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A Histochemical Study of Wound Periderm Formation

By

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In the present investigation, changes in activity of some biocatalysts responsible for the cellular oxidation and in amount of reserve substances, such as sugar, starch, protein and fat were histochemically studied during the wound periderm formation. The results obtained are reported below.

Materials and Method

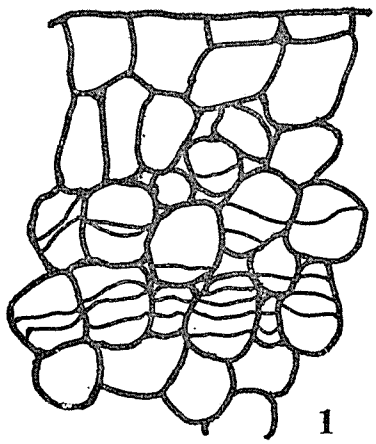
Roots of *Ipomoea Batatas*, *Dahlia pinnata*, *Arctium Lappa*, *Daucus Carota*, *Beta vulgaris* and *Raphanus sativus*; tubers of *Solanum tuberosum* and *Helianthus tuberosus*; corms of *Colocasia esculenta* and *Gladiolus gandavensis*, and stems of *Bryophyllum daigremontianum*, *Lycopersicon esculentum* and *Brassica oleracia* (Kohlrabiknollen) were used as materials for this investigation.

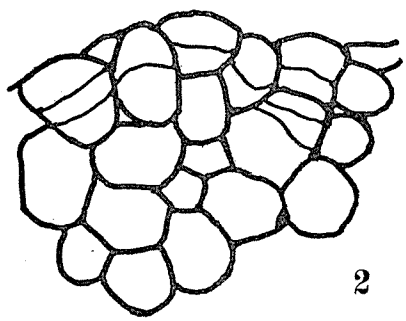
The materials, cut with a sharp knife making a cross thick section, were kept in a moist container at a temperature of about 30°C. for 24, 48, 72 and 96 hours. Longitudinal thin sections of the fresh tissue were made rectangular to the cut surface, and some of them were studied morphologically while others were treated with various histochemical reagents such as nadi reagent, guaiacol solution with or without hydrogen peroxide, pyrogallol solution, with or without hydrogen peroxide, pyrocathecol solution, ortho-and para-cresol solutions each with or without hydrogen peroxide, tyrosine solution, benzidine solution with hydrogen peroxide, potassium permanganate solution, acidified silver nitrate solution, Fehling's reagent, Barfoed's reagent, iodine potassium iodide solution, Millon's reagent and Sudan III (cf. Molish, 1923; Romeis, 1932; Ries, 1938; Sinke, Iijima and Hiraoka, 1947 and Glick, 1949).

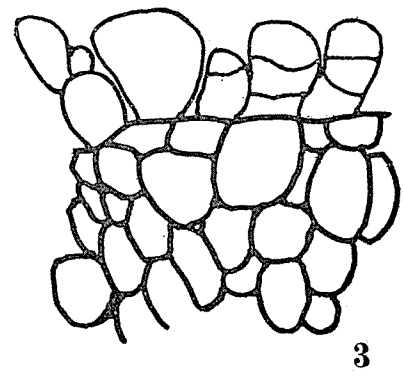
Similar longitudinal thin sections prepared from the materials immediately after the cutting by cross section were studied for the sake of comparison.

Observations

PRELIMINARY OBSERVATION: When the wound periderm is formed along the cut surface, the time required for the occurrence of cell division and elongation after the cutting differs according to different kinds of tissues for







Explanation of figures.

Longitudinal thin sections of storage tissues made recutangular to the cut surface 96 hours after the cutting by cross section.

Fig. 1, shows Type A (*Gladiolus gandavensis*).

Fig. 2, Type B (*Helianthus tuberosus*).

Fig. 3, Type C (*Beta vulgaris*).

(Magnification, 130X).

all the materials studied. The present investigation mainly deals with the wound periderm formation in parenchymatous tissue of cortex and pith.

In order to make our description clearer, morphological characteristics of the wound periderm are classified into three types. In type A, which, according to Krenke (1933), may be described as "wiederherstellende, exo- und endogene, längspolare Restitution", the wound periderms parallel to the cut surface are formed in the tissue several cell layers below the surface (Fig. 1). In type B, which may be regarded as "Schützherstellende, exo- und endogene, längspolare Restitution" after Krenke (1933), new cell divisions occur in the injured cells on the cut surface and the cell immediately below them, sometimes 2 or 3 in succession (Fig. 2). While in type C, the injured cells on the cut surface elongate themselves and transform themselves into projecting cells resembling bags.*) Cell divisions may also occur in those

*) Whether this type of the wound periderm formation belonged to "Restitution" or to "Reproduktion" after Krenke (1933) was not determined in the present investigation.

elongated cells (Fig. 3).

Table I. Results of histochemical tests on longitudinal thin sections immediately after the cutting by cross section.

Reagents \ Materials	*Ipomoea Batatas*	*Solanum tuberosum*	*Colocasia esculenta*	*Gladiolus gandavensis*	*Bryophyllum daigremontianum*	*Helianthus tuberosus*	*Dahlia pinnata*	*Arctium Lappa*	*Daucus Carota*	*Lycoperisicon esculentum*	*Brassica oleracia*	*Beta vulgaris*	*Raphanus sativus*
Nadi reagent	+	+	±	+	±	++	++	++	+	±	−	+	−
Guaiacol	−	−	−	−	−	−	−	−	−	−	−	−	−
Pyrogallol	+	+	−	−	−	±	−	−	−	−	−	+	−
Pyrocathecol	+	+	+	−	−	+	+	+	±	±	−	+	−
p-Cresol	−	−	−	−	−	−	−	−	−	−	−	−	−
o-Cresol	−	−	−	−	−	−	−	−	−	−	−	−	−
Tyrosin	−	++	−	−	−	−	−	−	−	−	−	+	−
Benzidine+H_2O_2	+	+	+	+	−	+	+	+	±	+	+	+	+
Guaiacol+H_2O_2	+	+	+	−	±	+	+	±	+	±	+	+	+
Pyrogallol+H_2O_2	+	+	+	+	−	+	±	±	+	±	+	+	+
p-Cresol+H_2O_2	−	±	±	−	−	±	±	−	−	−	−	−	−
o-Cresol+H_2O_2	±	±	±	±	−	±	±	±	±	±	±	±	±
K-permanganate	+	+	+	+	+	±	+	+	+	+	+	+	+
Acidified $AgNO_3$	−	−	−	−	−	−	−	−	−	−	−	−	−
Fehling's reagent	+	+	+	+	+	−	+	+	+	+	+	++	+
Barfoed's reagent	+	+	−	+	−	−	−	+	+	+	+	−	+
Iodine potassium iodide	++	++	++	++	−	−	−	−	±	−	−	−	−
Millon's reagent	±	±	±	±	±	±	±	±	±	±	±	±	±
Sudan III	±	±	±	±	±	±	±	±	±	±	±	±	±

Abbreviations: −, negative reaction; ±, slightly positive reaction; +, positive reaction; ++, strongly positive reaction.

The morphological characteristics of the wound periderm in the parenchymatous tissues may differ according to different kinds of materials. *Ipomoea Batatas*, *Solanum tuberosum*, *Colocasia esculenta*, *Gladiolus gandavensis* and *Bryophyllum daigremontianum*, in which the well differentiated wound periderms are formed along the cut surface within 72 hours after the cutting, usually belong to type A. *Helianthus tuberosus* and *Dahlia pinnata*, in which cell divisions occur along the greater part of the cut surface within 96 hours, frequently belong to type B, while *Beta vulgaris* and *Raphanus sativus*, in which cell elongation causing the periderm formation covers the greater part of the cut surface within 96 hours, belong to type C. In the materials other than those that are mentioned here, cell elongation and divisions cover the greater part of the cut surface within 96 hours after the cutting and the wound periderm formation belongs to an intermediary type between B and C (cf. Haberlandt, 1923).

HISTOCHEMICAL OBSERVATIONS: Histochemical tests for indophenol oxidase, polyphenol and monophenol oxidases, peroxidases, starch, protein, fat and reducing substances such as ascorbic acid and sugar were carried out mainly in parenchymatous tissues of cortex and pith during the wound periderm formation.

(*1*) *Observations on the longitudinal thin sections immediately after the cutting by cross section.*

When the tissues are treated with histochemical reagents immediately after the cutting, the results of the tests shown in Table 1 are obtained.

The tissues show a blue positive reaction colour to nadi reagent more or less strongly in all the materials except *Brassica oleracia* and *Raphanus sativus*.*)

The tests for polyphenol oxidase give different results, when different substances are used as substrates. In pyrogallol substrate, a yellow positive reaction colour develops itself in *Ipomoea Batatas*, *Solanum tuberosum*, *Helianthus tuberosus*, and *Beta vulgaris*, in pyrocathecol substrate, a greenish dark brown positive reaction colour in all the materials except *Gladiolus gandavensis*, *Bryophyllum daigremontianum*, *Brassica oleracia* and *Raphanus sativus*, while in guaiacol substrate no positive reaction has been noticed in all the materials studied.

The tests for monophenol oxidase show no positive reaction at all in all the materials studied, when para- and ortho-cresol are used as substrates. In the case where tyrosin is used as substrate, a black positive reaction colour appears in *Solanum tuberosum* and *Beta vulgaris*.

*) The activity of nadi oxidase is found stronger in the regions of cambiums and parenchymatous tissues lying close to vascular bundles than in the parenchymatous tissues remote from the bundles.

The test for peroxidase with benzidine and hydrogen peroxide gives a blue positive reaction colour in all the materials except *Bryophyllum daigremontianum*. The blue coloration turns yellow sooner or later. The test for peroxidase with guaiacol and hydrogen peroxide shows a positive reaction of orange colour in all the materials except *Gladiolus gandavensis* and that with pyrogallol and hydrogen peroxide a positive reaction of yellow colour in all the materials except *Bryophyllum daigremontianum*. While in the case where para-cresol and hydrogen peroxide are used as substrates, the test faintly gives a positive reaction colour of straw yellow in *Solanum tuberosum*, *Colocasia esculenta*, *Helianthus tuberosus* and *Dahlia pinnata*, and in the case where ortho-cresol and hydrogen peroxide are used as substrates, it feebly shows a positive reaction colour of cream yellow in all the materials except *Bryophyllum daigremontianum*.

Potassium permanganate is reduced in all the materials studied. Acidified silver nitrate is not reduced at all in the absence of light in all the materials studied. Fehling's reaction is positive in all the materials except *Helianthus tuberosus*, while Barfoed's reaction is positive in all the materials other than *Colocasia esculenta*, *Bryophyllum daigremontianum*, *Helianthus tuberosus*, *Dahlia pinnata* and *Beta vulgaris*. Starch is contained abundantly in the tissue of *Ipomoea Batatas*, *Solanum tuberosum*, *Colocasia esculenta* and *Gladiolus gandavensis*, but only in small amount in *Daucus Carota*. Both protein and fat are also found in small amount in all the materials studied.

(*2*) *Observations on the longitudinal thin sections 24, 48, 72 and 96 hours after the cutting by cross section.*

When the tissues are treated with the histochemical reagents 24, 48, 72 and 96 hours after the cutting by cross section, it is found that some remarkable changes in the activity of nadi oxidase and in the amount of starch take place during the wound periderm formation.

In all the materials except *Brassica oleracia* and *Raphanus sativus*, the activity of nadi oxidase in the wounded tissue increases previous to and during the occurrence of cell divisions in the regions in which cell divisions are to be and are observed, and the increased activity is maintained during the wound periderm formation. This increase in activity is evidently observed especially in the materials belonging to type A.

On the contrary, starch amount in the wounded tissue shows a sudden decrease in the region where new cell divisions are to take place previous to the occurrence of the cell divisions in *Ipomoea Batatas*, *Solanum tuberosum*, *Colocasia esculenta* and *Gladiolus gandavensis*, which belong to type A.

The results of our peroxidase tests also show that in the cases where a positive reaction of benzidine peroxidase is obtained, the blue reaction colour which has appeared in the tissues turns yellow and this discolouration occurs

more rapidly in the region of the wound periderm than in other regions.*) In the histochemical reactions other than those for nadi oxidase and starch, any change in activity of biocatalysts or in amount of reserve substances is not decidedly demonstrated during the wound periderm formation so far as the present investigation is concerned.

Conclusion

Nadi oxidase shows a positive reaction in the parenchymatous tissue of all the materials studied except *Brassica oleracia* and *Raphanus sativus*. It shows an increase in activity previous to and during the occurrence of cell divisions in the regions in which cell divisions are to be and are observed, and the activity is maintained in the regions mentioned above during the wound periderm formation. This phenomenon is shown clearly especially in the materials belonging to type A. The activity of nadi oxidase is stronger in the regions of cambiums and parenchymatous tissues lying close to vascular bundles, in which an active elongation or divisions of tissue cells are found, than in other parenchymatous tissues. These facts show that the activity of nadi oxidase has some close connection with the occurrence of cell divisions in the wounded tissues.

In *Ipomoea Batatas*, *Solanum tuberosum*, *Colocasia esculenta* and *Gladiolus gandavensis*, which belong to type A, starch decreases in amount in the wounded tissue previous to the wound periderm formation (Kabus, 1912; Nakano, 1924 and Krenke, 1933). Strasburger (1880), Schaede (1925) and Kuwada (1930) have observed that starch granules in staminate hairs and pollen grains of *Tradescantia* are dissolved during the cell division. Though no appreciable change in amount of protein and fat was recognizable in the present investigation, Friedrich (1908), Nakano (1924) and others have demonstrated a decrease in amount of protein and fat in wounded tissues. All these results of observations suggest that a decrease in amount of reserve substances is associated with the occurrence of cell division in the wounded tissues.

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*) This fact may be taken to suggest that the peroxidase activity increases in the course of the wound periderm formation.

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