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Histochemical Studies of Wound Periderm Formation
VI. Changes in Ascorbic Acid Content and Ascorbic Acid Oxidase Activity

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It is well known that Nadi-oxidase, polyphenol oxidase or peroxidase is capable of catalyzing the oxidation of ascorbic acid (TAUBER, 1936; JOHNSON and ZILVA, 1937; and ROBERTS, 1939). It is also known that the activity of polyphenol oxidase, which is activated when tissues are injured, is prevented by hydrogen donators such as ascorbic acid in intact tissues (REED and DUFRENOY, 1942; DUFRENOY, 1945; and MACDOUGAL and DUFRENOY, 1946). Therefore there seems to exist an intimate relation between these enzymes and ascorbic acid.

In cowpea seedlings, the ascorbic acid content in the tissue is in the highest concentration in the actively growing zones (REID, 1937), and in both bean and onion root tips, the meristem region contains the heaviest concentration of precipitated silver when the tissues are treated with a silver nitrate solution in the dark (CHAYEN *et al.*, 1953). Moreover, by analogy from the experiments of NEWCOMB (1951) and others, VAN FLEET (1952) has assumed that ascorbic acid oxidase plays some specific role in the growth of primary wall, and BONNER (1957) also has assumed that the enzyme is in some manner associated with the activities of cell wall formation during cell division.

The present author has found that the activities of Nadi-oxidase and pyrocatechol oxidase have some close connection with the occurrence of cell division during the wound periderm formation in *Solanum tuberosum* and *Helianthus tuberosus* (BABA, 1953, 1955 and 1958).

In view of the results of the investigation reviewed above, some changes may be expected in the content of ascorbic acid in tissues during the wound periderm formation. An attempt is made in the present study to see whether there is some relation between the occurrence of cell division and the changes of the ascorbic acid content in the tissue during the wound periderm formation.

Material and Methods

Tubers of *Solanum tuberosum* and *Helianthus tuberosus*, and roots of *Raphanus*

sativus were used as material. The material, cut with a sharp knife, was kept in a moist container at a temperature of 30°C for 24, 48, 72 and 96 hours. Tissues about 0.5 mm in thickness, were excised parallel to the cut surface. These freshly excised tissues were used for the assay of the ascorbic acid content and the ascorbic acid oxidase activity.

The excising, homogenizing or extracting process in the present experiment was carried out at room temperature approximately 15°C. The change of the room temperature from 5°C to 15°C had practically no effect on the value of the ascorbic acid content and the oxidase activity.

The method for determining the ascorbic acid[1)] content in the tissues which was employed in the present investigation was a modification of a TILLMANS method (TILLMANS *et al.*, 1932; FUJITA and IWATAKE, 1935). Five gm of the freshly excised tissues were mixed with acid-washed quarz sand and ground in a mortar with 30 ml of a 5% metaphosphoric acid solution (extracting liquid). Then, the homogenate was centrifuged, and the resulting clear extract was decanted. Another portion (10 ml) of the extracting liquid was used to wash the mortar and poured into the resulting sediment and stirred. The suspension was again centrifuged. These processes were repeated a second time (5 ml). All the acid extracts were combined and make up to a definite volume (45 ml). According to BESSEY and KING (1933), the combined extract contained practically all of the ascorbic acid. Twenty ml aliquots of 2, 6-dichlorophenolindophenol solution were titrated with the extract until a colourless end-point was obtained. The ascorbic acid content in the tissues was calculated from the strength of the indophenol solution expressed as mg ascorbic acid equivalent per ml reagent.

The total ascorbic acid[2)] content of the tissues was determined in the same way as mentioned above after reduction of dehydroascorbic acid by hydrogen sulfide.

For the ascorbic acid oxidase assays, 1 gm of the freshly excised tissue was homogenized in 9 ml of a M/30 phosphate buffer solution at pH 6.0. The ascorbic acid oxidase activity was measured with a Warburg manometric apparatus. The components added into the test vessel for the ascorbic acid oxidase measurement were as follows.

Vessel: 4 ml of the homogenate.
Side arm: 1 ml of M/30 phosphate buffer solution of pH 6 containing 3 mg *l*-ascorbic acid.
Midwell: 1 ml of 20% aqueous potassium hydroxide solution.

The same components with the exception of *l*-ascorbic acid were added into the control vessel.

The vessel, prepared as mentioned above, was equilibrated for 15 minutes

1) 2) In this paper, the name "ascorbic acid" is given to its reduced form only, while the oxidized form is written as "dehydroascorbic acid" (DIXON and WEBB, 1958), and "total ascorbic acid" shows ascorbic acid in the tissue after reduction of dehydroascorbic acid by hydrogen sulfide.

in a constant temperature bath at 30°C. Just before the measurement, the components in the side arm were added into the vessel. After the addition, the volume of oxygen consumed was followed at 10 minute intervals for an hour. The ascorbic acid oxidase activity was obtained by deducting the volume of oxygen consumed by the control sample from that of test sample, and the activity was expressed in terms of microliter of oxygen consumed by 1 ml of the homogenate for an hour.

Results

The results of the morphological observation of the tissues during the wound periderm formation were the same as those given in our previous paper (BABA, 1955), and the results may be briefly summarized here in order to make comparison with those obtained in the biochemical measurements. The cell divisions leading to the wound periderm were observed almost entirely along the cut surface at 48, 72 and 96 hours after the cutting in *Solanum tuberosum*, and the cell divisions were observed locally along the cut surface at 72 and 96 hours after the cutting in *Helianthus tuberosus* (BABA, 1955). In Diagram 1, 2 and 3, the arrows indicate the times at which cell divisions are first observed, whether locally or entirely along the cut surface. While, in *Raphanus sativus*, the cell divisions were not observed at all within 96 hours after the cutting (BABA, 1955).

I) *Ascorbic acid content.*

Immediately after the cutting, the ascorbic acid contents in *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus* were respectively 11.5, 11 and 12 mg per 100 gm tissues.

In *Solanum tuberosum*, the ascorbic acid content in the tissue decreased with the lapse of time after the cutting. In the tissues at 24 hours after the cutting, where cell division was not yet observed, the ascorbic acid content decreased about 2 mg per 100 gm tissues (Diagram 1). In the tissues at 48 hours after the cutting, where cell divisions were first observed, the ascorbic acid content decreased to about one half of that present immediately after the cutting (Diagram 1), and the content decreased accompanying repeated cell divisions, the content at 96 hours became only about one-fifth of that found immediately after the cutting (Diagram 1).

While, in *Helianthus tuberosus*, the ascorbic acid content also decreased with the lapse of time after the cutting (Diagram 1), and it measured only 2.5 mg per 100 gm of the tissues at 24 hours after the cutting, and finally ascorbic acid was found scarcely in the tissues at and after 72 hours.

On the contrary, in *Raphanus sativus*, little change in ascorbic acid content in the tissue was observed in the lapse of time after the cutting (Diagram 1).

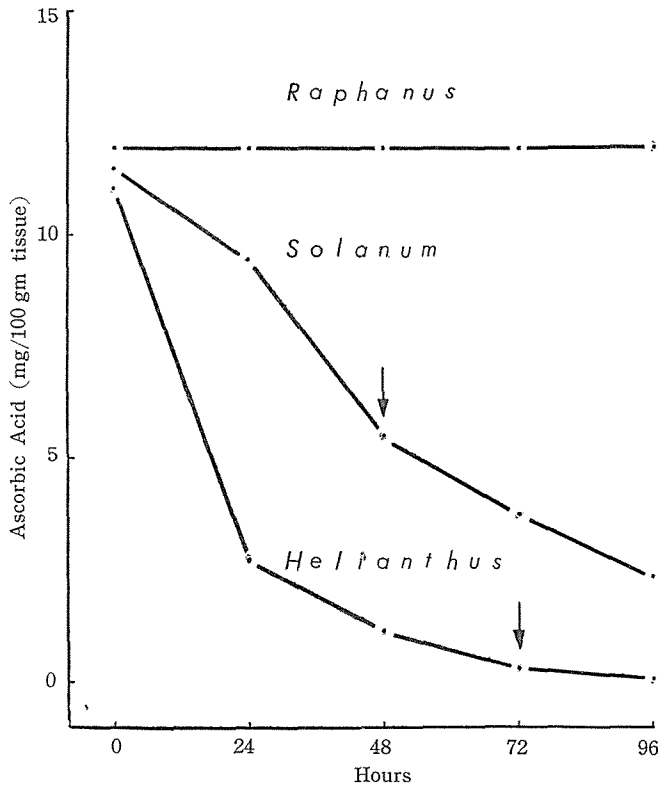

Diagram 1. Diagram showing the relation between the ascorbic acid content and the lapse of time after the cutting.
The ordinates represent the ascorbic acid contents, and the abscissas represent the times after the cutting.
The arrows mark the times at which cell divisions are first observed. Further explanation in text.

II) *Total ascorbic acid content.*

Diagram 2 shows changes in the total ascorbic acid content in the wounded tissues within 96 hours after the cutting in *Solanum tuberosum, Helianthus tuberosus* and *Raphanus sativus.*

As shown in this table, the initial total ascorbic acid contents in *Solanum tuberosum, Helianthus tuberosus* and *Raphanus sativus* were respectively 16.6, 16.5 and 16.0 mg per 100 gm tissues.

In *Solanum tuberosum* and *Helianthus tuberosus,* the total ascorbic acid content decreased in the lapse of time after the cutting and became respectively 11.4 and 12.6 mg per 100 gm tissues at 96 hours after the cutting. In *Raphanus sativus,* however, the total ascorbic acid content in the tissue remained unchanged

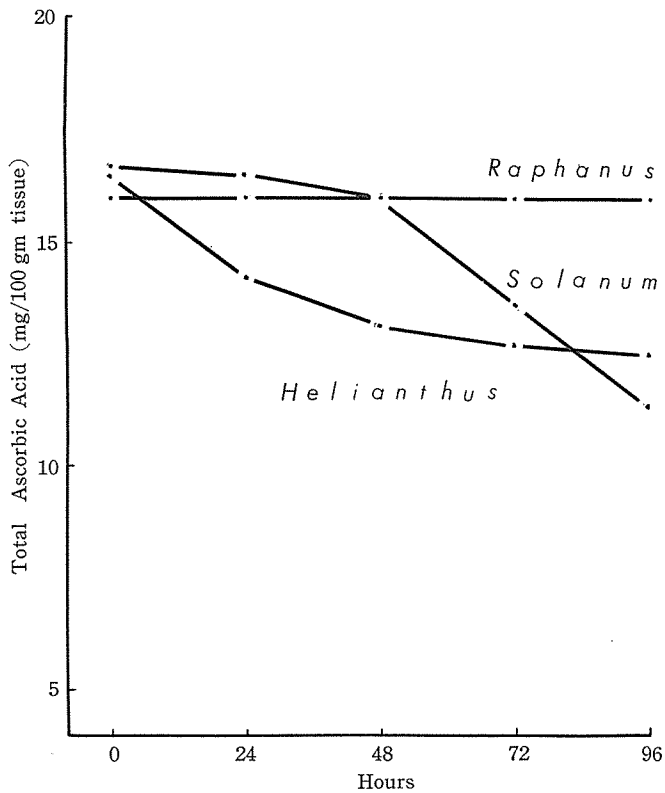

Diagram 2. Diagram showing the relation between the total ascorbic acid content and the lapse of time after the cutting.
The ordinates represent the total ascorbic acid contents, and the abscissas represent the times after the cutting.
Further explanation in text.

in the lapse of time after the cutting just as in the case of the ascorbic acid content.

III) *Ascorbic acid oxidase activity.*

The ascorbic acid oxidase activity in the tissue homogenate was expressed in microliters of oxygen used up in a Warburg apparatus, using *l*-ascorbic acid as a substrate. Diagram 3 shows the changes in ascorbic acid oxidase activity within 96 hours after the cutting in *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*.

In *Solanum tuberosum* and *Helianthus tuberosus*, the addition of *l*-ascorbic acid into the homogenate prepared immediately after the cutting resulted in an increase of oxygen consumption, however, in *Raphanus sativus* such an increase did not occur.

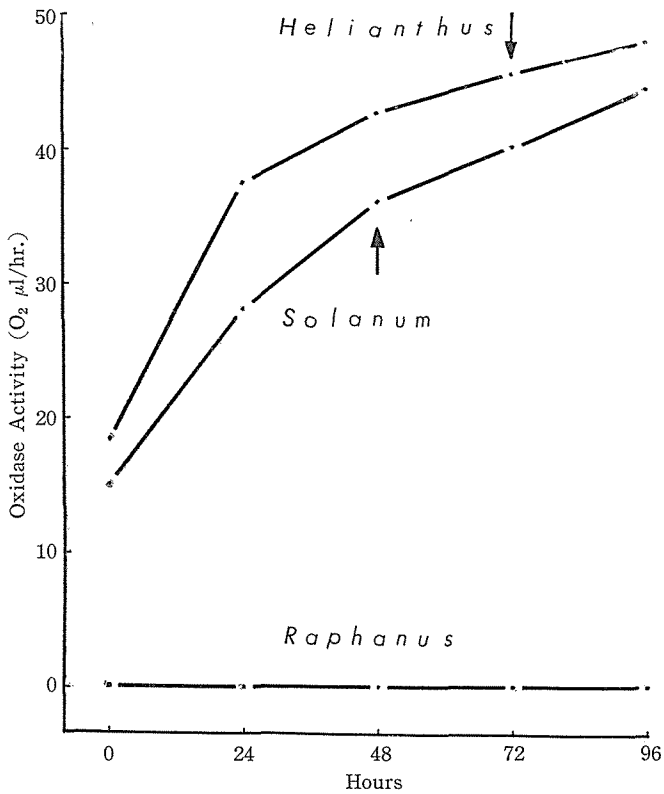

Diagram 3. Diagram showing the relation between the ascorbic acid oxidase activity and the lapse of time after the cutting.
The ordinates represent the ascorbic acid oxidase activities, and the abscissas represent the times after the cutting.
The arrows mark the times at which cell divisions are first observed. Further explanation in text.

The ascorbic acid oxidase activity in *Solanum tuberosum* and *Helianthus tuberosus* showed a marked increase with the lapse of time after the cutting as shown in Diagram 3. On the contrary, little increase was observed in the lapse of time after the cutting in *Raphanus sativus*.

Conclusion

In *Solanum tuberosum* and *Helianthus tuberosus*, the ascorbic acid content shows a remarkable decrease while the ascorbic acid oxidase activity shows a remarkable increase in the wounded tissues in the lapse of time after the cutting. In *Raphanus sativus*, however, little changes of both the ascorbic acid content and the ascorbic acid oxidase activity were observed in the lapse of time after

the cutting. Therefore, a causal relation may be recognized between the increase of ascorbic acid oxidase activity and the decrease of ascorbic acid content during the wound periderm formation in the present materials.

The ascorbic acid content decreases with the lapse of time after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*, in both of which cell divisions take place resulting in the wound periderm formation, however, little change in the ascorbic acid content is observed in *Raphanus sativus*, in which cell divisions are not observed within 96 hours after the cutting. Therefore, it may be concluded that the decrease of ascorbic acid content in the wounded tissues during the wound periderm formation may have some close connection with the occurrence of cell division during that period.

In our previous papers (BABA, 1953, 1955 and 1958), it has been concluded that the activities of Nadi-oxidase and pyrocatechol oxidase have some close connection with the occurrence of cell division. The peroxidase activity may also, to some extent, has a similar connection (*cf.* BABA, 1955). There seems to exist no contradiction between the results obtained in the present investigation and those previously reported.

Summary

1) The changes of ascorbic acid content and total ascorbic acid content in the wounded tissues were measured by employing a modified TILLMANS method within 96 hours after the cutting in the tubers of *Solanum tuberosum* and *Helianthus tuberosus* and the root of *Raphanus sativus*. The oxygen consumed by the homogenate prepared from the same materials in the presence of *l*-ascorbic acid was also measured by employing a manometric technique.

2) Both a remarkable decrease of ascorbic acid content and a remarkable increase of ascorbic acid oxidase activity were detectable in the lapse of time after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*, in both of these, the cell divisions took place resulting in the wound periderm formation. The total ascorbic acid content also showed a decrease in the lapse of time after the cutting in these materials.

On the contrary, in *Raphanus sativus*, in which cell divisions were not observed to occur within 96 hours after the cutting, little changes in the ascorbic acid content, total acid content and ascorbic acid oxidase activity were observed in the wounded tissues within 96 hours after the cutting.

Therefore, it may be concluded that the decrease of ascorbic acid content may have some close connection with the occurrence of cell division during the wound periderm formation.

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