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Histochemical Studies of Wound Periderm Formation.
III. Changes in Total Acidity, Hydrogen-ion Concentration and Oxidation-Reduction Potential.*

By

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It has been reported by SMALL (1929 and 1955) that the pH value of the phellogen is lower than that of the parenchyma.

ROBERTS (1950) and VAN FLEET (1954), on the other hand, have found differences in oxidation-reduction potentials between the meristems and the differentiated tissues.

The changes in cytoplasmic pH and rH values in wounded regions may also be expected to occur during a wound periderm formation. It is the purpose of the present study to see the relations between the occurrence of cell divisions and the changes of acidity, pH and rH in the cytoplasm during the course of the wound periderm formation.

Material and Methods

Tubers of *Solanum tuberosum* and *Helianthus tuberosus*, roots of *Raphanus sativus* were used as material for the present investigation.

Material, cut with a sharp knife making a thick cross section, was kept in a moist container at a temperature of about 30°C. for 24, 48, 72 and 96 hours.

For the determination of total acidity of tissues, tissues about 0.5 mm. in thickness, were excised parallel to the cut surface. Five g. of the freshly excised tissues were heat-killed at 100°C. for 5 minutes, and then were homogenized and extracted in 20 ml. of distilled water. The extracted fluid was squeezed from the homogenate through gauze. The process of the extraction was repeated twice on the residue. Then the extracted fluids were mixed, and volume of the mixture was measured. The mixture was titrated with a 0.045 N. barium hydroxide solution** (Cf. A. O. A. C. 1935).

Hydrogen ion concentration was determined by means of Small's range indicator method on frseh sections under the microscope (SMALL 1929 and 1955).

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** One ml. of 0.045 N. barium hydroxide solution corresponds to 0.045 mg. of ionizable hydrogen whether actually present as ions or combined with anions in form of molecules.

Thin sections of the injured tissues were made rectangular to the cut surface. The sections were placed in one of the aqueous solutions of the following indicators; bromo-phenol blue, bromo-cresol green, methyl red, bromo-cresol purple and bromo-tymol blue. The concentration was 0.02% for methyl red and 0.04% for all the others. The pH ranges were determined mainly in parenchymatous tissues.

For the determination of oxidation reduction potential the thin sections were placed in 0.01% aqueous solutions of brillant cresyl blue, methylen blue, nile blue, cresyl violet or neutral red. The faintly stained sections were washed with freshly distilled water, and then placed on a slide glass and kept under a coverslip in the water. The rH value was judged from the fact whether the discolouration of dyes in the cell took place or not.

Results

The results of the morphological observation during the wound periderm formation were the same as those given in the previous paper (BABA, 1955). For the sake of convenience, following abbreviations are used in the present paper.

Solanum tuberosum (Fig. 1, a)

A: The injured cells on the cut surface and cells immediately below the injured cells.

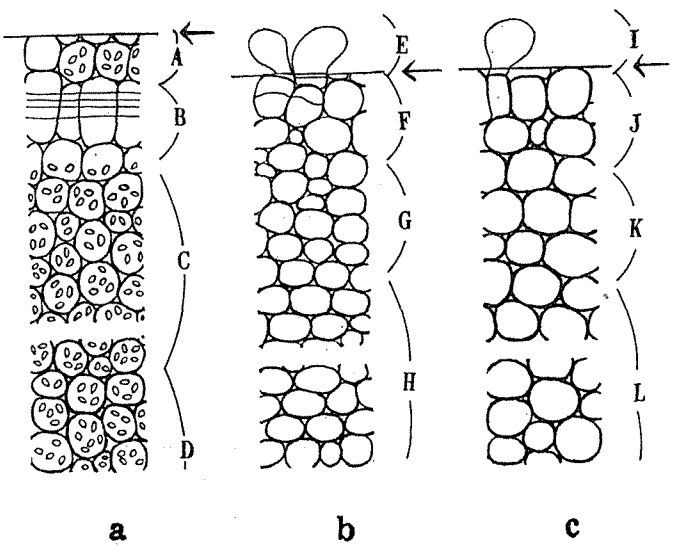

Figure 1. Schematic figures of thin sections of tissues made rectangular to the cut surface at 96 hours after the cutting.

Fig. 1-a, shows a schematic figure of parenchymatous tissue in *Solanum tuberosum*. 1-b, shows that in *Helianthus tuberosus*, and Fig. 1 c, shows that in *Raphanus sativus*. Arrows show the cut surface. Further explanation in text.

B: A few layers of cells which lie below A. In this region cell divisions leading to the wound periderm are observed within 48 hours after the cutting. (phellogen).
C: Several cell layers which lie below B.
D: The tissue which lies below C.

Helianthus tuberosus (Fig. 1, b)

E: Elongated cells on the cut surface. Some cells lying immediately below the cut surface elongate themselves and transform themselves into these elongated cells within 72 hours after the cutting.
F: The injured cells on the cut surface and cells immediatly below the injured cells. Cell division often takes place locally in this region within 72 hours after the cutting.
G: Several cell layers which lie below F.
H: The tissue which lies below G.

Raphanus sativus (Fig. 1. c)

I: Elongated cells on the cut surface. Similar to E.
J: The injured cells on the cut surface and cells immediately below the injured cells.
K: Several cell layers which lie below J.
L: The tissue which lies below K.

In the regions except those expressly pointed out, cell divisions were not observed within 96 hours after the cutting.

(A) Total acidity

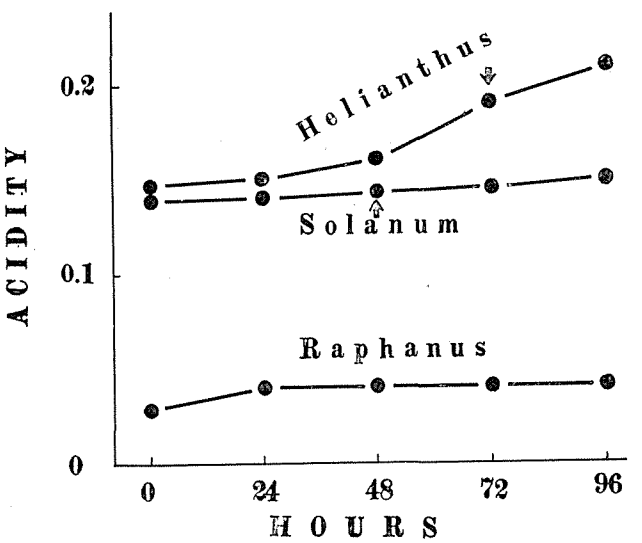

Diagram. 1 Diagram showing the relation between the amount of total acid in the tissues and the lapse of time after the cutting.

In this diagram, the axis of ordinates represents the amount of total acid in terms of mg. ionizable hydrogen, whether actually present as ions or combined anions in form of molecules, in 5 g. of fresh tissues. The axis of abscissas represents the lapse of time afer the cutting.

The time at which the cell division is observed, whether locally or entirely along the cut surface, is shown by arrows.

The changes in the total acidity during the wound periderm formation are given in Diagram 1. While the amount of total acid appeared to show a slight increase in *Solanum tuberosum*, a marked increase in amount was observed in *Helianthus tuberosus* within 96 hours after the cutting. In *Raphanus sativus*, however, a slight increase in total acidity was observed within 24 hours after the cutting, and thereafter the acidity did not show any increase at all.

(B) Hydrogen ion concentration

1) *Solanum tuberosum.* The cytoplasmic pH ranges during the course of wound periderm formation after the cutting, are summarized in Table 1.

Table 1. Changes in pH range during wound periderm formation in *Solanum tuberosum.*

Regions / Hours after the cutting	A	B	C	D
0	5.8 — 6.2	5.8 — 6.2	5.8 — 6.2	5.8 — 6.2
24 and 48	5.0 — 5.6	5.0 — 5.6	5.0 — 5.6	5.8 — 6.2
72 and 96	5.8 — 6.2	4.4 — 5.4	5.0 — 5.6	5.8 — 6.2

These pH ranges were determined with B. C. G., M. R., B. C. P. and B. T. B..

As shown in this table, the pH range in A lowers at 24 and 48 hours and recovers at 72 and 96 hours after the cutting. It is observed in B and C, however, that the pH ranges lower in the lapse of time after the cutting without recovery and that the lowering of the pH range is more marked in B than in C. The pH range in D, on the other hand, shows no change.

2) *Helianthus tuberosus.* The results obtained in *Helianthus tuberosus* are summarized in Table 2.

Table 2. Changes in pH range during wound peridem formation in *Helianthus tuberosus.*

Regions / Hours after the cutting	E	F	G	H
0 and 24	—	5.8 — 6.2	5.8 — 6.2	5.8 — 6.2
48, 72 and 96	5.8 — 6.2	5.0 — 5.6	5.8 — 6.2	5.8 — 6.2

These pH ranges were determined with B. C. G., M. R., B. C. P. and B. T. B..

It is seen in this table that the pH range in F in which cell divisions are

locally observed within 72 hours, lowers at 48, 72 and 96 hours after the cutting, while in E, G and H the pH range does not show a lowering in the lapse of time after the cutting.

3) *Raphanus sativus*. The results obtained in this material are summarized in Table 3.

Table 3. Changes in pH range after the cutting in *Raphanus sativus*.

Hours after the cutting. \ Regions	I	J	K	L
0 and 24	—	5.8 — 6.2	5.8 — 6.2	5.8 — 6.2
48, 72 and 96	5.8 — 6.2	5.4 — 6.2	5.8 — 6.2	5.8 — 6.2

These pH ranges were determined with B. C. G., M. R., B. C. G. and B. T. B..

As shown in this table, the pH range in J, in which cell divisions do not occur within 96 hours after the cutting, lowers little or only a little, and such a remarkable lowering in the pH range as mentioned in B of *Solanum tuberosum*, is not observed. In I, K and L, the pH range does not show a lowering in the lapse of time after the cutting.

Besides the employment of Small's range indicator method on tissue sections the following method was used in the present study: Freshly excised tissues were crushed with a mortor and pestle, and then the pH of the sap of tissues was determined with the color indicators employed in the above method. Though the pH values of the sap were lower than those obtained in the fresh thin sections, the result otbained in the former had a similar tendency to that of the latter.

(C) Oxidation reduction potential

In the materials of *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*, toluylene blue, cresyl blue and methylen blue, normal redox potentials (E_o) of which are 0.162, 0.078 and 0.047 volt respectively at pH 6.0 and 30°C.*, are reduced by the parenchymatous cells in the material immediately after the cutting.

Contrary to the above case, nile blue, cresyl violet, neutral red and neutral violet, E_o of which are −0.085, −0.123, −0.279 and −0.279 volt respectively at pH 6.0 and 30°C.*, are not reduced by these cells. Therefore, the oxidation reduction potential in these cells at pH 5.8-6.2, is lower than 0.056 volt* (E_o of methylen blue at pH 5.8) and higher than −0 098 volt* (E_o of nile blue at pH 6.2). If computed in terms of rH (CLARK and COHEN, 1923), the rH value in these cells may be between 13.7 and 9.2.

* HEWITT, 1950; RAPKINE et al, 1929; WURMSER, 1930; WURMSER et al, 1929.

Though the changes in value of pH was observed in the wounded regions of *Solanum tuberosum* and *Helianthus tuberosus*, those of rH value were not confirmed in the wounded regions during the wound periderm formation in all the materials studied.

Conclusion

The amount of total acid in the wounded regions in *Helianthus tuberosus* increases in the lapse of time after the cutting. In *Solanum tuberosum* and *Raphanus sativus*, however, there is little or a little increase of total acid during the wound periderm formation.

In *Solanum tuberosum* and *Helianthus tuberosus* in which the cell division takes place in the parenchymatous tissues within 96 hours, the pH value in parenchymatous tissues in which the cell divisions are to be and are observed lowers at 24 and 48 hours after the cutting. In the phellogen at 72 and 96 hours after the cutting in *Solanum tuberosum*, the pH value lowers most markedly. In *Raphanus sativus*, in which cell divisions do not take place, however, only a slight decrease in pH is observed in wounded regions in the lapse of time after the cutting. According to the results obtained by SMALL (1929), the pH value of the phellogen cells is lower than that of the parenchymatous cells in *Solanum tuberosum* and others. These results of observation suggest that a lowering in pH value may have some close connection with the occurrence of cell divisions in the wounded tissues.

It has been reported by ROBERTS (1950) and VAN FLEET (1954) that 2, 3, 5–triphenyltetrazolium chloride is reduced more strongly in the meristematic tissues than in the differenciated tissues of some higher plants. The results of the present investigation, however, show that the rH values do not change during the course of the wound periderm formation. Therefore, a relation between the occurrence of cell division and the change of the rH value remains as a further question.

Summary

1) The changes in amount of total acid, in pH and rH were determined during the course of the wound periderm formation in tubers of *Solanum tuberosum* and *Helianthus tuberosus* and roots of *Raphanus sativus*.

2) The amount of total acid increased in the wounded regions in the lapse of time after the cutting in *Helianthus tuberosus*, while little or a little increase was observed in *Solanum tuberosum* and *Raphanus sativus*.

3) The cytoplasmic pH was 5.8–6.2 in the parenchymatous tissues in all the plants stated above. In *Solanum tuberosum* and *Helianthus tuberosus*, a lowering of the pH value is observed in the part of tissues where cell divisions

are observed or expected to occur. In *Raphanus sativus*, on the contrary, neither the cell division nor the pH lowering was observed. It is assumed, therefore, that there seems to be some intimate relation between the occurrence of cell division and the lowering in the hydrogen ion concentration of the cells.

4) The changes in oxidation reduction potentials in the parenchymatous tissues were not observed during the course of the wound periderm formation of the plants stated above. The rH value observed was between 13.7 and 9.2.

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