without added fluoride, under constant light and temperature. Analyses were made on the chlorophylls, protochlorophyll, carotenoids, and other soluble magnesium compounds. The rate of change of the chlorophylls with respect to the rate of change of suspected chlorophyll precursors, and related compounds, was measured to investigate the mechanism of fluoride-induced chlorosis.

Sodium fluoride prevented the accumulation of chlorophyll a, chlorophyll b, and protochlorophyll in

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**Fig. 2.** The effect of fluoride on the content of protochlorophyll in bean leaves treated for 1, 2, and 3 days.

**Fig. 3.** The absorption spectra of etiolated leaves; fluoride treated (left) and untreated (right). The protochlorophyll peak is ca. 650 m$\mu$ and the chlorophyll peaks between 670 and 690 m$\mu$. 

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bean leaves which were etiolated at the initiation of the fluoride treatment. However, the protochlorophyll to chlorophyll a transformation apparently was not affected. Chlorophyll a and b, the carotenes, and total ether-soluble magnesium containing compounds were all affected proportionately. The similarity in magnitude of reduction of both the chlorophylls and total ether-soluble-magnesium containing compounds indicates there was no inhibition of pigment synthesis following the attachment of the magnesium atom to the ring structure.

These results suggest that if fluorides cause an inhibition of pigment synthesis, then this inhibition must occur very early in the synthesis of pigment components, or with some phase of basic metabolism necessary for their synthesis. The apparent dissolution of chloroplast structure that occurs concurrently with the appearance of chlorosis could explain the similarity in decrease of all pigments. Thus, fluorides may affect the early stages of pigment synthesis, or induce the degradation of chloroplast structure.

**Literature Cited**