A LIGHT ACTIVATED ACCUMULATION OF NICIN IN TOMATO LEAF DISKS

FREDERICK L. CRANE

Department of Botany, University of Michigan, Ann Arbor, Michigan

During the course of an investigation of the pathway of niacin biosynthesis in the leaves of higher plants, evidence has been obtained that light has a role in the process. This paper reports some experiments showing that niacin accumulates in tomato leaf disks when they are exposed to light.

Leaf disks were cut from healthy, fully expanded leaves taken from mature tomato plants grown in the greenhouse. Samples of twenty disks each were selected at random and placed in water during exposure to the experimental conditions. For determination of the initial niacin content, equal samples of disks were frozen immediately at -20°C. This freezing has been found (4) to protect the niacin content of plant tissue for several weeks. The samples exposed to experimental conditions were likewise frozen after a specific incubation time.

The fresh weight and dry weight were determined with separate samples of disks. Since preliminary experiments showed no significant change in weight occurred during short incubation periods (six hours or less), weight determinations were made only at the start of the experiment. For longer incubation periods weights were determined both before and after incubation.

To guard against possible bacterial contamination, all disks to be incubated for more than six hours were surface sterilized by soaking in 0.5% sodium hypochlorite for ten minutes before incubation.

The total niacin content of the disks was determined by the microbiological method (1) using Lactobacillus arabinosus 17-5.

Recovery of niacin was determined by assay of two equal samples of disks, to one of which a known amount of niacin had been added. Recoveries of 105, 99, and 94% were obtained in three experiments.

The significance of results was determined by comparison of the results obtained in a series of separate experiments. A repeated change in niacin content of 5 µg per gm dry weight, or about 10% of the initial niacin content, has been taken as a significant change. This is based on the fact that in 26 replicate samples from the first 50 experiments the difference in niacin content averaged 1.5 µg per gm dry weight, with a maximum difference of 6.1 and a minimum difference of 0.0 µg per gm dry weight. In four experiments in which samples were incubated in triplicate the largest standard error was ± 1.2 µg per gm dry weight.

The results of some representative experiments

Table I

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>INCUBATION CONDITIONS</th>
<th>NIACIN CONTENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (hrs)</td>
<td>Exposure</td>
</tr>
<tr>
<td>68</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Dark</td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Dark</td>
</tr>
<tr>
<td>73</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Dark</td>
</tr>
<tr>
<td>76</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>Dark</td>
</tr>
<tr>
<td>139</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Light</td>
</tr>
<tr>
<td>151</td>
<td>0</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Light</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Dark</td>
</tr>
</tbody>
</table>

Showing the accumulation of niacin in leaf disks exposed to light are presented in Table I. The light intensity at the base of the flasks containing the leaf disks was 50 ft, with illumination provided by a 100-watt tungsten bulb placed under a water bath, equidistant from all samples. Other flasks were kept in darkness. The temperature difference between light and dark treatments was always less than one degree C. Since preliminary experiments showed that vacuum infiltration of the disks with water did not affect the reaction, the disks were infiltrated to make

Table II

<table>
<thead>
<tr>
<th>LIGHT INTENSITY</th>
<th>NIACIN INCREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 HR INCUBATION</td>
</tr>
<tr>
<td>foot-candles</td>
<td>µg/gm dry wt</td>
</tr>
<tr>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>10</td>
<td>12.1</td>
</tr>
<tr>
<td>25</td>
<td>17.4</td>
</tr>
<tr>
<td>75</td>
<td>17.5</td>
</tr>
<tr>
<td>200</td>
<td>17.4</td>
</tr>
</tbody>
</table>

* Initial niacin content 48.9 µg/gm dry weight.
them translucent and to help provide even distribution of light.

A comparison of the niacin content of the incubated disks with that of the unincubated clearly shows that the niacin content increases in light either on a per disk or dry weight basis. There is no significant change in niacin content in the dark on either basis. These observations have been amply verified by the results of more than 50 experiments in which similar conditions prevailed.

The effect of light intensity on the rate of niacin increase was studied by suspending flasks with samples of leaf disks at increasing heights above two 100-watt bulbs, in positions where the light intensity had previously been determined with a model DW, G.E. light meter. The disks at zero intensity were in a light-tight container beside the incubation apparatus. A water bath was placed over the bulbs to prevent temperature changes in the samples. The results of this experiment are shown in table II.

The fact that the niacin increase during the first three hours is the same at all light intensities above 25 fc indicates that the reaction has become light saturated at about that intensity. A light saturation at such a low light intensity is not generally characteristic of the process of photosynthesis (6), but exchange of gases might limit the process in this experiment.

Another experiment of this type was run, with the same results. It has also been found that leaf disks exposed to full sunlight do not increase in niacin content any faster than disks exposed to 50 fc.

To determine if the light activation was caused by photosynthetic production of sugar, samples of disks were incubated for four hours in light and dark after infiltration with either 1 or 2% sucrose solutions. The sucrose did not cause niacin accumulation in dark, nor did it increase the amount formed in light.

The heat lability of the system was determined by boiling samples of disks for 2 to 3 minutes in flasks and then incubating them in light for periods of from 3 to 16 hours. The niacin content of the boiled samples after incubation was compared to the niacin content of samples incubated without pretreatment, and of samples frozen without incubation. The niacin content of the boiled disks did not change during incubation, which indicates that the system responsible for accumulation is thermostable.

To investigate the effect of temperature on reaction rate, samples of disks were incubated in water baths at 26° and 37°C at a light intensity of approximately 75 fc. The results of one experiment are presented in table III.

At both temperatures the rate of niacin accumulation tends to decrease after several hours incubation. This rate decrease has been observed in other experiments. The Q₁₀ of the reaction over this temperature range is 2.2 with an incubation time of 3 hours. In another experiment the Q₁₀ was 2.0 over the same temperature range with an incubation time of 4 hours. The effect of temperature on the reaction indicates that thermochemical reactions are involved, presumably enzyme catalyzed.

As further experiments were run, it was found that within any group of tomato plants of the same age the niacin content of the leaves increased during the morning, and decreased during the afternoon and evening. The increase of niacin in leaf disks, however, was found to occur regardless of the time of cutting, and was not limited to periods when an increase of niacin normally occurs in leaves attached to the plant. Thus the synthesis of niacin in leaf disks would not be primarily a function of the normal diurnal variation.

The evidence that the niacin increase in leaf disks was not an expression of a similar increase in leaves attached to the plant led to an investigation of the wounding effect caused by cutting the leaf disks. It is well known that wounding plant tissue causes an increase in respiration (7); and a hormone which stimulates cell division has been isolated from wounded tissue (3). LaRue (5) has shown that wound hormones which stimulate the rooting of cuttings can be extracted from tomato leaves.

To evaluate the effect of wounding, the increase of niacin in intact detached leaflets was studied. Six pairs of leaflets from the upper leaves of tomato plants were cut and weighed. By this method the wounding caused by disk cutting is largely eliminated. Six of the leaflets were incubated in light for 3.5 hours, while the other six members of the matched pairs were frozen immediately. In two separate experiments the niacin content of the incubated leaflets was 22 and 35% higher than that of the unincubated. The increase of niacin content in these leaflets is taken as an indication that wounding during disk cutting is not the cause of niacin synthesis in leaf disks, but that the accumulation observed is initiated by detaching the leaf. Detached leaflets incubated in the dark did not increase in niacin content.

As a further check on wounding effect, disks were washed more than usual before incubation to see if the niacin increase was reduced. Several experiments were run in which disks were washed with running tap water, then soaked in tap water at 5°C for as long as three days before they were incubated. In every instance the synthesis in the washed disks was not significantly different from that in disks from the same sampling incubated immediately after cutting. The fact that synthesis was not decreased serves to

**TABLE III**

| Effect of Temperature on the Accumulation of Niacin in Leaf Disks Incubated in Light* |
|----------------------------------------|-----------|-----------|
| **INCUBATION TIME**                   | **NIACIN INCREASE** | **26°C** | **37°C** |
|                                       | µg/gm dry wt |           |           |
| hrs                                    |            |           |           |
| 3                                      | 4.4        | 10.5      |
| 10                                     | 10.5       | 15.9      |
| 22                                     | 18.8       |           |

* Initial niacin content 33.0 µg/gm dry weight.
support the conclusion above that niacin increase is not initiated by wounding.

It is emphasized that the increase of niacin in leaf disks must be considered as a net result of synthesis minus destruction during the incubation period. It is clear from the results presented that a rapid synthesis of niacin occurs in tomato leaf disks incubated in light. Whether the actual rate of synthesis is lowered by concurrent destruction cannot be determined, but experience with leaf disks in the dark indicates that niacin destruction, in darkness at least, is a rather slow process.

The synthesis of niacin in the light is apparently not related to the photosynthetic production of sugar, since the light intensity at which the reaction reaches its maximum rate is too low to cause appreciable photosynthesis in the leaf disks. This is further indicated by the fact that addition of sucrose will not cause synthesis in the dark. A study of the action spectrum of the reaction, among other things, would be desirable to eliminate photosynthesis completely from consideration.

The magnitude of the reaction, in some cases resulting in a doubling of the niacin content of the leaf disks in 18 hours, is sufficient to be considered important in the niacin economy of the plant. Since the synthesis is apparently not a result of wounding during disk cutting, the most obvious suggestion is that the niacin accumulates because the normal translocation to other parts of the plant is interrupted. The fact that the roots of many plants, including certain varieties of tomato (2), are dependent on an external source of niacin for continued growth when excised from the plant may be taken as an indication that the leaves could be the natural source of the vitamin for the rest of the plant. Furthermore, the increased niacin content of tomato plants under long photoperiods recently reported by Gustafson (4), as well as the increase of niacin during the morning in leaves attached to the plant, observed in conjunction with the present experiments, may be a manifestation of a light activated niacin producing system in normal operation.

This paper constitutes a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan in 1953. The work was made possible by an F. C. and Susan Eastman Newcombe Fellowship in Plant Physiology, and the author is especially grateful to Professor F. G. Gustafson for his advice and criticism in this investigation.

LITERATURE CITED


B VITAMINS IN STarchy AND SUGARY MAIZE ENdOSPERMs

HOWARD J. TEAS

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Study of the interrelationship between genes and metabolism has provided many examples of biochemical reactions under hereditary control. In some cases it has been possible to assign a fairly simple role to the chemical result of gene action, for instance where a single chemical difference predominates as in many growth-requiring mutants of Neurospora, or as in flower color mutants where a difference in structure of the pigment molecule may be the sole obvious chemical change associated with a gene change (6).

Received June 8, 1953.

1 This work was supported by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, United States Navy (Contract N6-onr-244, Task Order 5, Project NR-164-340).

2 Present address, Federal Experiment Station, Mayaguez, Puerto Rico.

In some instances, as in the starchy-sugary alleles, a gene difference can condition such a series of effects that analysis of the primary gene action becomes difficult. Quantitative chemical differences between starchy (Su) and sugary (su) maize have been reported for a variety of substances. Sugary kernels or endosperms have a higher content of: protein, fat (16), water soluble polysaccharides, reducing sugars, sucrose (10), tryptophan (28), indoleacetic acid (2), and niacin (3). Recent attempts to define the action of the starchy-sugary alleles have been predicated on the hypothesis that one gene-caused biochemical difference gives rise to the whole biochemical and morphological expression of the character by secondary reactions; that is, a single chemical effect interacting with a variety of systems gives rise to the various pleiotropic effects of the sugary gene. The problem