MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXII, No. 1, Article 9. 1955

# A Histochemical Study of Wound Periderm Formation
## II. Changes in Activity of Nadi-Oxidase, Dehydrogenase, Peroxidase and Catalase

By

**Sango BABA**

Botanical Institute, Faculty of Science, Kyoto University

(Received August 31, 1955)

It has been indicated by WIESNER (1892) and others that there is a causal connection between an injury of cells and the occurrence of cell divisions in the neighbourhood of the injured cells. HABERLANDT (1902, 1914, 1923 and 1928) has demonstrated such effects in a series of his well known experiments with tissue pieces from *Solanum tuberosum* and others. It has been suggested that the effects depend on an "wound hormone" and the hormone is active in normal periderm formation and exerts an influence upon an occurrence of cell division. Recently, BONNER et al. (1937, 1938, 1939 a and b) have extracted and purified an active substance, termed "traumatic acid", from *Phaseolus vulgaris*. They have indicated that this substance induces a periderm formation in washed discs of tubers of *Solanum tuberosum*.

In these experiments mentioned above, phenomena in connection with the recovery of the meristematic activity in permanent tissues were studied. Studies in physiological and chemical changes, which precede and follow the beginning of cell divisions during the recovery of the meristematic activity in permanent tissues, are necessary to obtain a clearer knowledge on the causal analyses of cell divisions.

In the previous work (BABA, 1953), changes in activity of some biocatalysts have microscopically been observed during the wonnd periderm formation and it has been found that the activity of nadi-oxidase has some close connection with the occurrence of cell divisions. In the present paper, further investigations on the activities of nadi-oxidase, dehydrogenase, peroxidase and catalase during the wound periderm formation, are reported.

## Materials and Methods

Tubers of *Solanum tuberosum*, which had been stored from 2 to 6 months after the harvest, and those of *Helianthus tuberosus* and roots of *Raphanus sativus* immediately after the harvest from field, were used from September to January as materials for the present investigation.

Materials, cut with a sharp knife making a thick cross section, were kept in a moist container at a temperature of about 30°C. for 12, 24, 48, 72 and 96 hours.

For morphological observations, longitudinal thin sections of the tissues were made rectangular to the cut surface, and were observed with a microscope.

For the determination of enzyme activity, the materials immediately after the cutting, and those kept in the above mentioned conditions after the cutting, both were used. Tissues, about 0.5 mm. in thickness, were cut parallel to the previous cut surface. These excised tissues were weighed and homogenized in a buffer solution with a mortar and pestle. Methods of determination of enzyme activities will be described in the appropriate places in this paper.

The materials kept in the moist container intact at the temperature mentioned above for 12, 24, 48, 72 and 96 hours were used as control samples.

## Results

### (A) MORPHOLOGICAL OBSERVATION

1) *Solanum tuberosum*

In parenchymatous tissues, except those lying around the vascular bundles, the morphological characteristics found in the course of the wound periderm formation usually belong to type A as mentioned in the previous paper (BABA, 1953). The development of the wound periderm in the tissues at 24, 48, 72 and 96 hours after the cutting is uaually as follows:

No morphological changes of the wounded tissues found within 24 hours. Within 48 hours a decrease in amount of starch is observed in the tissues one or more cell layers below the cut surface. In this stage, cell divisions are observed in some of the cells lying in cortex and outer layers of the pith (Fig. 1), while they are seldom observed in the cells lying in the inner layers of the pith. Within 72 hours, one or more cell layers of the divided cells are observed along the entire cut surface (Fig. 2). At 96 hours, the formation of wound periderm is more conspicuous than those found at 72 hours (Fig. 3). It is observed, moreover, a suberization of the cell wall is often completed in cortex and the outer layers of the pith.

The type of the wound periderm formation in parenchymatous tissues which lie within and around the vascular bundles, is somewhat different from the above case. In these tissues, cell divisions leading to the wound periderm formation, are observed in the cells immediatly below the cut surface and their neighbouring cells. It is noted, moreover, that some of the injured cells elongate on the cut surface. Figure 4 shows an example of these wound periderm at 96 hours after the cutting. In these tissues, cell divisions with or without elongation take place more frequently and earliar, after the cutting, than in the parenchymatous tissues which are remote from the vascular bundles.

2) *Helianthus tuberosus*

It has been reported in the previous paper (BABA, 1953) that the type of the wound periderm formation in the parenchymatous tissues, except lying around vascular bundles in this plant, belongs to type B. The result obtained in the present experiment, however, is somewhat different from that reported in the paper.

Cell divisions leading to the wound periderm formation in this plant, often take place locally in the injured cells on the cut surface and the cells immediatly below them (type B). Moreover, elongated cells *) from the injured cells are often observed locally on the cut surface (type C). Therefore, the type of the wound periderm formation in this case, may belong to an intermediary type between B and C (cf. BABA, 1953). The development of the wound periderm in the tissue at 24, 48, 72 and 96 hours after the cutting, is usually as follows:

There are no morphological changes in the wounded tissues within 24 hours. Within 48 hours, divided and elongated cells are observed in some tissues especially in outer layers of the pith, but these cells in pith and divided cells in cortex are rarely and sporadically observed. At 72 hours, divided and elongated cells in pith and divided cells in cortex are observed more extensively than at 48 hours. This tendency becomes more distinct at 96 hours (Fig. 5). In this stage, it is often observed that both divided cells and elongated cells in some regions, and either divided cells or elongated cells in other regions, are observed on the cut surface in the middle part of the pith. In the other regions of the part of the pith, however, neither divided cells nor elongated cells are found on the cut surface.

In parenchymatous tissues which lie within and around the vascular bundles, divided cells immediately below the cut surface and elongated cells on it are usually observed within 48 hours. The elongated and the divided cells form a big projection on the cut surface within 96 hours as shown in Figure 6.

3) *Raphanus sativus*

The morphological characteristics found in the course of wound periderm formation in parenchymatous tissues except those lying around the vascular bundles, belong to type C (BABA, 1953). The elongated cells from the injured cells on the cut surface, however, are observed rarely and sporadically within 96 hours after the cutting (Fig. 7).

In parenchymatous tissues which lie within and around the vascular bundles, elongated cells from the injured cells are usually observed. These cells are found more numerously and earlier than in the parenchymatous tissues stated above. These elongated cells are usually formed within 48 hours and they become more numerous in the lapse of 72 and 96 hours after the cutting (Fig. 8).

(B) ENZYME ACTIVITY

1) *Nadi-Oxidase*

*) In the present material of *Helianthus tuberosus*, the water content of the tuber is about 81%. The elongated cells on the cut surface in parenchymatous tissues are not observed within 96 hours when the water content become lower than 65% or thereabouts.

Nadi-oxidase shows a blue positive reaction colour when nadi reagent is used as a substrate. The activity of the oxidase was determined by the amount of the blue coloured product when the reagent was oxidized.

One gm. of freshly excised tissues was homogenized in 9 ml. of McIlvaine's phosphate-citrate buffer solution at pH 6.0. Following reagents were added successively in a small Erlenmeyer flask; 1 ml. of 1% alpha-naphthol solution in 50% alcohol, 1 ml. of 0.75% aqueous solution of para-phenylendiamine and 7 ml. of the buffer solution. Then 1 ml. of the tissue homogenate was added to the mixture of the above reagents. The flask was kept under a constant aeration for 10 minutes at 30°C.. The blue coloured product there produced was extracted with 20 ml. of acetone. The oxidase activity was determined by the measurement of the density of the coloured product in acetone by means of a photoelectric

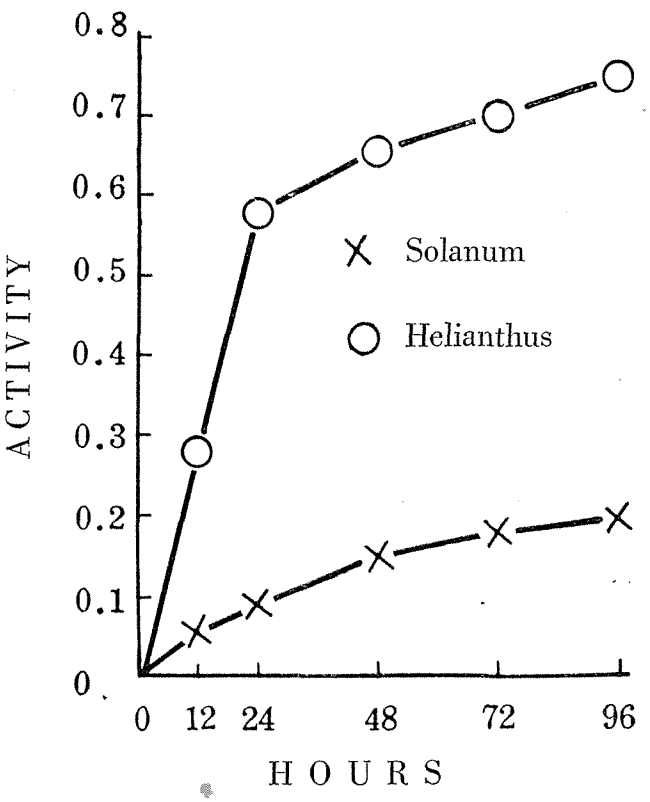



Diagram. 1 Diagram showing the relation between the nadi-oxidase activity and the lapse of time after the cutting during the wound periderm formation.

In this diagram, the axis of ordinates represents the oxidase activity in terms of optical density.

Table 1. Time Required for Decolorization of Methylene Blue during Wound Periderm Formation

| Hours After Cutting | Time Required for Decolorization (minutes) | | |
|---|---|---|---|
| | *Solanum tuberosum* | *Helianthus tuberosus* | *Raphanus sativus* |
| 0 | 45 | 15 | 18 |
| 12 | 20 | 14 | 15 |
| 24 | 9.5 | 18 | 10 |
| 48 | 8.8 | 33 | 14 |
| 72 | 12 | 50 | 17 |
| 96 | 15 | 150 | 18 |

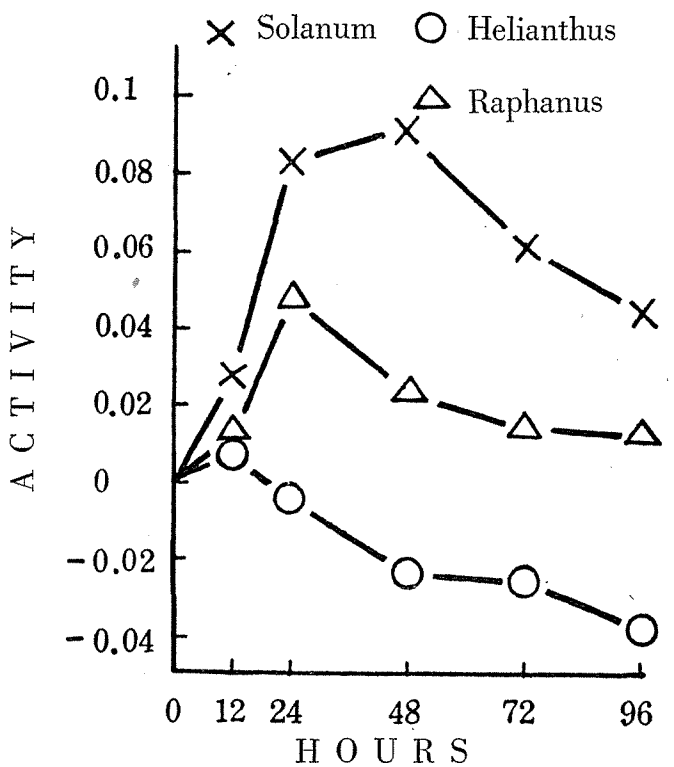



Diagram. 2 Diagram showing the relation between the dehydrogenase activity and the lapse of time after the cutting during the wound periderm formation.

In this diagram, the axis of ordinates represents the dehydrogenase activity in terms of reciprocal of the time required for the decolorization of methylene blue.

tube colorimeter.

In *Solanum tuberosum* and *Helianthus tuberosus*, nadi-oxidase activity is weak in the homogenate immediately after the cutting. While *Raphanus sativus* does not show activity immediatly after the cutting. In *Solanum tuberosum* and *Helianthus tuberosus*, the activity increases in the lapse of time after the cutting. These results are summarized in Diagram 1. Contrary to the case mentioned above, in *Raphanus sativus*, the activity remains unchanged with the lapse of time after the cutting.

2) *Dehydrogenase*

Dehydrogenase activity was determined by the methylene blue method after THUNBERG (1920) in an anaerobic condition.

In *Solanum tuberosum*, 2 gm. of freshly excised tissues were homogenized in 8 ml. of Sörensen's phosphate buffer solution containing potassium cyanide at pH 8.0. The concentration of potassium cyanide in the mixture was 0.01 Mol.. In the case of *Helianthus tuberosus* and *Raphanus sativus*, 3 gm. of the tissues were homogenized in 7 ml. of the buffer solution. 1.8 ml. of the homogenate and 0.2 ml. of 0.1 mM. methylene blue were added into a Thunberg tube. The tube was evacuated by a mechanical pump with vigorous shaking, and was kept in a bath regulated at 30°C.. The time required for the decolorization of methylene blue by these homogenate during the wound periderm formation is summarized in the Table 1.

Dehydrogenase activity expressed as reciprocal of the time required for the decolorization is shown in Diagram 2. In Table 1 and Diagram 2, it is seen

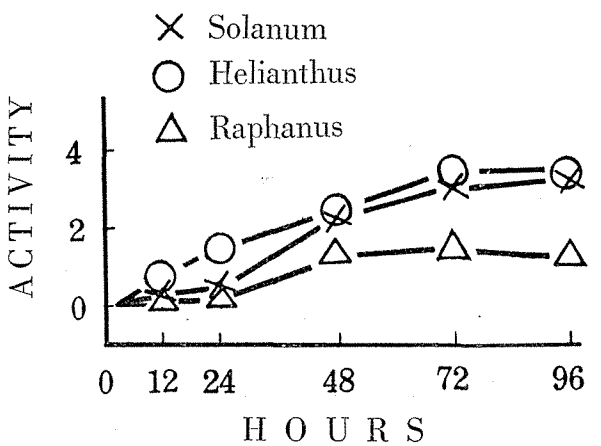



Diagram. 3 Diagram showing the relation between the peroxidase activity and the lapse of time after the cutting during the wound periderm formation.

In this diagram, the axis of ordinates represents the enzyme activity in terms of mg. of purpurogallin produced by 1 ml. of the homogenate.

that within 48 hours after the cutting in *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*, the dehydrogenase becomes very active, and thereafter the activity decreases.

3) *Peroxidase*

Peroxidase activity was measured by examining the amount of purpurogallin produced by the enzyme using pyrogallol as a substrate in the presence of hydrogen peroxide (WILLSTÄTTER et al. 1918, 1923). One gm. of freshly excised tissues was homogenized in 9 ml. of McIlvaine's phosphase-citrate buffer solution at pH 6.0. One ml. of 5% aqueous solution of pyrogallol, 0.1 ml. of 0.166 M. aqueous solution of hydrogen peroxide, 7.9 ml. of the buffer solution and finally 1 ml. of the homogenate, were mixed in a small Erlenmeyer flask. The mixture was kept at 30°C. for 10 minutes; then the enzyme was inactivated by adding 5 ml. of 20% sulfuric acid. The purpurogallin which had been thus produced was extracted with 50 ml. of ether, and that extract was compared, with the aid of a photoelectric tube colorimeter, with a standard solution of purpurogallin dissolved in ether.

In *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus* immediately after the cutting, about 20 mg., 21 mg. and 22 mg. of purpurogallin are produced respectively by 1 ml. of their homogenates. The amount of purpurogallin produced increases little by little with the lapse of time after the cutting. These results are shown in Diagram 3.

4) *Catalase*

Catalase activity was determined by examining the amount of hydrogen peroxide decomposed by the enzyme (EULER and JOSEPHSON, 1927).

Two gm. of freshly excised tissues were homogenized in 8 ml. of Sörensen's phosphate buffer solution at pH 6.8. In a small Erlenmeyer flask containing 0.5 ml. of 0.166 M. aquous solution of hydrogen peroxide and 8.5 ml. of the buffer solution, 1 ml. of the homogenate was added and mixed. The mixture was kept at 1.5°C. for 10 minutes, and then the enzyme was inactivated with 10 ml. of 20% sulfric acid. The mixture was titrated with a 0.0969 N. potassium permanganate solution.

In *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*, immediately after the cutting, hydrogen peroxide equivalent to about 0.63 ml., 0.82 ml. and 0.58 ml. of a 0.0969 N. potassium permanganate solution respectively is decomposed by the enzyme present in 1 ml. of the homogenate. Changes of catalase activity during the wound periderm formation are shown in Diagram 4. The activity in *Solanum tuberosum* at 24 hours after the cutting, shows a sudden increase and the activity in *Helianthus tuberoses* and *Raphanus sativus* also show an increase with the lapse of time after the cutting. The activity in *Solanum tuberosum* at 96 hours, however, shows a decrease as compared with that at 72 hours.

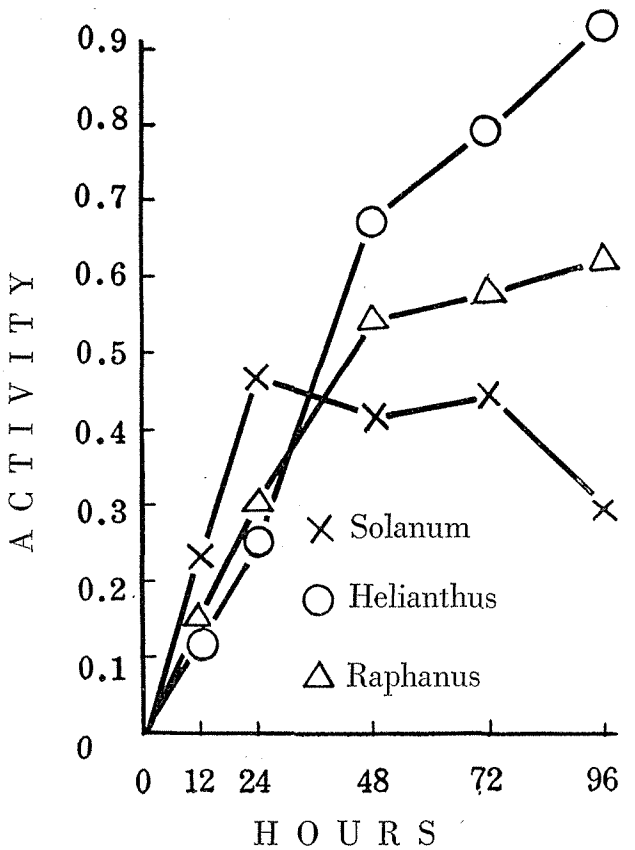



Diagram. 4 Diagram showing the relation between the catalase activity and the lapse of time after the cutting during the wound periderm formation.

In this diagram, the axis of ordinates represents the enzyme activity in terms of ml. of 0.0969 N. potassium permanganate equivalent to the hydrogen peroxide decomposed by the catalase.

## Conclusion

In parenchymatous tissues except those which lie around the vascular bundles, the morphological characteristics found in the course of the wound periderm formation in *Solanum tuberosum* and *Raphanus sativus* are usually the same respectively as those have been stated in the previous paper (BABA, 1953). While, in *Helianthus tuberosus*, cell divisions immediately below the cut surface and elongations of the injured cells on the cut surface often take place in some localities (cf. BABA, 1953). These elongations of the cells are not observed within 96 hours when the water content of the tuber is lower than 65% or thereabouts.

It may be assumed, therefore, that the occurrence of the elongated cells may have a connection with the water content of the tuber.

On the cut surface of the parenchymatous tissues which lie within and around vascular bundles, divided cells and elongated celles in *Solanum tuberosum* and *Helianthus tuberosus* and elongated cells in *Raphanus sativus*, are observed more numerously and earlier than in the parenchymatous tissues remote from the bundles. Further in *Solanum tuberosum* and *Helianthus tuberosus*, divided cells or divided and elongated cells are observed at first in parenchymatous tissues immediately near the vascular bundles, and then these cells are extensively towards the pith. All these facts mentioned above may support HABERLANDT's view of "Leptohormone" (HABERLANDT, 1930).

The activity of nadi-oxidase increases with the lapse of time after the cutting in *Solanum tuberosum* and *Helianthus tuberosus* in which cell divisions take place during the wound periderm formation, while, the activity of the enzyme in *Raphanus sativus*, in which cell divisions do not take place, does not increase. This fact agrees with the result obtained in our previous work (BABA, 1953) that the activity of nadi-oxidase has some close connection with the occurrence of cell division in the wounded tissues.

Dehydrogenase is most active within 48 hours after the cutting in *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*. Thereafter, the activity decreases with the lapse of time after the cutting.

The activity of peroxidase in *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus* and that of catalase in *Helianthus tuberosus* and *Raphanus sativus* increase with the lapse of time after the cutting. The catalase activity in *Solanum tuberosum* shows a sudden increase at 24 hours after the cutting. At 96 hours, when suberization of the cell wall often takes place in the most part of cortex and some part of pith, the enzyme activity decreases as compared with the activity at 72 hours after the cutting.

## Summary

(1) In the materials of *Solanum tuberosum*, *Helianthus tuberosus* and *Raphanus sativus*, morphological changes in the course of the wound periderm formation were microscopically observed, while the activities of nadi-oxidase, dehydrogenase, peroxidase and catalase were determined in vitro during the wound periderm formation.

(2) In the parenchymatous tissues lying remote from the vascular bundles, divided cells in *Solanum tuberosum*, divided cells and elongated cells in *Helianthus tuberosus* and elongated cells in *Raphanus sativus*, were often observed on the cut surface during the wound periderm formation.

(3) In the parenchymatous tissues lying within and around the vascular bundles, elongated cells or divided cells on the cut surface, were found more numerously and earlier than in the parenchymatous tissues remote from the

vascular bundles in all the materials studied.

(4) The activity of nadi-oxidase has some close connection with the occurrence of cell divisions.

(5) Dehydrogenase is most active within 48 hours after the cutting in all the materials studied.

(6) The activity of peroxidase in all the materials studied and that of catalase in *Helianthus tuberosus* and *Raphanus sativus*, increase with the lapse of time after the cutting. In *Solanum tuberosum*, however, the activity of catalase suddenly increases at 24 hours after the cutting, and decreases at 96 hours after the cutting.

## Literature cited

BABA, S.: Mem. Coll. Sci., Kyoto Univ. B. 20 (1953).
BONNER, J. and ENGLISH, J.: Science 86 (1937).
BONNER, J. and ENGLISH, J.: Plant Physiol., 13 (1938).
ENGLISH, J. and BONNER, J.: J. Biol. Chem., 121 (1937).
ENGLISH, J., BONNER, J. and HAAGEN-SMIT, A. T.: Proc. Nat. Acad. Sci., 25 (1939, a).
ENGLISH, J., BONNER, J. and HAAGEN-SMIT, A. T.: Science 90 (1939, b).
EULER H. von and JOSEPHSON, K.: Ann. Chem. 452 (1927).
HABERLANDT, G.: Sitzgsber. Akad. Wiss. Wien, Math.-nat. Klasse. (1902). Cited by KRENKE N. P.
HABERLANDT, G.: Sitzgsber. Akad. Wiss. Berlin, Physik.-math. Klasse. (1914). Cited by BLOCH R.
HABERLANDT, G.: Beitr. Allg. Bot., 2 (1923).
HABERLANDT, G.: Sitzgsber. Akad. Wiss. Physik. -math. Klasse. (1928) Cited by KRENKE N. P.
HABERLANDT, G.: Scientia (1930, a). Cited by KRENKE N. P.
THUNBERG, T.: Skand. Arch. Physiol., 40 (1920)
WIESNER, J.: Die Elementarstruktur und das Wachstum der lebenden Substanz. Wien (1892, a). Cited by KRENKE N. P..
WILLSTÄTTER, R. and POLLINGER, A.: Ann. Chem. 430 (1923).
WILLSTÄTTER, R. and STOLL, A.: Ann. Chem. 416 (1918).

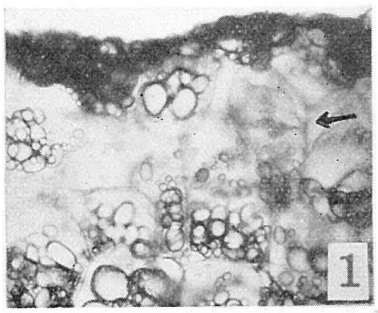
## Explanation of Plate

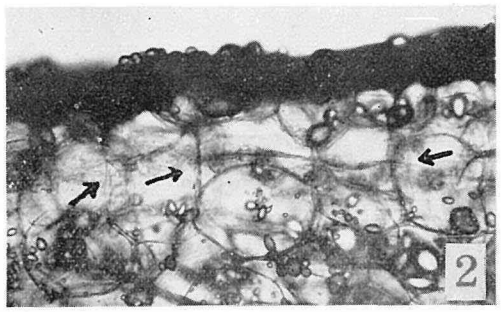
All the figures are photomicrographs of the thin longitudinal sections of tissues made rectangular to the cut surface after the cutting by cross section.

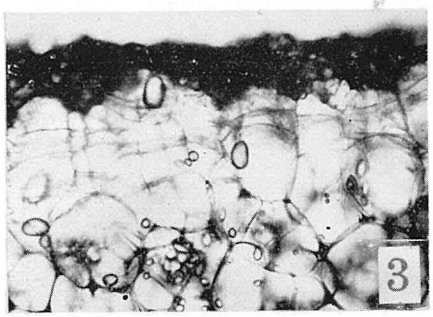
Figs 1–4 show an usual course of the wound periderm formation in *Solanum tuberosum*. (Magnification, 90 ×). Fig. 1 shows a section of parenchymatous tissue at 48 hours. The divided cell indicated by an arrow. Fig. 2 shows that at 72 hours. The cell membranes of the divided cells are indicated by arrows. Fig. 3 shows that at 96 hours after the cutting. Fig. 4 shows a section through the region of the vascular bundle at 96 hours after the cutting.

Fig. 5 and 6 show one of the forms of the wound periderm formation, which are frequently observed at 96 hours after the cutting in *Helianthus tuberosus*. (Magnification, 150 ×). Fig. 5 shows a section of parenchymatous tissue of the pith and Fig. 6 shows a section through the region of the vascular bundle.

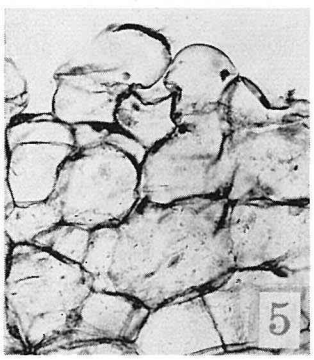
Fig. 7 and 8 show one of the forms of the wound periderm formation at 96 hours after the cutting in *Raphanus sativus*. (Mangnification, 220 ×). Fig. 7 shows a section of parenchymatous tissue of the pith and Fig. 8 shows a section through the region of the vascular bundle.

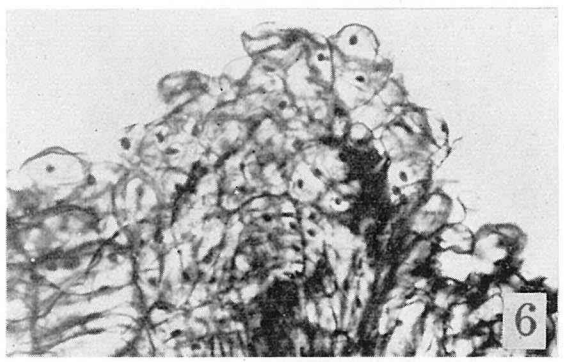

1

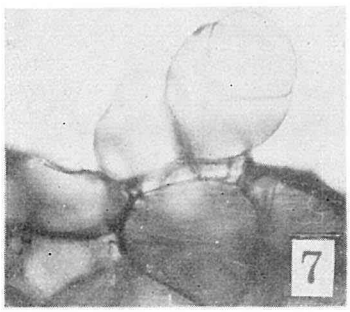

2

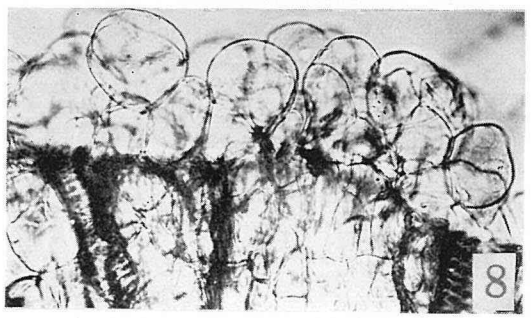

3


4


5


6


7


8