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Histochemical Studies of Wound Periderm Formation
V. Changes in Respiratory Activity of Wounded Tissues

By

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The relationship of respiration to cell divisions has been studied in fertilized eggs of invertebrates or of algae by several investigators. Some authors have found that cell divisions are not directly related to oxygen consumption (GRAY, 1931; WHITAKER, 1932 b and 1933, and others), while, others have concluded that cell divisions involve an oxidative process dependent on oxygen consumption (WHITAKER, 1932 a, c and 1933; CLOWES *et al.*, 1937 and 1940; KRAHL *et al.*, 1937, 1940 and 1941, and others). In some higher plants, BEATTY (1946) has found a correlation between oxygen consumption and mitotic frequency in young leaves, and RUHLAND *et al.* (1938) have shown that the meristematic tissue in stems has high values of respiratory quotient (R. Q.).

The present author has studied cell divisions during the wound periderm formation, and has found that cell divisions leading to the wound periderm are observed within 96 hours after the cutting in *Solanum tuberosum* and *Helianthus tuberosus*, but they are not observed within that period in *Raphanus sativus* (BABA, 1953, 1955 and 1958). In *Solanum* and some other plants, injured tubers or tissues show a greatly increased respiratory activity and this activity, after reaching a maximum, falls gradually to a normal rate as the wound heals over (RICHARDS, 1896; HOPKINS, 1927; APPLEMAN and BROWN, 1946; SAID and SHISHINY, 1947, and others). The author has also demonstrated that the activities of nadi oxidase, pyrocatechol oxidase and peroxidase have some close connection with the occurrence of cell divisions during the wound periderm formation in *Solanum tuberosum* and *Helianthus tuberosus* (BABA, 1955 and 1958).

From the facts mentioned above, the investigation of changes in the respiratory activity, the R. Q. and the respiratory inhibition caused by some inhibitors during the wound periderm formation seems important for the causal analysis of cell divisions. Therefore, the present study is aimed at determining whether the occurrence of cell divisions has some relationships to such changes or not.

Material and Methods

Tubers of *Solanum tuberosum* and *Helianthus tuberosus*, roots of *Raphanus sativus* were used as materials. Materials, cut with a sharp knife, were kept in a moist container at a temperature of about 30°C for 24, 48, 72 and 96 hours. From the materials, tissue slices about 0.5 mm in thickness, were excised parallel to the cut surface. One gm of these freshly excised slices suspended in 3.0 ml of M/30 phosphate buffer solution at pH 6.0 was used to measure rates of respiration, R. Q. and rates of respiratory inhibition by some respiratory inhibitors.

The respiration rates were determined by the standard WARBURG technique at 30°C (*cf.* UMBREIT *et al.*, 1949), and they were expressed as microliters oxygen taken up per hour by 1.0 gm of the freshly excised tissues.

Methods to determine respiratory inhibitions caused by respiratory inhibitors were all the same as mentioned above with the exception that each phosphate buffer solution contained one of the inhibitors. The respiratory inhibitors used in this study as well as their final concentrations are given in the following list. The phosphate buffer solution which contained an inhibitor was adjusted at pH 6.0 except in the case of potassium cyanide, which was adjusted at pH 7.0.

No.	Names of respiratory inhibitors	Final concentrations
1.	Potassium cyanide	M/1,000
2.	Sodium azide	M/2,000
3.	95% Carbon monoxide+5% oxygen	Saturate
4.	Sodium diethyldithiocarbamate	M/300
5.	Salicylaldoxime	M/300
6.	8-Hydroxyquinoline	M/1,000
7.	Potassium xanthogenate	M/1,000
8.	Malonic acid	M/100
9.	Malachite green	M/10,000
10.	2,4-Dinitrophenol	M/1,000
11.	Phloridzin	M/1,000
12.	Potassium arsenite	M/300
13.	Sodium fluoride	M/100
14.	Monoiodoacetic acid	M/1,000
15.	α, α'-Dipyridyl	M/1,000
16.	o-Phenanthroline	M/1,000
17.	Benzensulfonic acid	M/500
18.	Urethan	M/300

For the studies of photoreversible inhibition caused by CO, 1 gm of the excised tissue slices was suspended in 3.0 ml of M/30 phosphate buffer solution (pH 6.0), which was saturated with a gas mixture of 95% CO and 5% O_2, and the air in a WARBURG vessel was replaced by the gas mixture. Then the respiration rates of the tissue were determined both in the dark and also under strong illumination. The respiration rates of control samples in a gas mixture

of 95% N_2 and 5% O_2 were determined simultaneously.

Both the respiration rates and the inhibitions caused by the inhibitors in the present study were calculated from data taking in an average of more than 10 experiments of 2 hours period.

Results

The cell divisions leading to the wound periderm were observed along the cut surface within 96 hours after the cutting in *Solanum* and *Helianthus*, while,

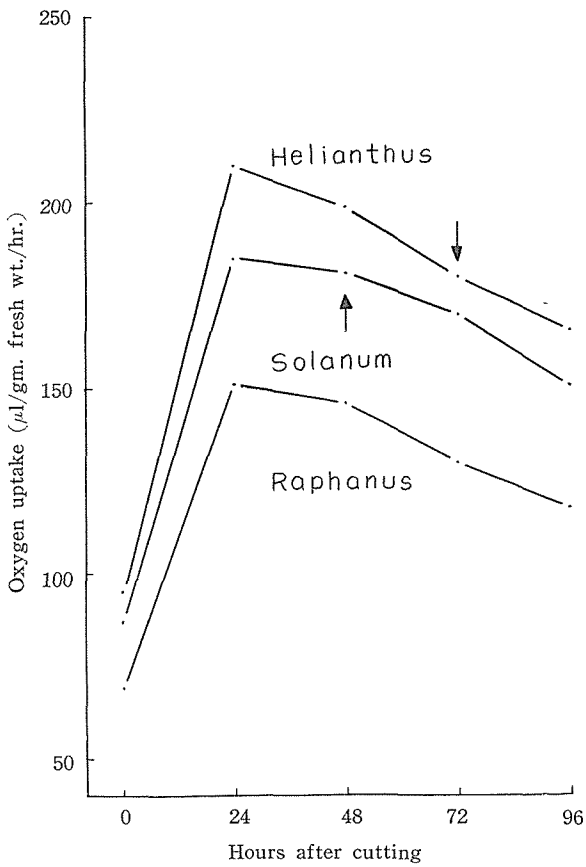

Diagram 1. Diagram showing relation between oxygen consumption and lapse of time after cutting.

The ordinates represent oxygen consumption, and the abscissas represent times after cutting.

The arrows mark the times at which cell divisions first observed.

such cell divisions were not observed in that period in *Raphanus*. These results of the morphological observation of the tissues during the wound periderm formation were the same as those given in the previous paper (BABA, 1955). In Diagram 1, the arrows mark the times at which cell divisions are first observed whether locally or entirely along the cut surface.

I) *Respiration rates.*

Immediately after the cutting, the respiration rates of the tissues in *Solanum*, *Helianthus* and *Raphanus* were approximately 87, 95 and 69 μl O_2/gm fresh weight/hour respectively.

The respiration rates at 24 hours after the cutting showed the maximum values in respective plants during the wound periderm formation and they were 185, 210 and 151 μl O_2/gm fresh weight/hour in *Solanum*, *Helianthus* and *Raphanus* respectively. Afterwards, the rates dropped with the lapse of time after the cutting, and the values at 96 hours after the cutting reached 151, 166 and 118 μl O_2/gm fresh weight/hour respectively for *Solanum*, *Helianthus* and *Raphanus*. Thus, the respiratory activities in all the materials used showed similar drifts which are shown in Diagram 1.

II) *Respiratory quotient.*

The R. Q. values in *Solanum*, *Helianthus* and *Raphanus* immediately after the cutting were 0.99, 0.94 and 0.96 respectively. The changes of R. Q. during the wound periderm formation in these plants are presented in Diagram 2. From this Diagram, it will be observed that the R. Q. in *Solanum* and *Helianthus* showed a slight increase until 48 hours after the cutting to reach about 1.15

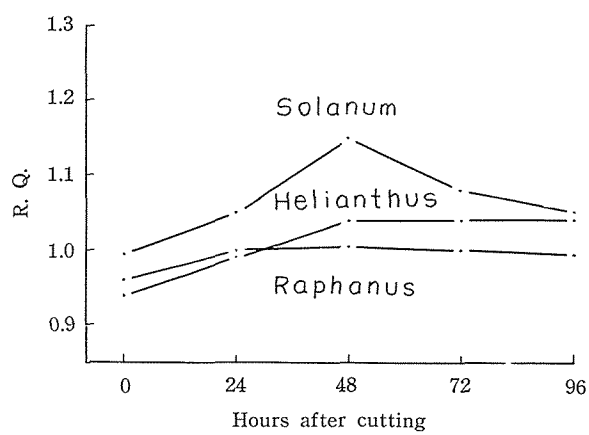

Diagram 2. Diagram showing relation between R. Q. and lapse of time after cutting.
The ordinates represent R. Q. values, and the abscissas represent times after cutting.

and 1.04 respectively, then they showed a slight decrease in *Solanum* and remained unchanged in *Helianthus*. At 96 hours after the cutting, the R. Q. values were about 1.05 in *Solanum* and 1.04 in *Helianthus*. The R. Q. values in *Raphanus* changed from 0.96 to 1.01 within 96 hours after the cutting, and no remarkable change was observed during that period.

III) *Effects of some respiratory inhibitors on the respiration.*

The effects of respiratory inhibitors on the respiration of the freshly excised tissues during the wound periderm formation in *Solanum*, *Helianthus* and *Raphanus* are summarized in Tables 1, 2 and 3.

From these tables, it is clear that in all the materials used the maximum inhibition was observed immediately after the cutting, and there occurred a tendency of decreasing inhibition with the lapse of time after the cutting. The results of these experiments which must be noted here are as follows:

(1) The inhibition caused by all the inhibitors used did not show a remarkable change prior to and following the first cell divisions which were observed at 48 hours in *Solanum* and at 72 hours in *Helianthus*.

Table 1. Effects of respiratory inhibitors on the respiration in *Solanum tuberosum* during wound periderm formation.

Inhibitors	Inhibition (%)				
	0*	24*	48*	72*	96*
1. K. cyanide	55	40	40	38	33
2. Na azide	52	42	41	38	32
3. CO (in dark)	53	38	38	38	31
(in light)	0	0	0	0	0
4. Na diethyldithiocarbamate	38	30	30	28	27
5. salicylaldoxime	35	31	30	26	23
6. 8-hydroxyquinoline	17	15	15	14	10
7. K xanthogenate	8	7	7	7	5
8. malonic acid	30	30	30	25	20
9. malachite green	50	50	48	40	30
10. 2,4-dinitrophenol	35	35	34	29	21
11. phloridzin	0	0	0	0	0
12. K arsenite	32	32	32	27	25
13. Na fluoride	40	28	28	25	22
14. monoiodoacetic acid	43	40	35	32	30
15. α, α'-dipyridyl	32	27	24	23	20
16. o-phenanthroline	15	13	13	12	9
17. benzensulfonic acid	5	3	3	3	3
18. urethan	0	0	0	0	0

*) These numbers show the lapse of time (in hours) after the cutting.

Table 2. Effects of respiratory inhibitors on the respiration in *Helianthus tuberosus* during wound periderm formation.

Inhibitors	Inhibition (%)				
	0*	24*	48*	72*	96*
1. K cyanide	55	50	48	45	43
2. Na azide	45	39	35	31	30
3. CO (in dark)	42	41	40	40	38
(in light)	0	0	0	0	0
4. Na diethyldithiocarbamate	25	25	24	23	22
5. salicylaldoxime	24	18	15	15	15
6. 8-hydroxyquinoline	30	30	29	29	28
7. K xanthogenate	5	5	4	4	4
8. malonic acid	30	30	30	30	29
9. malachite green	61	61	60	55	50
10. 2,4-dinitrophenol	37	36	33	27	25
11. phloridzin	−30	−30	−30	−30	−30
12. K arsenite	25	24	24	23	23
13. Na fluoride	45	34	34	34	32
14. monoiodoacetic acid	55	51	51	50	48
15. α, α'-dipyridyl	49	48	48	47	46
16. o-phenanthroline	35	27	22	22	21
17. benzensulfonic acid	6	4	4	4	4
18. urethan	0	0	0	0	0

*) These numbers show the lapse of time (in hours) after the cutting.

Table 3. Effects of respiratory inhibitors on the respiration in *Raphanus sativus* within 96 hours after the cutting.

Inhibitors	Inhibition (%)				
	0*	24*	48*	72*	96*
1. K cyanide	65	57	57	56	56
2. Na azide	64	59	59	57	57
3. CO (in dark)	40	37	37	36	36
(in light)	9	9	9	9	9
4. Na diethyldithiocarbamate	3	3	3	3	3
5. salicylaldoxime	5	5	5	5	5
6. 8-hydroxyquinoline	1	1	1	1	1
7. K xanthogenate	1	1	1	1	1
8. malonic acid	7	7	7	6	6
9. malachite green	52	51	50	48	47
10. 2,4-dinitrophenol	55	53	53	52	51
11. phloridzin	−10	−10	−10	−10	−10
12. K arsenite	33	31	30	30	30
13. Na fluoride	12	12	12	12	12
14. monoiodoacetic acid	56	55	54	53	53
15. α, α'-dipyridyl	20	19	19	19	19
16. o-phenanthroline	15	14	14	14	14
17. benzensulfonic acid	1	1	1	1	1
18. urethan	0	0	0	0	0

*) These numbers show the lapse of time (in hours) after the cutting.

(2) The reagents from No. 4 to 8 in Tables 1, 2 and 3 gave a considerable inhibition of respiration in *Solanum* and *Helianthus* during the course of the wound periderm formation, while they gave little inhibition in *Raphanus*.

(3) The inhibition caused by CO in the dark in *Solanum* and *Helianthus* within 96 hours after the cutting was completely or nearly completely recovered by light, while, that in *Raphanus* was not completely recovered.

(4) Phloridzin (No. 11) and urethan (No. 18) gave little inhibition on respiratory activity in all the materials studied, but phloridzin promoted the respiration in *Helianthus* and *Raphanus*.

Discussions and Conclusions

Dividing eggs of invertebrates and algae offer some of the best opportunities for a physiological study of cell division, especially in their first cleavage after fertilization (*cf.* KRAHL, 1950; BULLOUGH, 1952). WHITAKER with *Fucus* and *Nereis* (1932 a, c) and with *Arbacia* (1933) and KRAHL *et al.* with sea urchin eggs (1937, 1940 and 1941) have concluded that cell divisions are dependent on an oxidative process in the cell, on the contrary GRAY with *Echinus* (1931), WHITAKER with *Cumingia* (1932 b) and with *Chaetopterus* (1933) have found that the cell divisions are not directly related to oxygen consumption. In higher plants, BEATTY (1946) has found a correlation between oxygen consumption and mitotic frequency in the intact young leaves of *Ligustrum* and *Hedera* and MATSUMURA (1957) has reported that oxygen consumption shows a marked increase in a stage where active free nuclear divisions are observed in the endosperm of *Lilium*.

An injury given to tubers of *Solanum* and some other plant tissues causes an increased respiration (RICHARDS, 1896; HOPKINS, 1927; APPLEMAN and BROWN, 1946; SAID and SHISHINY, 1947, and others). In harmony with these results, the present investigation also reveals that the respiration rates show maximum values at 24 hours after the cutting in *Solanum*, *Helianthus* and *Raphanus*, and thereafter the rates decrease with the lapse of time. In the previous study, however, it has been reported that the cell divisions leading to the wound periderm are first observed at 48 hours in *Solanum* and at 72 hours in *Helianthus* while in *Raphanus* such cell divisions are not observed within 96 hours after the cutting (BABA, 1955). Therefore, it is highly probable to conclude that the over all respiration does not show a direct correlation with the occurrence of cell divisions during the wound periderm formation in the present materials.

The R. Q. in *Solanum*, *Helianthus* and *Raphanus* immediately after the cutting is 0.99, 0.94 and 0.96 respectively. These values show a close agreement with the data given by HANES and BARKER (1931), PRINGSHEIM (1935), SINGH and MATHUR (1938), PLATENIUS (1942) and SCHADE *et al.* (1949). As to the relation of R. Q. to mitotic activity, RUHLAND *et al.* (1938) have found that the meristematic tissue of the stems of *Syringa* and others has high R. Q. values. In

the present study, the R. Q. value in *Solanum* and *Helianthus* shows a slight increase until the cell divisions first appear along the cut surface, and thereafter, in the former material it shows a slight decrease while in the latter material it remains unchanged in the lapse of time. On the contrary in *Raphanus*, in which the cell divisions are not observed, the R. Q. does not show a remarkable change during the whole period of observation. The R. Q. values in the part of tissue where cell divisions are observed or expected to occur during the wound periderm formation are slightly high, but not so high as in the case of RUHLAND *et al.* (1938). It may be due to a difference of the materials used.

Among the respiratory inhibitors used in the present study, K cyanide (No. 1), Na azide (No. 2) and CO (No. 3) have been considered to be the inhibitors for the terminal oxidases which contain iron and copper atoms in their prosthetic groups (WARBURG, 1949), and Na diethyldithiocarbamate (No. 4), salicylaldoxime (No. 5), 8-hydroxyquinoline (No. 6) and K xanthogenate (No. 7) the inhibitors for the oxidases which contain copper atoms in their prosthetic groups (KUBOWITZ, 1937; KING, 1939; JAMES and GARTON, 1952). Malonic acid (No. 8) and malachite green (No. 9) have been regarded as the inhibitors for dehydrogenases (QUASTEL and WHEATLEY, 1931, and others), and 2,4-dinitrophenol (No. 10), phloridzin (No. 11) and K arsenite (No. 12) are uncouplers (LOOMIS and LIPMANN, 1948, and others), and Na fluoride (No. 13) and monoiodoacetic acid (No. 14) are reagents for inhibiting glycolysis (WARBURG and CHRISTIAN, 1942, and others).

The maximum inhibition of respiration are observed immediately after the cutting in all the materials studied, and the inhibitory rate decreases gradually with the lapse of time after the cutting. This fact may be due to the changes of physiological condition of the tissues by cutting (*cf.* BOSWELL *et al.*, 1938; SCHADE *et al.*, 1948 and 1949; BOSWELL, 1950), in part, due to the changes in respiratory system caused by the cutting, or in part, due to the lignification of cell membrane which proceeds in the lapse of time after the cutting.

The degree of respiratory inhibition caused by all the inhibitors used does not show a remarkable change prior to, during and following the first cell divisions in *Solanum* and *Helianthus*. It must be noted that the inhibitors for the oxidases which contain copper atoms in their prosthetic groups and the inhibitor for the isocitric dehydrogenase give a considerable inhibition in these two materials, and that these inhibitors, stated above, give little respiratory inhibition in *Raphanus* in which cell divisions are not observed. The above mentioned behavior of the inhibitors for copper containing oxidases may be supported by the fact that the pyrocatechol oxidase shows a positive reaction in *Solanum* and *Helianthus* but does not in *Raphanus* (BABA, 1958).

Though the inhibition of respiration by CO in the dark was completely or nearly completely recovered by light in *Solanum* and *Helianthus*, it was not completely recovered in *Raphanus*. It is a well known fact that cytochrome

oxidase is inhibited by CO in the dark and this inhibition is recovered by light (*cf.* WARBURG, 1949). Therefore, the results obtained in the present study agree with the result that nadi oxidase shows a positive reaction in *Solanum* and *Helianthus* but does not in *Raphanus* (BABA, 1955).

Summary

1) The rates of respiration, respiratory quotient and respiratory inhibition by some respiratory inhibitors were studied by employing the manometric technique using the tubers of *Solanum tuberosum* and *Helianthus tuberosus* and the roots of *Raphanus sativus* within 96 hours after the cutting.

2) The respiratory rates at 24 hours after the cutting show maximum values within 96 hours after the cutting in all the materials studied. While, the cell divisions leading to the wound periderm are first observed at 48 and 72 hours after the cutting in *Solanum* and *Helianthus* respectively, such cell divisions were not observed within 96 hours after the cutting in *Raphanus* (*cf.* BABA, 1955). From these facts, it seems that the over all respiration does not show a direct correlation with the occurrence of cell division during the wound periderm formation in the present materials.

3) Though the R. Q. in *Solanum* and *Helianthus* shows a slight increase during the wound periderm formation, in *Raphanus* it does not show a remarkable change within 96 hours.

4) The respiratory inhibition of the tissues is maximum immediately after the cutting in all the materials used, and there is a tendency of decreasing in inhibition with the lapse of time after the cutting.

5) The inhibitors for copper containing oxidases and that for iso-citric dehydrogenase give a considerable inhibition in *Solanum* and *Helianthus*, while they give little respiratory inhibition in *Raphanus*.

6) Though the inhibition by carbon monoxide in the dark is completely or nearly completely recovered by light in *Solanum* and *Helianthus*, it is not completely recovered in *Raphanus*.

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