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# RESPIRATION OF DEVELOPING ROSE PETALS[1]


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Some of the earliest investigations on the relation of respiration to development were carried out on flowers and floral structures. The Araceae were found particularly suitable for this purpose because of the high metabolic rates of such structures as the spadix and appendix. Respiratory activity in the aroids was found by van Herk (1, 2, 3) and later by James and Beevers (4) to exhibit a sudden rise followed by a decline. The maximum rate of respiration occurred when the appendix or spadix had reached its maximal size.

The purpose of this study was to examine the over-all gas exchange and some specific metabolic reactions in relation to growth and senescence of rose petals. The energy for expansion and unfolding of the petals is no doubt supplied by respiration. Attempts were made, therefore, to investigate the relation between the respiratory and the energy processes during the course of development of rose petals.

## Materials and Methods

*Rosa hybrida*, cultivar Better Times grown in a commercial greenhouse was used in all these experiments. The flowers were picked each morning and only the five outermost petals were used for respiration measurements.

Gas exchange measurements were made in the Warburg apparatus at 25° C with a shaking rate of 112 cycles per minute and a 3-cm amplitude. The results are reported as $Q_{O_2}$, $Q_{O_2}(N)$, and $Q_{O_2}(P)$, which designate oxygen uptake in cubic millimeters per hour per mg dry weight, mg nitrogen, or mg phosphorus, respectively, and $Q_{CO_2}$, which designates carbon dioxide production in cubic millimeters per hour per mg dry weight. The rose petals from 8 flowers were randomized, cut into 12-mm$^2$ pieces with scissors, and five pieces which varied in dry weight from 15 to 30 mg were placed in Warburg vessels with 3 ml of 0.05 M phosphate or McIlvaines buffer. Penetration of substrates or inhibitors was aided by vacuum infiltration (5). Preliminary experiments showed that the infiltration procedure had no effect on the respiration rate. Homogenate preparation is described in the text.

The photoreversibility of carbon monoxide was examined by irradiating with a no. 2 Mazda photoflood lamp immersed in the bath 10 cm from the side of the Warburg flask. Cyanide inhibition was measured using the center well solutions of Robbie (6) and Laties (7). 2,4-dinitrophenol (DNP) of the appropriate concentrations was prepared in 0.067 M phosphate buffer and then adjusted to pH 4.3. The other inhibitors were prepared in 0.05 M phosphate buffer and then adjusted to pH 4.5. Insoluble phosphorus was determined by the method of Krotkov and Helson (8) and nitrogen was determined by the semi-


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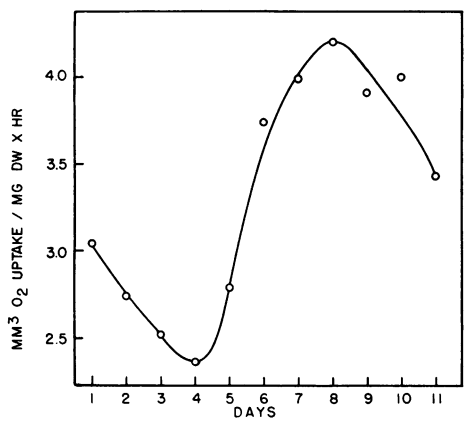
micro Kjeldahl method. All values reported are means of at least duplicate determinations with a maximum variation of 8 % between samples.

## Results

About 250 rose buds of uniform size were selected on the plants so that we could follow changes in respiration of the petals as they developed. At about 8 A.M. each day a sample of flowers was picked. Sampling began when the diameter of the flower buds reached 15 mm. The initial respiration rates were measured daily; the drift in respiration in relation to unfolding is presented by figure 1 and a diagram of the stages of development of the rose petal is shown in figure 2. Each stage in this figure represents about two days. The stage of slow petal expansion (fig 2, A and B) is characterized by a continual decline in $Q_{O_2}$ to a minimum value. The stage of rapid petal expansion (fig 2, C) is characterized by a sudden increase in $Q_{O_2}$. The stage of the full petal expansion (fig 2, D through F) shows a slow decline in the $Q_{O_2}$ from a maximal value.



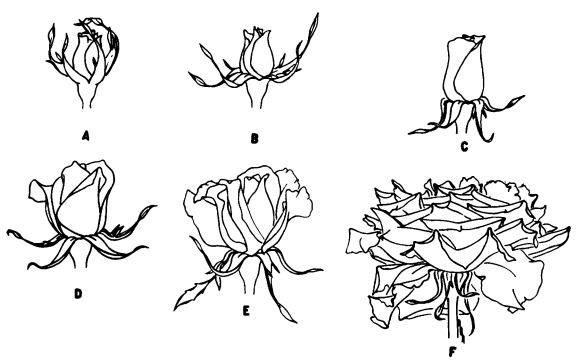
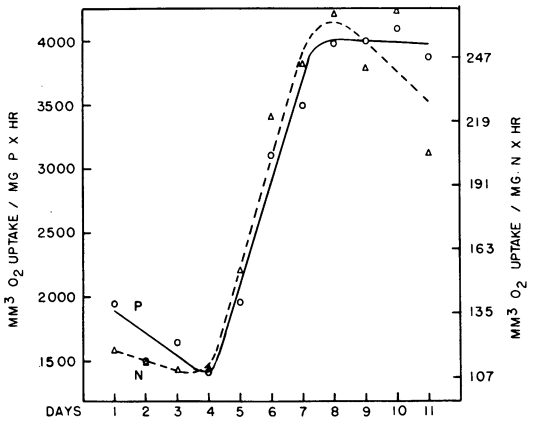




Fig. 1 (*above*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on dry weight.

Fig. 2 (*below*). Development of the entire rose flower during the period when respiration measurements were made. The stages shown are about 2 days apart (see text for details).



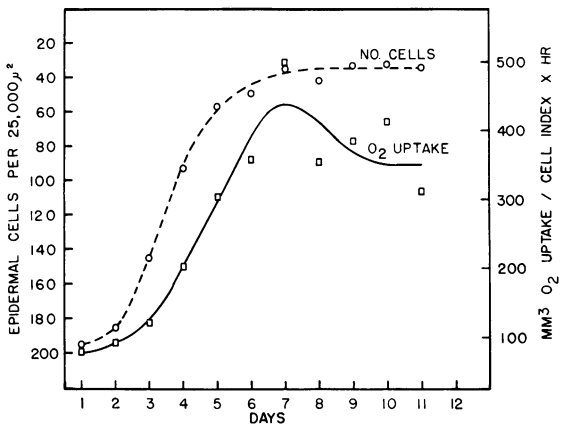
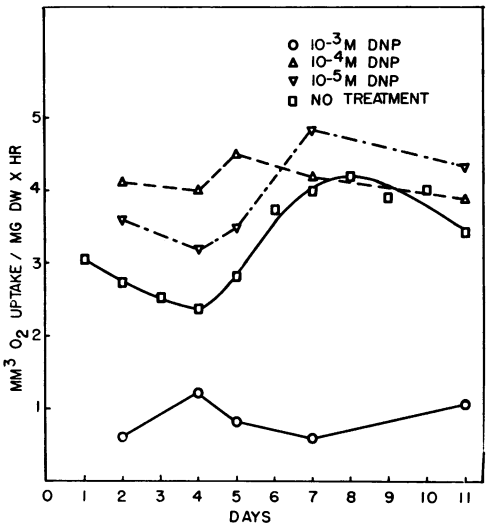




Fig. 3 (*above*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on total nitrogen (N) and insoluble phosphorus (P).

Fig. 4 (*below*). Drift of the initial oxygen uptake of pieces from the 5 outer rose petals with development based on a cell index. The decreasing number of epidermal cells per 25,000 $\mu^2$ with development was used to determine the cell index.

The drift in respiration was also examined using total nitrogen and insoluble phosphorus as the base for oxygen uptake. This represents an attempt to base respiratory measurements on an active fraction of the cell constituents. Respiration calculated on a nitrogen or insoluble phosphorus basis has a similar pattern (fig 3) to that shown by respiration based on dry weight (fig 1).

The petal area used for each respiration measurement was approximately constant. A count of the number of upper epidermal cells per unit area provided a means of expressing respiration on a cellular basis, on the assumption that the ratio of epidermal to mesophyll cells remains constant throughout growth



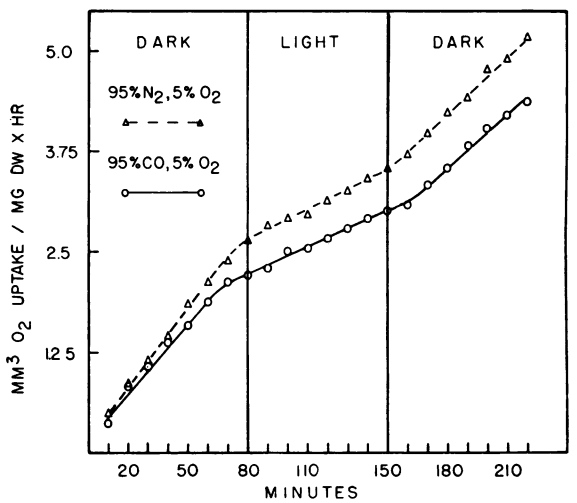




FIG. 5 (*above*). Effect of 2,4-dinitrophenol (DNP) on the initial oxygen uptake of pieces from the 5 outermost rose petals with development.

FIG. 6 (*below*). Oxygen uptake of pieces from the 5 outer rose petals during slow petal expansion in the presence of carbon monoxide (95 % CO + 5 % $O_2$) and in its absence (95 % $N_2$ + 5 % $O_2$).

due to cell expansion. No cell divisions were observed during petal expansion. The number of cells in a unit area decreased as the petals increased in size. The oxygen uptake on a cell index basis (fig 4) resembled the pattern of the respiratory drift based on dry weight, nitrogen and insoluble phosphorus. This was calculated by dividing the oxygen uptake of the petal area used by the total number of upper epidermal cells normalized by dividing by the number of upper epidermal cells of the first day.

The nature of the metabolic changes associated with development was examined in experiments with 2,4-dinitrophenol (DNP). It was shown above that the respiration of rose petals varied with their stage of development. It was of interest, therefore, to see whether the rate of oxygen uptake by this material could be stimulated by DNP and whether the degree of stimulation is related to the stage of development. The respiratory rates of rose petal pieces subjected to different concentrations of DNP are plotted against time in figure 5. The curves are compared with a normal respiratory curve of developing rose petals. The stimulatory effect of DNP at $10^{-5}$ M and $10^{-4}$ M was more pronounced during the stage of slow petal expansion (fig 2, A and B) than during the stage of full expansion (fig 2, D through F). The importance of selecting the proper DNP concentration for demonstrating stimulation of oxidative activity has been shown for several tissues (9). In the case of avocado fruit slices, for example, it was found (10) that $10^{-4}$ M DNP was most effective in bringing about an increase in oxygen uptake, while $10^{-3}$ M DNP was definitely inhibitory. With the rose petals, the greatest percentage of increase of oxidative activity was brought about by $10^{-4}$ DNP and took place during the period preceding full petal expansion.

A comparison of the metabolism of the rose petals was attempted at the stages of slow and full petal expansion. The respiratory quotient was found to be 1.0 at both stages. Increasing the oxygen concentration from 20.9 % (air) to 100 % had no effect on respiration. Evidently the terminal oxidase system was saturated with respect to oxygen at 20.9 %. Data of table I indicate that during the slow petal expansion stage (fig 1, A and B) the petals had a lower rate of anaerobic $CO_2$ production than during the fully expanded stage (fig 1, D through F). Fluoride at 0.03 M inhibited the anaerobic $CO_2$ production of petals moderately in the stage of slow petal expansion and strongly during the fully expanded stage (table I).

Characterization of the terminal oxidase of the petals was attempted by the use of the heavy metal inhibitors azide, cyanide, and carbon monoxide. At the lower concentration, cyanide and azide produced a slight stimulation of oxygen uptake in the slowly expanding petals (table II). They were moderately to severely inhibitory at the higher concentration. The fully expanded petals were strongly inhibited at

TABLE I

EFFECT OF FLUORIDE ON THE ANAEROBIC $CO_2$ PRODUCTION OF ROSE-PETAL PIECES

| STAGE OF PETAL EXPANSION AND INHIBITOR | $Q_{CO_2}$ | % INHIBITION |
|---|---|---|
| Slow (fig 1, A and B) | | |
| None | 0.77 | ... |
| 0.03 M NaF | 0.47 | 39 |
| Full (fig 1, D–F) | | |
| None | 2.1 | ... |
| 0.03 M NaF | 0 | 100 |

TABLE II

EFFECT OF CYANIDE AND AZIDE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES

| STAGE OF PETAL EXPANSION AND INHIBITOR | CONCENTRATION | $Q_{O_2}$ | % INHIBITION |
|---|---|---|---|
| Slow (fig 1, A and B) | | | |
| None | ..... | 2.4 | .. |
| KCN | $1 \times 10^{-4}$ M | 2.7 | -13 |
| KCN | $5 \times 10^{-3}$ M | 0.6 | 75 |
| None | ..... | 2.3 | .. |
| $NaN_3$ | $1.7 \times 10^{-4}$ M | 3.1 | -35 |
| $NaN_3$ | $1.7 \times 10^{-3}$ M | 1.8 | 22 |
| Full (fig 1, D–F) | | | |
| None | ..... | 4.1 | .. |
| KCN | $1 \times 10^{-4}$ M | 1.3 | 68 |
| KCN | $5 \times 10^{-3}$ M | 0.12 | 97 |
| None | ..... | 3.4 | .. |
| $NaN_3$ | $1.7 \times 10^{-4}$ M | 0.55 | 84 |
| $NaN_3$ | $1.7 \times 10^{-3}$ M | 0.01 | 100 |

the lower concentration of cyanide and azide and completely inhibited at the higher concentrations. The oxygen uptake of the slowly expanding petals was inhibited only about 10 % in the dark by carbon monoxide (95 % CO + 5 % $O_2$) compared to the oxygen uptake of similar petals in 95 % $N_2$ + 5 % $O_2$ (fig 6). Irradiation of the flasks caused a decrease in oxygen uptake compared with the dark period. The inhibition in light compared with dark was about 55 %. The effect of light on decreasing oxygen uptake was not due to carbon monoxide since the control flasks responded similarly. The oxygen uptake of fully expanded petals was inhibited by carbon monoxide in the dark and this inhibition was partially reversed by light (fig 7).

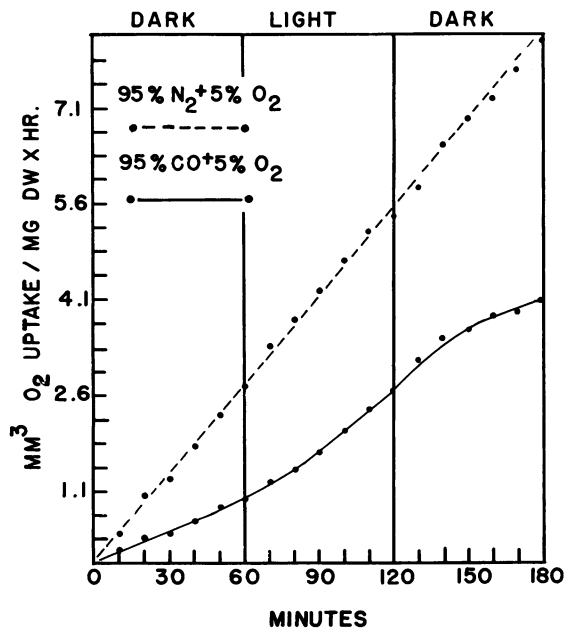



FIG. 7. Oxygen uptake of pieces from the 1st 5 outer fully-expanded rose petals in the presence of carbon monoxide (95 % CO + 5 % $O_2$) and in its absence (95 % $N_2$ + 5 % $O_2$).

The influence of malonate on the initial respiration of rose petals was examined at the slow and full petal expansion stages. The petals in the slow expansion stage were only weakly inhibited by malonate (table III). Increasing the concentration of malonate caused an increase in the percentage of inhibition, but inhibition at high concentrations is probably non-specific. The respiration of the fully expanded petals was markedly inhibited by 0.05 M malonate (table III) but not by 0.01 M malonate. The inhibition of respiration caused by 0.05 M malonate was not reversed by pyruvate or fumarate.

TABLE III

EFFECT OF SOME ORGANIC ACIDS AND GLUCOSE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES

| % INHIBITION | TREATMENT | $Q_{O_2}$ | STAGE OF PETAL EXPANSION |
|---|---|---|---|
| Slow (fig 1, A and B) | None | 2.7 | .. |
| | 0.05 M malonate | 2.3 | 15 |
| | None | 3.3 | .. |
| | 0.04 M malonate | 2.9 | 12 |
| | 0.06 M malonate | 2.3 | 30 |
| | 0.08 M malonate | 2.0 | 39 |
| | 0.10 M malonate | 1.8 | 45 |
| Full (fig 1, D–F) | None | 3.2 | .. |
| | 0.05 M malonate | 1.2 | 62 |
| | 0.03 M pyruvate | 3.7 | -16 |
| | 0.05 M malonate + 0.03 M pyruvate | 1.4 | 56 |
| | None | 3.3 | .. |
| | 0.05 M malonate | 1.0 | 70 |
| | 0.05 M fumarate | 2.9 | 12 |
| | 0.05 M malonate + 0.05 M fumarate | 0.7 | 79 |
| | 0.01 M malonate | 3.2 | 3 |
| | 0.03 M pyruvate | 3.9 | -18 |
| | 0.01 M malonate + 0.03 M pyruvate | 3.2 | 3 |
| Slow (fig 1, A and B) | None | 3.4 | .. |
| | 0.03 M pyruvate | 3.2 | 6 |
| | 0.05 M glucose | 3.4 | 0 |
| | 0.06 M succinate | 3.7 | - 9 |
| Full (fig 1, D–F) | None | 3.1 | .. |
| | 0.03 M pyruvate | 3.9 | -26 |
| | 0.05 M glucose | 3.7 | -19 |
| | 0.06 M succinate | 3.6 | -16 |

Several substrates were infiltrated into rose petals at the slow and full petal expansion stages. During the slow petal expansion stage, no substrate tested elicited any extra respiration (table III). Fully expanded petals had a slightly increased respiration after infiltration of several substrates. Starvation of the excised petals by holding for 24 or 48 hours at 25° C did not change their response to the added substrate.

A comparison of the oxygen uptake of a homogenate from rose petals and rose-petal pieces was made. Homogenates were prepared by grinding 5 g

of rose petals with 25 ml of 0.10 M phosphate buffer pH 7.4 in a chilled mortar with sand. The endogenous oxygen uptake of homogenates prepared from rose petals was linear for about 30 minutes and then gradually declined. Cyanide at $5 \times 10^{-3}$ M did not inhibit the oxygen uptake of a homogenate prepared from petals at the stage of the slow petal expansion or at the stage of full petal expansion (table IV). However, oxygen uptake of the petal pieces of either stage was almost completely inhibited at $5 \times 10^{-3}$ M cyanide.

The results of the heavy metal inhibitor studies on fully expanded petals suggested that cytochrome oxidase might be operative as the terminal oxidase. Attempts to assay for cytochrome oxidase with homogenates were not successful. Further attempts to demonstrate cytochrome oxidase in the particulate matter obtained from high-speed centrifugation were not successful.

It is obvious from the experiments with heavy metal inhibitors and DNP that considerable metabolic changes occur during the growth and development of rose petals. In order to carry out further studies on the changes of the enzymatic activities and phosphorylations, attempts were made to prepare an active mitochondrial fraction from rose-petal homogenates. Homogenates of rose petals were made by blending 30 g of tissue for one minute with 60 ml of a solution 0.4 M in sucrose and 0.1 M in phosphate adjusted to pH 7.1. The blendor was operated at 50 volts. The homogenate was centrifuged first at 500 $\times$ G for five minutes, and the supernatant solution from this centrifugation was further centrifuged at 17,000 $\times$ G for 15 minutes to obtain the mitochondrial fraction. The mitochondrial fraction so obtained stained with Janus green, but did not catalyze the oxidation of any acids in the tricarboxylic acid cycle. The pH and temperature of the preparation were carefully controlled. Throughout the preparation, the pH was kept between 6.8 and 7.1 and the temperature was between 0° and 3° C. Tonicity of the medium was varied (0.25 to 0.75 M sucrose). However, the mitochondrial fractions were metabolically inactive. Therefore, it appears that inactivity of the preparations cannot be attributed to the factors of pH, temperature, or tonicity in the range examined. The possibility of the release of an inhibiting substance during the preparation of the mitochondria was tested. The rose-petal supernatant solution was added to preparations of avocado and pea mitochondria. The avocado particles were prepared according to the procedure of Biale et al (11), while the pea mitochondria were obtained by a method similar to that of Millerd et al (12) for mung beans. The oxidation of succinate by pea and avocado mitochondria was inhibited by the rose-petal supernatant solution (table V). A similar inhibition was obtained for the oxidation of $\alpha$-ketoglutarate and malate. These results suggest that the damage to the rose-petal mitochondria was irreversible and took place during the course of preparation.

TABLE IV

EFFECT OF CYANIDE ON THE OXYGEN UPTAKE OF ROSE-PETAL PIECES AND HOMOGENATES

| STAGE OF PETAL EXPANSION, INHIBITOR AND TYPE OF MATERIAL | $Q_{O_2}(N)$ | % INHIBITION |
|---|---|---|
| Slow (fig 1, A and B) | | |
| No inhibitor | | |
| Piece | 110 | .. |
| Homogenate | 140 | .. |
| $5 \times 10^{-3}$ M KCN | | |
| Piece | 23 | 79 |
| Homogenate | 130 | 7 |
| Full (fig 1, D–F) | | |
| No inhibitor | | |
| Piece | 250 | .. |
| Homogenate | 70 | .. |
| $5 \times 10^{-3}$ M KCN | | |
| Piece | 10 | 96 |
| Homogenate | 100 | -43 |

TABLE V

EFFECT OF ROSE SUPERNATANT SOLUTION ON THE OXYGEN UPTAKE OF AVOCADO AND PEA MITOCHONDRIA

| COMPONENTS IN REACTION MIXTURE * | OXYGEN UPTAKE IN MM³/VESSEL/HOUR | |
|---|---|---|
| | AVOCADO MITOCHONDRIA | PEA MITOCHONDRIA |
| Mitochondria + succinic acid | 200 | 181 |
| Mitochondria + succinic acid + rose supernatant | 70 | 90 |
| Mitochondria + rose supernatant | 35 | 54 |
| Rose supernatant + succinic acid | 24 | ... |
| Rose supernatant | 23 | 15 |

* Reaction mixture: 60 micromoles of succinic acid, 30 micromoles of phosphate, 3 micromoles of ATP, 12 micromoles of Mg, and 0.5 ml mitochondrial enzyme of avocado or pea as indicated, with or without 1.0 ml of rose supernatant as indicated in 3.0 ml of 0.5 M sucrose solution adjusted to pH 7.1 at 20° C.

## DISCUSSION

The course of respiration in developing rose petals followed a definite pattern resembling that of the climacteric rise in fruits (13). There was a marked rise in respiration during the expansion of the rose petals. In the case of fruits, the respiratory rise, which is associated with the ripening process, takes place normally after the fruits are fully expanded and mature. In the case of developing rose petals, the respiratory rise accompanies development and maturation, characterized morphologically by the rapid expansion and unfolding of the petals. From a morphological viewpoint, the respiratory rise of rose petals

is not comparable to the climacteric rise of fruits. Respiration studies of entire flowers did not reveal a respiratory rise at any time after commercial harvest (14). The centripetal development of the petals in a multipetaled flower probably confounds the respiratory rise observed here in one whorl of petals. Bonner and Honda (15) found no indication of a respiratory rise in petals of mature Camellia flowers already at maximum size.

The stimulatory effect of certain DNP concentrations was found to be at a minimum when the endogenous respiration of the rose petal was at its peak. The respiration of the early stage of growth can effectively be raised by eliminating its dependence on phosphorylation with the aid of DNP. The pronounced DNP effects in the early developing stage of rose petals suggest that the phosphorylative capacity is limiting, perhaps due to relatively low levels of phosphate acceptor. As unfolding progresses the increased synthetic activity might be responsible for a greater turn-over of the acceptor. An analysis for adenylates, however, is needed before any definite conclusions can be drawn.

Along with changes in the respiratory pattern associated with the growth of rose petals, observations were made on changes in the metabolism by inhibitor studies. The results of these inhibitor studies suggest that the terminal oxidase of the full expansion stage of the rose petals is principally a metal enzyme and probably cytochrome oxidase. However, the nature of the terminal oxidase of the slow expansion stage of the rose petals was not ascertained.

The high rate of oxygen consumption by the homogenate was striking. The cyanide insensitivity of these preparations resembles that of the spinach chloroplast preparation of Laties (16). It appears that the homogenization process rendered the cyanide-sensitive system inoperative.

The inhibitions of enolase by fluoride has been amply demonstrated in plants (5, 17). The anaerobic $CO_2$ production of the rose petals was markedly inhibited by fluoride, and this fluoride inhibition is more pronounced at the full expansion stage than at the slow expansion stage. The studies on the effect of malonate also indicate that possible changes have taken place in the aerobic metabolism with the respiratory rise. Pyruvate, succinate, and glucose all increased the oxygen uptake of the rose petals in the full-expansion stage, but they were without any marked effect on the rose petals in the slow-expansion stage.

There was a rise in the anaerobic $CO_2$ production along with the respiratory rise in the developing rose petals. The rate of the anaerobic $CO_2$ production of the petals in the full-expansion stage was 2.7 times as high as the rate of the petals of the slow-expansion stage. Thus, the ratio of the anaerobic $CO_2$ production to the aerobic $CO_2$ production was 0.31 for the petals in the slow-expansion stage and 0.52 for the petals in the full-expansion stage. Apparently, a Pasteur effect occurs only in the full-expansion stage.

In recent years, cytoplasmic particles, believed to be mitochondria, have been isolated from a number of plant tissues. The mitochondria are known to be the site of oxidation of Krebs-cycle acids and are able to carry out phosphorylation coupled with oxidation (18). The failure to obtain metabolically active mitochondrial preparations from the rose petals prevented further enzymatic characterization of the respiratory pattern of rose petals. The supernatant solution from rose petals was shown to be inhibitory to the normal oxidative activities of mitochondria from avocado and pea. This inhibitory effect suggests the possibility that there is an inhibitor present in the cell sap of rose petals, which interferes with the isolation of an active mitochondrial preparation.

## Summary

The respiration of rose petals was studied in relation to growth and development. A definite respiratory pattern was found in the developing rose petals. It consisted of a respiratory rise before the opening of the flower, a respiratory peak when the flower fully opens, and a respiratory drop following the complete opening of the flower.

DNP at concentrations of $10^{-5}$ M and $10^{-4}$ M increased and at $10^{-3}$ M inhibited the respiration of the developing rose petals. The stimulatory effect of DNP was more pronounced during the early stage of petal unfolding than during the stage of full expansion.

Metabolic changes accompanied the growth and development of the rose petals. While the nature of the terminal oxidase from inhibitor studies was not defined during the stage of slow petal expansion, it appeared that cytochrome oxidase participated in the respiration during full-petal-expansion stage. Fluoride was an inhibitor of anaerobic carbon dioxide production and it was more effective during the stage of full petal expansion. Malonate was only a respiratory inhibitor during the full-petal-expansion stage, and the inhibition was not reversible on the addition of fumarate or pyruvate. Pyruvate, succinate and glucose increased the respiration during the full-petal-expansion stage only. The oxygen uptake of the homogenate was cyanide insensitive. Attempts to study metabolic changes in the developing rose by mitochondrial preparation were not successful. Indications were obtained that the supernatant solution from rose petals inhibited the activity of the mitochondria.

The authors are indebted to Dr. F. M. Scott for the cell counts of the rose petals during development.

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# PHOTOCONTROL OF ALCOHOL, ALDEHYDE, AND ANTHOCYANIN PRODUCTION IN APPLE SKIN[1]

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While examining the influence of light on anthocyanin synthesis in pieces of apple skin floating on sucrose solutions, we noticed that light strongly inhibited the formation of the characteristic apple odor (10). In the present work the influence of visible radiation on aldehyde, alcohol, and anthocyanin formation was examined in detail. Radiation was found to suppress oxygen consumption markedly with accompanying sparing of sugar, suggestive of the Pasteur effect. One of the aromatic rings in the anthocyanin molecule is derived from acetate (1, 3, 4, 11), and anthocyanin synthesis in many plants requires radiation. The action spectra suggest that the photoreceptive pigment is the oxidized form of an acyl coenzyme A dehydrogenase (9, 10), in accord with the utilization of acetate. Further evidence that two photoreactions with the same photoreceptor are required for anthocyanin synthesis in apple skin is presented here.

## MATERIALS AND METHODS

Early-harvested Jonathan and Arkansas apples were held at 0° C after being sealed in 1-bushel bags made of 0.38-mm polyethylene. Narrow strips of green areas of the apple skin were removed with a White Mountain apple peeler. The strips were cut into 7- × 7-mm pieces with razor blades mounted in a holder. The pieces, which had some cortical tissue attached, were washed twice with 0.3 M sucrose and then for one hour in running tap water. Fifty-one pieces (25 $cm^2$) of apple skin were placed cuticle side up in a 10-cm Petri dish containing 15 ml of 0.3 M sucrose. The Petri dishes were held in darkness or irradiated with a cool white fluorescent source of radiant energy, giving a maximum illumination of about 2000 foot-candles (ft-c).

All inhibitors and substrates used were dissolved in 0.3 M sucrose and the final solution was adjusted to pH 4. All temperatures reported were measured in the sucrose solution in which the pieces of apple skin floated.

In all experiments in which anthocyanin synthesis was examined, the Petri dishes containing the apple-skin pieces were held 24 hours in darkness after irradiation at a temperature three degrees lower than that during the irradiation period.

The anthocyanin content of the pieces of apple skin was determined on an extract made at 1° C in

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