

POLAR DISTRIBUTION OF RESPIRATORY RATE IN THE ONION ROOT TIP

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Introduction

Since the root tip of *Allium cepa* is being used more and more in physiological investigations, it was considered of value to investigate and compare the respiratory rate of different regions in this polar tissue. LUND and KENYON (8) observed that the region of active mitotic division in the root tip had the greater rate of reduction of methylene blue when compared to more basal regions and that this apical region was electropositive to the higher regions of the root. They also found that there was the same polar distribution in the production of acid (CO_2) when tested with phenol red. These factors were given as evidence in support of the theory of the origin of bioelectric potentials as oxidative-reductive systems in flux equilibrium. MARSH (9) showed that the electrical potential difference between apical and basal contacts on the root could be depressed by KCN, and ROSENE and LUND (11) found that the displacement of oxygen with hydrogen around the root also lowered the E.M.F. The readmission of oxygen gave a "rebound" effect in the p.d. of the root which was interpreted as resulting from a greater accumulation of oxidizable material in the apex as compared to the base. BERRY and HOYT (1, 2) observed that an onion root under anaerobic conditions could not be stimulated when direct or alternating current was passed through the root. ROSENE (13) has recently reported the inhibiting effect of KCN on the rate of exudation in excised onion roots. The present paper evaluates the normal respiratory rate of three segments of the root and the problems associated with measurements of this type. There is also included a preliminary estimate of the effects of cyanide and methylene blue on oxygen consumption in these segments.

Method

The WARBURG manometric method (5) was used for measuring the oxygen consumption of from 34-61 root segments. The dry weights varied from 3-14 mg. and the final values are expressed as cubic millimeters per root per hour. Respiration was also calculated as cu. mm. of oxygen consumed per mg. dry weight per hour (Q_{O_2}) but the results were no more reproducible than those reported below. For this reason the former values alone are reported in this paper. Average values obtained in this way were checked on single roots using the SCHOLANDER microrespirometer (15).

The roots were grown as usual in an aerated tap-water aquarium from bulbs 10-15 mm. in diameter. The bulbs were supported on a paraffined wooden board so that the roots could grow freely into the water through holes in the board. In preliminary experiments they were permitted to grow down into open glass tubes with the bulb supported on a flanged end. More

uniform aeration of all roots was obtained when no glass tubes were used. A thermoregulator maintained the aquarium temperature at $25^{\circ} \pm 1^{\circ}$ C. Roots 30–60 mm. in length were used and a total growth period of 72 ± 8 hours was employed.

Root segments were cut the desired length with small dissecting scissors having a rotating compass point soldered to one blade. For minimum injury the segments were placed immediately on moistened filter paper and transferred to the flasks by spatula. Roots from 8–15 bulbs were used, each flask having the same number of tips from each onion, with either equal lengths or 5, 10, and 15-mm. lengths used in the different flasks for one experimental run. The time required to prepare tissue limited the number of experimental flasks to three. With the temperature of the water bath at 25° C. and shaking speed at 100 oscillations per minute, the respiratory rate was constant for three hours or more and then began to decline. Only the rates of oxygen consumption measured during the initial three hours are, therefore, reported in this paper. After the experimental run, roots were dried in an electric oven at $60\text{--}70^{\circ}$ C. and weighed to 0.5 mg.

Results

NORMAL RESPIRATION

Table IA summarizes the results of Warburg manometric measurements made with three different lengths of root tips, as shown in column one. The range of values calculated as cubic millimeters of oxygen consumed per root per hour is given in column four and the mean value and probable error for each segment are shown in column five. From these results it is possible to evaluate and compare the rates of oxygen consumption for the arbitrarily selected segments of root designated in column six. The values as they appear in column seven are obtained by finding the difference between the mean values in column five. The apical 5-mm. root tip has an oxygen consumption

TABLE I

NORMAL RESPIRATION OF ONION ROOT TIP
A. WARBURG MANOMETERS

LENGTH OF ROOT TIP	NUMBER OF EXPS.	NUMBER OF ROOTS PER EXP.	MM. ³ O ₂ /ROOT/HR. (RANGE OF VALUES)	MM. ³ O ₂ /ROOT/HR. (MEAN)	SEGMENT OF ROOT	MM. ³ O ₂ /ROOT/HR. (BY DIFFERENCE)	% OF APICAL 5 MM.
I	II	III	IV	V	VI	VII	VIII
<i>mm.</i>					<i>mm.</i>		%
0-5	31	46-61	0.783-1.189	0.997 ± .075	0-5	(0.997)	100.0
0-10	41	36-56	1.349-2.116	1.678 ± .144	5-10	0.681	68.3
0-15	39	34-60	1.870-2.793	2.255 ± .137	10-15	0.577	57.8
B. SCHOLANDER MICRORESPIROMETER							
0-5	2	1	0.768-1.081	0.925 ± .105	0-5	(0.925)	100.0
0-10	5	1	1.371-1.678	1.522 ± .074	5-10	0.597	64.5
0-15	7	1	1.567-2.351	1.977 ± .162	10-15	0.455	49.2

over 30 per cent. greater than the adjoining 5-mm. length and 40 per cent. greater than the segment 10–15 mm. above the tip (column right). The values thus obtained seem more reliable than actual measurements on root segments with two cut surfaces. It is reasonable to assume that the shortest segments of roots cut 5 mm. above the tip suffered proportionally greater stimulation and protoplasmic injury than those cut 10 to 15 mm. above the tip. If the increase in respiration from stimulation exceeds the decrease due to injury to the cells, the observed differences in respiratory rate of the polar segments would slightly exceed the true values. There is, however, nothing in these data that indicates the nature of the error introduced by cutting.

Results obtained with the Scholander microrespirometer are given under the same headings in Table IB. In order to test the significance of the difference between means shown in column seven, the "t" test was employed (7). This test is particularly useful for small samples and measures the probability level at which the difference in means might occur by chance. If the probability is 5 per cent., the difference is statistically significant and 1 per cent. is highly significant statistically. Both values found here gave a probability of less than 1 per cent. Oxygen consumption of individual roots was 80–86 per cent. of that obtained by the Warburg technique with roots grown on the same days; however, it is clear that the same type of polar distribution of oxygen consumption is obtained. The percentage values listed in the last column agree within 9 per cent. with those derived from Warburg measurements.

It was originally hoped that the use of a large number of segments in each manometer flask would cancel the variations in the respiratory rate of individual roots. Lack of reproducibility was observed, however, and certain environmental factors which might influence these variations were controlled. The aquarium in which the roots were grown was kept at constant temperature and the roots were permitted to develop unrestricted by tubes in an aerated aquarium in order to maintain a reasonably uniform oxygen tension. Some roots were grown in test tubes but results from these were also variable. With 10 per cent. Knop's solution substituted for tap water in the aquarium, the reproducibility of respiratory rates was not improved. It is significant, however, that in experiments set up in duplicate or triplicate on a given day with the same number of roots from a given bulb in each flask, the respiratory rates fell within the approximate limits of error of the method, less than 5 per cent., in 16 out of 20 cases. Of the four exceptions, all of which fell within 10 per cent., two were found in five tests with 5-mm. lengths of root; one in nine tests with 10-mm. lengths and one in six tests with 15-mm. lengths. These results would suggest, therefore, that the sampling for a given determination was adequate to balance out the variations in respiration found in roots of the same bulb and in roots of different bulbs subjected to approximately identical growth conditions. There are no data on the range that might be found in the oxygen consumption of roots from the same bulb but preliminary measurements show that roots reaching a

length of 30 mm. after two days' growth had 35-45 per cent. greater oxygen consumption than roots permitted to grow for four days. Since all roots at least 30 mm. long were used in each experiment and since some of these roots may have been younger than others, it may be that a comparable range of variation might be expected. ROSENE (12) has found that the velocity of water absorption measured as cubic millimeters of water per square millimeter of root surface per hour may show considerable difference in roots from the same bulb.

Growth conditions not controlled from day to day were room temperature and illumination. The temperature varied from approximately 14° to 21° C. and even though the bulbs were immediately above a thermostatically controlled water bath, it may be that they were influenced to some degree by this range in temperature. The roots, on the other hand, were always subject to some change in temperature during the period required to prepare them for study. There is not sufficient data available, however, to permit an accurate analysis of this effect. Since WENTRAUB (16) has recently shown that light of low intensities inhibits the growth of roots, there is reason to believe that constant illumination of the growth aquarium might lead to more uniform respiration.

EFFECT OF POTASSIUM CYANIDE ON RESPIRATION

After a period of normal respiration potassium cyanide, 1/1000 M final concentration, was added to manometer flasks. All of these measurements were made in Warburg manometers, using 40 or more roots in each determination. Results are summarized in table II, where columns three and four give, respectively, the range and the mean with probable error of cubic mm. of oxygen consumed per root per hour. The average oxygen consumption of the same three 5-mm. segments was calculated by difference of the means with the results shown in column six. When the differences of the means were subjected to the "t" test they were found to be highly significant with a probability of less than 1 in 100 that the differences shown would occur by chance. When these rates are compared with the normal rate of oxygen consumption for the corresponding segment, it is seen that the first 5 mm.

TABLE II

RESPIRATION OF CYANIDE POISONED ONION ROOT TIPS

LENGTH OF ROOT TIP	NUMBER OF EXPS.	MM. ³ O ₂ /ROOT/HR. (RANGE)	MM. ³ O ₂ /ROOT/HR. (MEAN)	SEGMENT OF ROOT TIP	MM. ³ O ₂ /ROOT/HR. (BY DIFFERENCE)	% OF NORMAL*
I	II	III	IV	V	VI	VII
<i>mm.</i>				<i>mm.</i>		%
0-5	4	0.341-0.492	0.445 ± .042	0-5	(0.445)	44.6
0-10	4	0.724-0.934	0.819 ± .051	5-10	0.374	55.0
0-15	3	1.234-1.416	1.318 ± .051	10-15	0.499	86.5

* This figure is the quotient of column VI with the corresponding value in Table IA column VII.

of the root tip has only about 45 per cent. of normal respiration in cyanide, whereas, the 5- to 10-mm. segment and the 10- to 15-mm. segment have 55 per cent. and 86 per cent., respectively, of normal respiration in cyanide. The metabolic activity of the cells in a polar tissue like the root seems, therefore, differentially inhibited by the action of cyanide. These results may be compared to those reported by ROSENE (13) in which Webber had found 41.55 per cent. inhibition of oxygen consumption in onion roots with 0.0025 per cent. KCN. These measurements were made with the Winkler technique over a 48-hour period at 24° C. The length of root tips employed was not specified but the value corresponds closely with our 10-mm. lengths. Webber also obtained reversible inhibition (amount not given) with 0.005 M KCN in both apical and basal segments, using the Warburg method. As COMMONER (4) has emphasized, however, the average percentage inhibition by KCN does not necessarily indicate the relative activity of cyanide-sensitive and cyanide-stable respiration. It is necessary to utilize a range of cyanide concentrations in order to find the maximum inhibition. Under these conditions, comparisons of greater validity can be made.

RESPIRATION IN CYANIDE AND METHYLENE BLUE

Since the greatest effect of cyanide was produced on the first 5-mm. segment and the minimum effect on the last 5-mm. segment, it was decided to test the combined action of cyanide and methylene blue on 5-mm. and 15-mm. lengths of root tip. The mean results, with probable errors, from seven experiments in which various sequences were followed are shown in table III.

TABLE III

ONION ROOT TIP RESPIRATION IN CYANIDE AND METHYLENE BLUE

LENGTH OF ROOT TIP	NUMBER OF EXPS.	RESPIRATION IN		RESPIRATION IN		RESPIRATION IN	
		CN	MB	CN	MB	CN	MB
I	II	III		IV		V	
mm.		%		%		%	
0-5	4	125.6 ± 1.68		75.5 ± 5.00		66.7 ± 1.00	
0-15	3	114.7 ± 3.48		77.4 ± 4.80		73.4 ± 2.60	
						RESPIRATION IN MB NORMAL	
						VI	
						%	
						109.7 ± 6.01	
						102.1 ± 5.24	

In some cases the cyanide was added previous to the addition of 0.002 per cent. methylene blue or the methylene blue was added before the cyanide. In this way it was possible to show that methylene blue increased the oxygen consumption of the first five mm. of the root tip poisoned by cyanide more than it did in basal segments as shown in column three of table III. The presence of methylene blue in cyanide-poisoned roots, for example, produced an increase of approximately 30 per cent. in respiration of the apical 5 mm., as shown in column four (when compared to column seven of table II). The less sensitive basal region showed essentially no response to the methylene blue since most of the changes seen in the 0-15-mm. segments were probably due to the apical cells. Therefore, under the conditions of these experiments

the cyanide-sensitive respiration of the apical region was approximately 50 per cent. restored to normal by the addition of the methylene blue (from 45 per cent. to 75 per cent.). Methylene blue alone produced a slight elevation above normal respiration, but this increase approaches the limit of accuracy of the Warburg technique. This and the small number of experiments render the increase of doubtful significance.

Discussion

The oxygen consumption of individual roots might be expected to show considerable variation, not only because of inherent differences, but also because of inconstant growth conditions. The fact that measurements with samples of 40 or more roots failed to compensate for these variations from day to day but were effective on a given day emphasizes the importance of the environmental factors. Since light intensity seems to be at least one factor not rigorously controlled in these experiments, it is interesting to note the rather inconsistent effects of light on respiration of colorless plant tissues reported in the literature. CANNON (3) compared the volume of oxygen removed from water by roots when the shoots were in direct sunlight and in dense shade. Using *Salix* and *Helianthus*, he found that in 69 per cent. of the cases less oxygen was absorbed in light. FÖCKLER (6), on the other hand, found that light accelerates respiration in a variety of colorless plant tissues, especially at high intensities. In no case was a retarding effect observed. The oxygen consumption of *Pistia* roots was reported by RANJAN (10) to be unaffected by light. It is thus apparent that additional work is required before any valid conclusion can be drawn regarding the rôle of light in root respiration in general and especially as it may apply to onion roots.

The effect of temperature on oxidative metabolism is well known. The roots were grown at the same temperature that was used for measuring oxygen consumption but, since the bulbs may have been subject to some cooling and since the preparation of roots for experimentation necessitated a brief exposure to room temperature, an alteration in the mean level of respiration may have occurred from day to day. The data, however, do not permit a valid conclusion on this point. The lack of uniformity encountered in these experiments is not unique since other workers using adequate samples have also observed a wide range of respiratory values. ROSS (14), for example, reported a range of 20 to 30 for Q_{O_2} 's of *Nitella*, based on one gram wet weight (corresponding to 25 mg. dry weight). This is comparable to the results observed with the onion root.

The differences in oxygen consumption which were calculated for the three 5-mm. segments of root tip and were found statistically to be highly significant might be expected from cytological evidence alone. The apical segment contains the active mitotic cells with few vacuoles and a maximum content of protoplasm while the two more basal segments have the large sap vacuoles, very little meristematic tissue and highly differentiated cells. The

mass of protoplasm becomes progressively less in going from the tip to more basal segments. Thus a greater oxygen consumption does not necessarily reveal a greater intensity of oxidative metabolism in apical cells but rather a greater quantity of metabolizing material. The differential inhibition of oxygen consumption by cyanide suggests, however, that there is a polar difference in the oxidative metabolism of the onion root. This is further borne out by the greater increase in oxygen consumption of the cyanide poisoned root when methylene blue is added to apical segments as compared to longer lengths of root. There is, therefore, direct experimental evidence for the polar difference in oxidative metabolism which has been offered as explanation for the electrical behavior of onion roots under various environmental conditions (1, 2, 8, 9, 11). The ultimate proof of such a polar difference must await, however, more extensive and carefully controlled investigations.

Summary

1. The average oxygen consumption in cubic millimeters per root per hour, as measured with 34-61 roots by the Warburg manometric method, is 0.997 for 5-mm., 1.678 for 10-mm., and 2.255 for 15-mm. lengths of onion root. Similar results were obtained on individual lengths of root by the Scholander microrespirometer but the average values were 80 to 85 per cent. of the above.

2. There was as much as 50 per cent. variation in day to day values in all lengths of roots studied, but the results agreed within 10 per cent. in experiments set up in duplicate or triplicate on a given day. The maintenance of certain constant growth conditions failed to eliminate the variability but room temperature and illumination were not controlled.

3. A polar difference in cyanide inhibition and in the antagonistic effect of methylene blue for cyanide poisoning was found. This was interpreted as suggesting a fundamental difference in the oxidative metabolism of the different segments of the root.

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