STUDIES OF ONION ROOT RESPIRATION

I. VELOCITY OF OXYGEN CONSUMPTION IN DIFFERENT SEGMENTS OF ROOT AT DIFFERENT TEMPERATURES AS A FUNCTION OF PARTIAL PRESSURE OF OXYGEN*

by

L. JOE BERRY AND W. E. NORRIS JR

Biological Laboratories, Bryn Mawr College, Bryn Mawr, Pa (U.S.A.)

The relationship between oxygen tension and respiratory rate has been studied for a number of animal and plant species. TANG[1] reviewed the literature up to 1933 and found that hyperbolic curves were usually obtained when rate of oxygen consumption was plotted against pressure of oxygen. Beyond a certain critical pressure respiration was constant. There are three possible explanations for these results: (1) there is a direct mass action dependency on oxygen concentration (pressure) in the kinetics of respiration (KEMPNER[2]); (2) diffusion alone is limiting and the diminished rate of respiration at progressively lower oxygen pressures is due to an increasing core of anaerobiosis in the system (WARBURG[3], GERARD[4]); and (3) to a combination of (1) and (2). Because diffusion can act as a limiting factor in respiratory rate, it is now standard procedure to cut tissue slices within a maximum thickness so as to eliminate diffusion as a complication in studies on oxidative metabolism. In contrast to this *in vitro* problem, KROGH[5] has calculated that capillary supply in vertebrate organisms is such that diffusion in all probability adequately supplies the body cells with oxygen.

The relationship between oxygen pressure and rate of oxygen consumption in higher plants has been less thoroughly investigated. Many publications are available in which the rate of carbon dioxide evolution has been measured at different oxygen pressures. MACK[6] gives an extensive review of the earlier work of this type and more recently CHOUDHURY[7], PLATENIUS[8], and BIALE AND YOUNG[9] have made important contributions. These studies are valuable in arriving at optimum conditions for storage, etc., but offer little evidence of the dynamics of gaseous exchange. The change in rate of production of carbon dioxide at pressures of oxygen lower than air is dependent primarily upon the anaerobic rate compared with the aerobic rate. There seems to be considerable variation in this from one species of plant to another (CHOUDHURY[7]). Thus if the carbon dioxide output were the same both aerobically and anaerobically, respiration, evaluated in this way, would appear to be independent of oxygen pressure. VLAMIS AND DAVIS[10] measured both oxygen consumed and carbon dioxide produced by rice and barley seedlings. Oxygen uptake *vs* oxygen pressure curves were typically hyperbolic

---

* Supported by a Grant-in-Aid from the Sigma Xi Research Fund and by the Madge Miller Research Fund of Bryn Mawr College.

but at the lowest oxygen pressure investigated, 0.2%, oxygen consumption was only 15% of that in air, while carbon dioxide output was about 50%. In similar studies, TAYLOR[11] found the oxygen consumption of very young wheat and rice seedlings to decrease rapidly with lowered oxygen pressure but carbon dioxide evolution declined under the same conditions for wheat and increased for rice. This would account for the wide range of oxygen pressures at which the minimum rate of respiration, evaluated by carbon dioxide production, occurs in different tissues.

Measurements of the velocity of oxygen consumption in young roots as a function of oxygen pressure have been reported by WANNER[12] AND BERRY[13]. WANNER, using a polarographic technique, concluded that the rate of diffusion of oxygen was the limiting factor in the respiration of roots and that this was dependent upon temperature. BERRY employed the WARBURG manometric procedure and found at 25° C that the limiting pressure of oxygen depended upon the segment of root under investigation. It was further shown that the anaerobic production of carbon dioxide was less than it was aerobically in each of the three segments investigated. A differential rate of respiration for various polar zones of roots had previously been shown to exist for roots of wheat and onion by WANNER[14], for barley by MACHLIS[15], for onion by BERRY AND BROCK[16], and for barley, rape, corn, onion, and bean by PREVOT[17].

The present investigation was undertaken in order to analyse more completely the relationships between diffusion and temperature in the oxygen consumption of different zones along the axis of onion roots. These data strongly suggest that diffusion becomes limiting at progressively higher oxygen tensions as temperature increases and that the temperature characteristic of respiration assumes a value typical of diffusion when diffusion is predominantly limiting.

METHOD

Roots were grown and prepared for respiratory measurements according to the methods described in detail by BERRY[13]. All experiments were carried out in an airconditioned dark room with neon lights made of ruby glass tubing as the only illumination. A small flashlight was used for reading the manometers. The temperature in the room varied between 20–25° C. This was less constant than previously reported[13] because of poorly controlled heating during the winter months. For respiratory measurements below room temperature, the water bath in which the reaction vessels were immersed was cooled to 20° C by circulating tap water through coils of copper tubing and to reach 15° C ice water was circulated by means of a centrifugal pump.

Two-day old roots were used exclusively in these experiments and the number of segments added to each manometer flask varied with the temperature at which gaseous exchange was being measured. As many as 130 roots were needed at 15° C to give pressure changes comparable to 30 or 40 roots at 30° or 35° C. Thus by having essentially the same manometer readings at the different temperatures, a possible source of variable error is eliminated. The duration of each run was one or two hours.

Different partial pressures of oxygen were obtained by the evacuation method described by UMBREIT, BURRIS, AND STAUFFER[18], using commercial tank oxygen and nitrogen without further purification. Calculations show that this yields final values accurate to approximately 0.1 %.

Rates of respiration are given as cubic millimeters of oxygen consumed per root segment per hour. These values may be converted to Q_{O_2} per gram wet weight or per gram dry weight by multiplication with the conversion factors shown in Table I. The average weights were determined by a series of weighings on a Kuhlmann microbalance.

All experiments were carried out with root segments cut 5, 10, or 15 mm above the tip. The respiratory rate for each of the two zones, 5–10 mm, and 10–15 mm above the tip could then be calculated by difference. The apical 5 mm is essentially a zone of mitotic activity, the next zone is one of elongation and differentiation and the third region, 10–15 mm above the tip, consists predominantly of mature cells. In this procedure only one cut is required in arriving at the velocity of oxygen uptake for each of the three zones of root tip. BERRY[13] has shown that the values obtained in this way differ from those found when each segment is employed directly by amounts that fall within the limits of accuracy of the manometric technique.

TABLE I

Root segment	Conversion factor to change µl O_2/root/h to Q_{O_2} per g	
	wet weight	dry weight
0– 5	501	8520
5–10	548	13940
10–15	591	18640

RESULTS

1. *Respiratory rate as a function of oxygen tension at 15° C*

Fig. 1 shows the respiratory rate in µl of oxygen per root per hour plotted against percent of oxygen in the manometer vessel for each of the three zones. Every point is the average of from three to six separate determinations. The maximum probable error occurred with the point at 5% oxygen for the 0–5 mm zone and amounted to slightly less than 8% of the value plotted. Most probable errors fell within the 3–5% range. The curves show that a rapid decrease in the rate of oxygen consumption occurs in each segment when a certain critical oxygen pressure is reached. At pressures higher than this, the respiratory rate is constant regardless of the oxygen tension. For this temperature, breaks in the curves fall at 10% oxygen for both the 5–10 mm and 10–15 mm regions above the tip and at 15% oxygen for the apical 5 mm. Since a low temperature is generally believed to depress metabolism more than it does a physical process such as diffusion (see Moelwyn-Hughes[19]), one might expect to reduce the importance of diffusion to a minimum when the temperature is low.

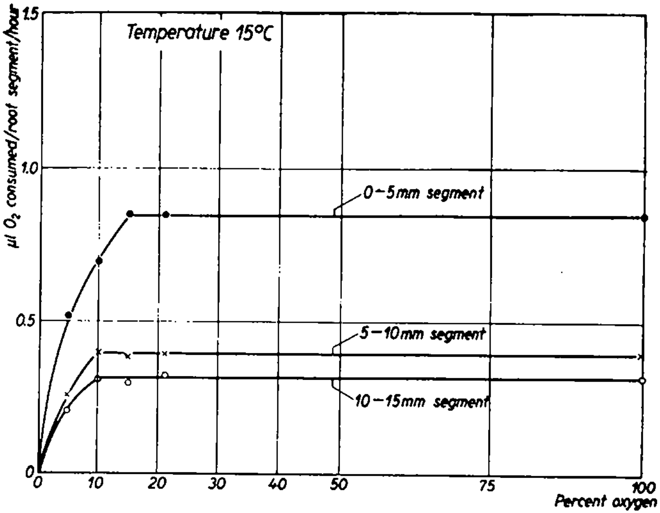


Fig. 1. Oxygen consumed in different root zones as a function of oxygen pressure at 15° C

Table II (columns 2 and 3) shows the values of carbon dioxide production for the 0–5 mm zone under the same conditions. Both volume of carbon dioxide in µl evolved per root per hour as well as the values for respiratory quotient are given. It can be seen that the total output is reduced at pressures of oxygen below the critical pressure and that fermentation is clearly evident at 5% oxygen. R.Q. values so near unity suggest that essentially pure carbohydrate serves as substrate. Even though there is a reduction in carbon dioxide evolution below the critical pressure (15% oxygen) for this zone of the root, the relative magnitude of reduction is less than that observed for oxygen. Respiratory rate measured by carbon dioxide production would show, however, at this temperature, the same dependency on oxygen pressure as that shown in Fig. 1. Values for carbon dioxide in the 5–10 mm and 10–15 mm segments were found to be quite variable, less so at 15° C than at the higher temperatures. Because of this difficulty, it is believed that little significance could be attached to these results at this time. They clearly indicate at this temperature, however, the same trend as that shown for the most apical 5 mm. The R.Q.'s are about 1.2 at 5% oxygen.

TABLE II

CO_2 OUTPUT AND RESPIRATORY QUOTIENTS FOR THE 0–5 mm SEGMENT OF ROOT AT DIFFERENT TEMPERATURES AND OXYGEN PRESSURES

(1) Pressure of oxygen	15° C		20° C		30° C		35° C	
	μl/root /hour	(3) R. Q.	μl/root /hour	(5) R. Q.	μl/root /hour	(7) R. Q.	μl/root /hour	(9) R. Q.
5%	0.60	1.15	0.84	1.38	1.59	2.12	1.90	2.83
10%	0.69	0.98	1.02	1.13	1.69	1.26	2.51	2.24
15%	0.86	1.00	1.15	0.99	1.89	1.16	2.51	1.55
21% (air)	0.86	1.01	1.28	1.04	2.18	1.11	2.86	1.43
40%							3.40	1.35
45%					2.85	1.03		
50%					2.90	1.04	3.27	1.23
100%	0.88	1.04	1.22	0.97	2.89	1.05	3.18	1.19

2. *Respiratory rate as a function of oxygen tension at 20° C*

Fig. 2 summarizes the data on oxygen consumption for each root zone at 20° C. Each point is the mean of from 4–16 separate determinations and the probable error in all cases is less than 3% of the average value plotted. The critical pressures occur in air for the 0–5 mm segment, at 15% oxygen for the 5–10 mm segment and at 10% oxygen for the 10–15 mm zone. This is an increase for the two more apical segments over the values found at 15° C. Attention is also called to the slight depression in the 10% oxygen value for the 10–15 mm segment. This point is just enough lower to be within the approximate limit of accuracy of the method. The increase in rate of oxygen consumption with temperature can be seen by comparing the constant levels in Figs 1 and 2. Each zone shows approximately the same relative increment. The values for carbon dioxide at 20° C are given in Table II, columns 4 and 5 for the 0–5 mm zone. The R.Q.'s are again approximately 1.00 except at the two lowest oxygen pressures where fermentation becomes significant. The decrease in volume of carbon dioxide evolved per root per hour as a function of oxygen tension again coincides with that for oxygen consumption but the percentage decrease in respiration based on carbon dioxide values is less than that for oxygen.

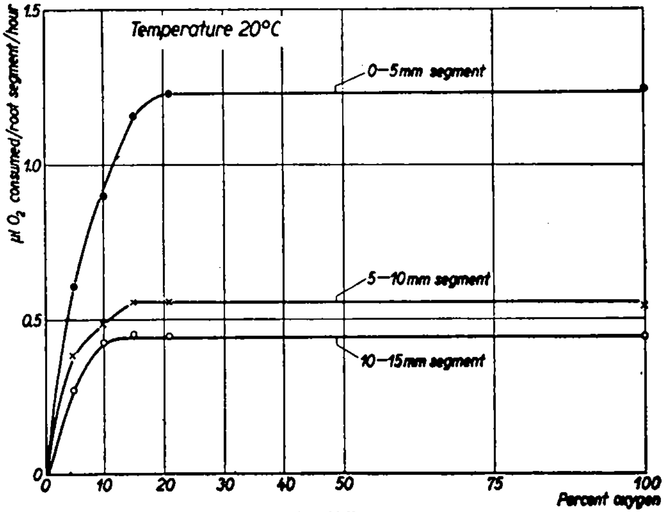


Fig. 2. Oxygen consumed in different root zones as a function of oxygen pressure at 20° C

3. *Respiratory rate as a function of oxygen tension at 30° C*

(The values for 25° C have been reported by BERRY[13]).

The curves in Fig. 3 summarize the results at 30° C. Each point is the average of from three to eight separate determinations and the probable errors range from 5% of the value at 45% oxygen in the 0–5 mm zone to 0.1% for the same zone at 100% oxygen. Most fall between 3% and 4%. The critical pressures are 45% oxygen for the 0–5 mm segment, 21% (air) for the 5–10 mm segment and 10% oxygen for the 10–15 mm segment. Large increases in the maximum rate of oxygen consumption are easily seen for each segment as compared to the rates at 15° C and 20° C.

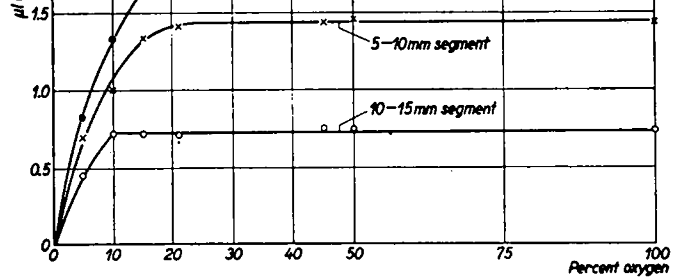


Fig. 3. Oxygen consumed in different root zones as a function of oxygen pressure at 30° C

The carbon dioxide values and R.Q.'s are listed in Table II, columns 6 and 7. The parallelism between these results and those for oxygen are again apparent. The relative decrease in carbon dioxide evolved at 5% oxygen compared with that at 100% oxygen is much less than the decrease in oxygen consumption at the same partial pressures. The R.Q.'s show the progressive increase in anaerobiosis and fermentation that accompanies the diminishing percentage of oxygen.

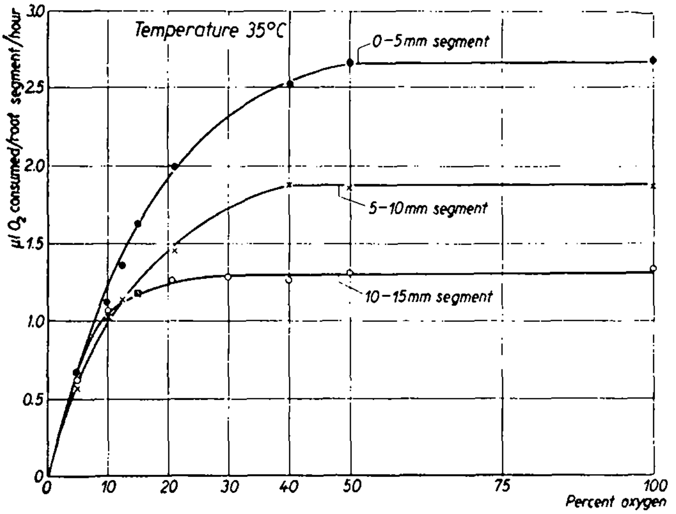


Fig. 4. Oxygen consumed in different root zones as a function of oxygen pressure at 35° C

4. *Respiratory rate as a function of oxygen tension at 35° C*

The results at this temperature are given in Fig. 4. From 3–9 separate determinations were averaged for each point and the largest probable error (6%) occurred with 9 determinations in air for the 5–10 mm segment. The critical pressures are 50% oxygen for the 0–5 mm region of root, 40% oxygen for the 5–10 mm segment and air for the 10–15 mm zone. It should be noted that the higher critical pressure at this temperature for the most apical 5 mm of root is not accompanied by as high a mean rate of oxygen consumption, calculated on an hourly basis, as that found for 30° C (2.66 µl/root/hour versus 2.76 µl/root/hour). However, as Fig. 5 shows, there is a progressive decline in the rate of respiration with time at 35° C in an atmosphere of pure oxygen. These curves were drawn with each point representing the average value of six separate experiments. A decrease in rate with time was observed only at this temperature for the periods of measurement used in these experiments. When the oxygen consumed during the first quarter hour is used to calculate the hourly rate of respiration for this segment, an uptake of 3.06 µl/root/hour is found. This is higher than the value at 30° C even though at 35° C an inactivation of enzymes seems to occur. A smaller decline in rate is also to be found in the 5–10 mm segment, but it becomes apparent only after a longer period than that required with the apical 5 mm. The basal segment has a constant rate of oxygen consumption during this period. It has also been found that carbon dioxide evolution

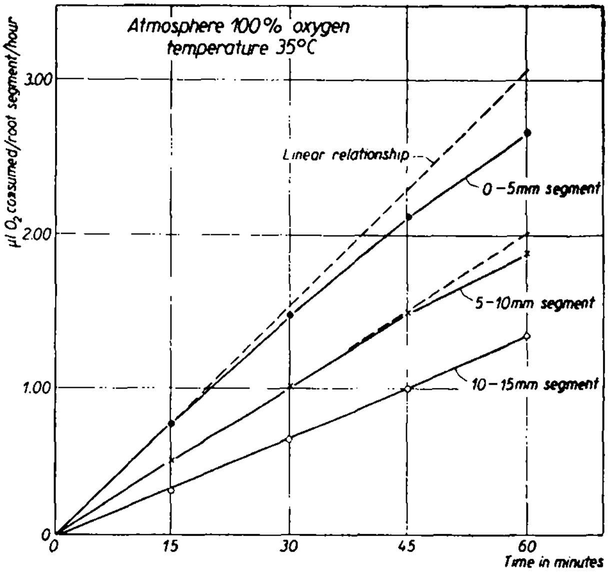


Fig. 5. Oxygen consumed in different root zones as a function of time at 35° C, and in an atmosphere of pure oxygen

References p. 605/606.

diminishes with time in the first segment but it is less than that for oxygen consumption and is virtually absent in the two more basal segments. The constant rates of respiration for these segments show considerable increase over the rates at 30° C (compare Figs 4 and 3) so that they are not strictly comparable with the apex.

Reference to Table II, columns 8 and 9, further brings out the abnormal behaviour of the apex at 35° C. The R.Q. is 1.2 even at oxygen pressures above the critical level and rises steadily to 2.8 at 5% as the percentage of oxygen declines. A greater sensitivity to heat inactivation of those enzymes reponsible for the utilization of molecular oxygen rather than the enzymes involved in fermentation might account for the high R.Q. at this temperature in the presence of pure oxygen. This was not at all true for the 10–15 mm segment which had normal R.Q. values down to 15% oxygen. Carbon dioxide production in the apex dropped only at pressures lower than 40% (50% critical pressure) and, as was true for all other temperatures, there was a smaller decrease in rate of output of carbon dioxide at 5% oxygen than was seen for oxygen consumption.

When the rate of oxygen consumption by the different zones of the root at 35° C is converted to Q_{O_2} by the conversion factors in Table I, very high values are obtained. A comparison with those listed by GODDARD[20] for a variety of animal and plant tissues shows that the meristematic region of the onion root is among the highest. This fact emphasizes the intense energy production that occurs in these cells as well as in those of animal origin.

The similarity of the respiratory rates at which certain pressures of oxygen become limiting is of interest. At three different temperatures air is the critical pressure. This was found for the 0–5 mm zone at 20° C, for the 5–10 mm zone at 30° C, and for the 10–15 mm zone at 35° C. The hourly rate was 1.23, 1.45, and 1.28 μl of oxygen per root respectively. It thus appears that for a given pressure of oxygen, only a certain velocity of respiration can be supported, independent of the root zone involved. This is again indicated by the 1.87 μl/root/hour rate shown by the 5–10 mm segment at 35° C, where the critical pressure is 40% oxygen and the 1.73 μl/root/hour for the 0.5 mm segment at 25° C where calculations show the critical pressure to be about 30% or more oxygen (see BERRY[13]). It also shows the difficulty that might be expected in accurately determining the limiting pressure for those segments with low oxygen uptake. The 10–15 mm segment had the same critical pressure (10%) at 15° C, 20° C, and 30° C, even though the respiratory rate more than doubled over this temperature interval. The accuracy of the 30° C value might be called into question but three separate determinations at 10% oxygen checked with a probable error much less than 1% of the average rate. This was also true for the other two segments under the same conditions. However, 15% oxygen was the critical pressure for the 0–5 mm segment at 15° C with a rate of 0.85 μl of oxygen per root per hour and also for the 5–10 mm segment at 20° C with a rate of 0.55 μl per root per hour. The rate for the 10–15 mm segment at the same oxygen pressure at 30° C was 0.73 μl per root per hour. For more precise determinations, oxygen pressure changes in steps less than 5% should be used with the lower respiratory rates but then one is confronted with the problem of precisely measuring a small but significant change in rate of oxygen consumption.

5. *Temperature characteristics as a function of oxygen pressure*

In spite of the criticisms[21, 22], aimed at the use of temperature characteristics in elucidating the underlying mechanisms of biological processes, it is believed that by calculating the μ value in the Arrhenius equation, the information obtained can be used in evaluating the results reported above. For this purpose the curves in Fig. 6 should first be examined since these represent the data for each root zone obtained in an atmosphere of 100% oxygen. The process of gaseous diffusion into the locus of change in the root should not be a limiting process in pure oxygen. The μ values were calculated from those points falling on the curves and the agreement in the numerical values for the slope of each curve is good for data of this type. The point shown at 35° C (1/T = 3.25·10^{-3}) for the 0–5 mm segment is the value derived when the maximum rate (3.06 μl of oxygen per root per hour) was calculated from the initial 15 minute uptake (see Fig. 5) and it falls only slightly below the expected value. All curves were drawn by inspection to give the best fit for the points. No suggestion of a break in any curve can be seen except between 30° C and 35° C in the one for the 0–5 mm segment. If any significance can be attached to these results, it would be to suggest that comparable reactions are proceeding in each of the root zones.

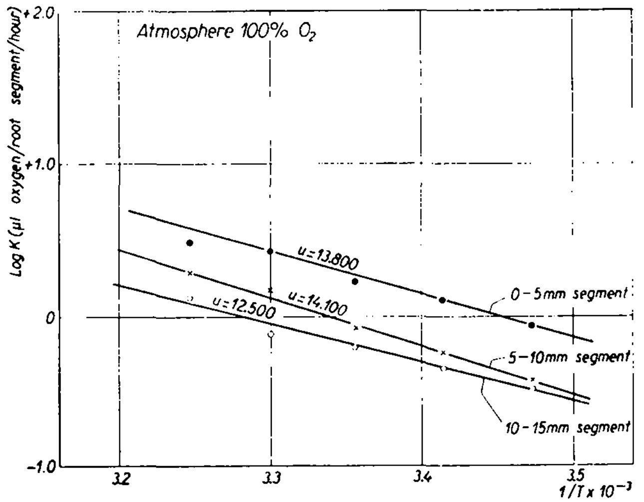


Fig. 6. Log K (μl of oxygen consumed /root segment/ h) plotted against the reciprocal of the absolute temperature, for different root zones in an atmosphere of pure oxygen

If reference is now made to the curves in Fig. 7, drawn for the rate of oxygen consumption in each root segment at 5% oxygen, a pronounced difference is seen between the one for the 0–5 mm segment and those for the more basal segments of root. The slope (μ value) for the apex is now equivalent to 4300 calories per mole, and for the other two curves it is 11 300. SIZER[23] gives the temperature characteristic of diffusion as approximately 3000 calories; GLASSTONE, LAIDLER, AND EYRING[24] report values lying between 6600 calories and 3800 calories, depending upon the temperature at which the measurement is made as well as upon the actual substances involved; and MOELWYN-HUGHES[19] states that under conditions where the rate of a heterogeneous reaction is determined by a diffusion

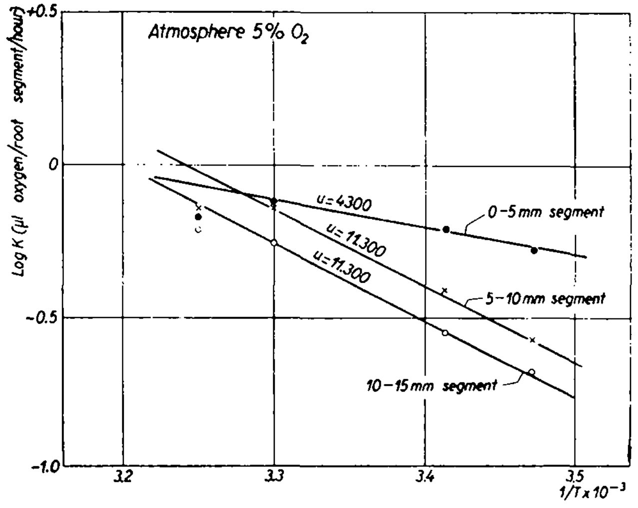
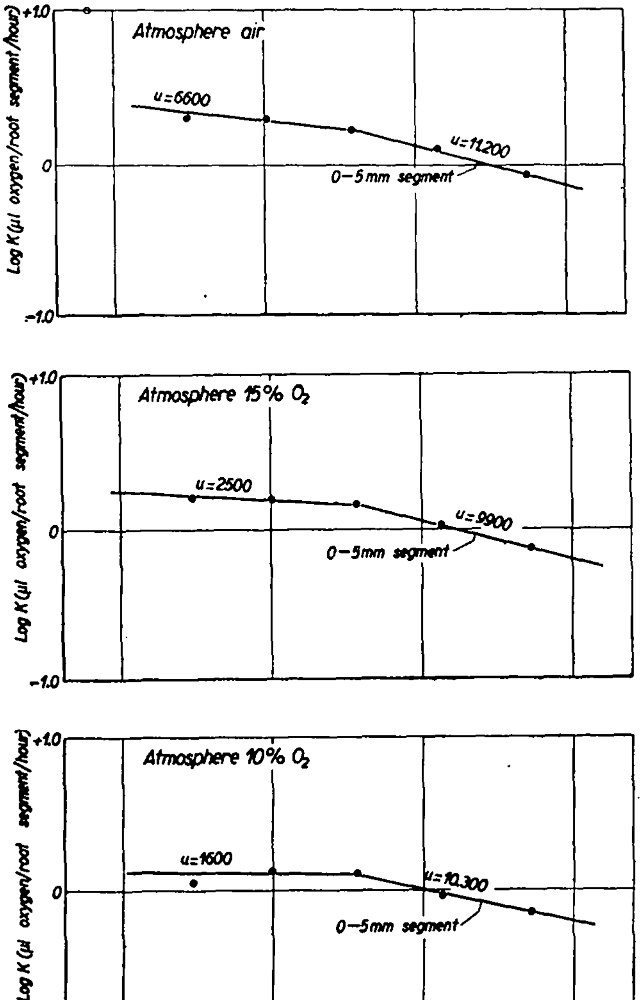


Fig. 7. Log K (μl of oxygen consumed /root segment/ h) plotted against the reciprocal of the absolute temperature for different root zones in an atmosphere of 5% oxygen

process, the critical increment should be about 4500 calories at 25° C. 4300 calories is in particularly close agreement with the value given by Moelwyn-Hughes. The question naturally arises as to the basis for the large slopes seen in the other two curves. In the first place, the points at 35° C are completely off the curves and might have been included as breaks in the curves with different slopes. By inspection, it is seen that a line joining the points at 30° C and 35° C for the 10–15 mm segment of root would be parallel with the curve for the 0–5 mm segment. The points at each of these temperatures for the 5–10 mm segment fall almost on a horizontal line (slope = 0) and may be due to the inactivation of the respiratory mechanism at the higher temperature. At temperatures below 30° C the fraction of the total respiring volume of root that might be dependent upon diffusion is probably small compared to that for the apical 5 mm. It has been calculated (see Berry and Norris[25]) that in 5% oxygen at 30° C more than one-half of the 5–10% mm segment is aerobic. Even though the pressure of oxygen at 5% is a limiting factor in the metabolic rate, it is limiting only a small part of the whole and its contribution might be masked by the small decrease in slope that can be seen for the two basal curves at this oxygen pressure compared with those at 100% oxygen. This suggestion is in line with the criterion set up by Burton[26] for the relative velocity of the "master" reaction. It must be only 1/10 as fast as the next slowest link in a catenary system or else the observed velocity may be an average of several reactions proceeding in sequence.



Figs 8, 9, and 10. Log K (μl of oxygen consumed /root segment/ h) plotted against the reciprocal of the absolute temperature for the 0–5 mm zone of the root in an atmosphere of 10% oxygen, 15% oxygen, and 21% oxygen (air)

Since only the 0–5 mm segment was found to undergo a major change in temperature characteristic at 5% oxygen, the results at successively higher oxygen pressures are shown only for this segment of the root. At 10% oxygen the points fall on straight lines, as shown in Fig. 8, with a break occurring at 25° C. Similar curves are shown in Figs 9 and 10 for 15% and 21% (air) oxygen, respectively. In all three figures a break is seen at 25° C and the slope of the left hand portion of the curves progressively increases from 1600 calories at 10% oxygen to 2500 calories at 15% and 6600 calories in air. The right hand curves increase from about 10000 calories to 11200 calories. The last value agrees within extremely narrow limits with those found for the two more basal regions of root at 5% oxygen (Fig. 7).

DISCUSSION

The hyperbolic curves relating pressure of oxygen to rate of respiration for each segment of root at different temperatures are consistent with most of the experimental data found in the literature. The pressure of oxygen, below which oxidative metabolism becomes diminished, is generally dependent upon three factors: (1) the size of the material under investigation; (2) the velocity of oxygen consumption per unit of tissue; and (3) the permeability of the tissue to oxygen. Both (1) and (2) are in direct proportion to the critical pressure while (3) is inverse. In the present experiments the size remains constant under all conditions and (2) varies with temperature. Figs 1, 2, 3, and 4 show, by comparison, an increasing critical pressure as the respiratory rate is elevated. The importance of permeability is considered in some detail by BERRY AND NORRIS[25], but there is evidence to support the assertion that considerable differences may be expected between cells and tissues of various origin.

It is particularly interesting to note that at temperatures of 25° C (see BERRY[13]) or above, the apical 5 mm of root are partially anaerobic in air and that this condition extends up into the 5–10 mm zone at temperatures above 30° C. From an ecological point of view, the question naturally arises as to the significance of this fact and its bearing on the economy of the plant. Do the optimum conditions for growth depend at least in part upon this relationship? It could possibly account for the long recognized dependency of root growth on oxygen tension, and the standard procedure of growing onion roots in an aerated aquarium. So far as the authors are aware no data are available for the relative growth rates of onion roots at different oxygen tensions, but there is the general impression (frequently of doubtful significance) that more rapid growth occurs when the medium is aerated. It was observed, moreover, throughout the duration of the present experiments that when the growth temperature of the onion roots fell below 25° C, thicker roots were obtained. Since the onion root has been used extensively in working out the relationship between inherent electrical potentials and cellular oxidation (for bibliography see ROSENE[28]), the most favourable temperatures may not always have been employed for this purpose. In fact, BERRY AND HOYT[29] found that the magnitude of externally applied current, which caused the apex of the root to become electronegative to a basal region of the root as measured in an external circuit (instead of its usual positivity), was reduced at lower temperatures and that at about 30° C no such effect could be produced. Whether this is dependent upon an anaerobic core, upon the velocity of oxidation, or some other factor(s) remains unanswered.

Two facts point to diffusion as the primary limiting factor in rate of oxygen consumption by the root segments at oxygen tensions below the critical pressure. First, the respiratory quotients consistently exceed unity under these conditions, indicating that anaerobic carbon dioxide is being produced. Second, the temperature characteristics of those curves in which the rate of respiration was most significantly lowered by a reduced oxygen pressure are in general agreement with values for diffusion[19, 23, 24]. It is obvious that we are dealing with more than a simple physical system in which diffusion alone is involved. An attempt to visualize the condition in the 0–5 mm segment at 15° C in 5% oxygen will illustrate the complexity. Oxygen diffuses into the cylinder of cells and is consumed as it passes toward the axis. Therefore an outer sleeve of aerobic cells extending into the root a distance X from the surface surrounds a cylindrical core of anaerobic cells in the center. The rate of respiration as measured manometrically depends upon the size of X (*i.e.*, the number of aerobic cells), the rate of diffusion (how fast molecular oxygen reaches the loci of combination), and, possibly, on the mass action relationship between concentration of oxygen and concentration of enzyme with which it combines. This last suggestion implies an interaction between molecular oxygen, as substrate, and an aerobic oxidase (such as cytochrome oxidase) comparable to that known to hold for the majority of enzymatic changes[30]. However, it is generally assumed (cf. 20) that the rate of oxidation of the enzymes directly linked with molecular oxygen is independent of the concentration of oxygen even though an experimental proof is lacking. In those enzyme reactions where mass action does apply, essentially the same type of hyperbolic curves as those seen in Figs 1, 2, 3, and 4 as well as in the curve for the 0–5 mm segment in Fig. 5 would result. In each case a Michaelis "constant"[30] could be calculated, but it would not be obtained under conditions that would permit such a calculation and would be without meaning.

When the temperature is raised around the apical segment in 5% oxygen, an increased respiratory rate is obtained but the proportionate increase is less than it is in 100% oxygen. If the enzyme concentration is constant, and if the rate of oxygen consumption is independent of oxygen concentration, the only explanation for the experimental observation is to assume that X is smaller. The limiting factor in determining the magnitude of change in velocity of respiration would seem to be, therefore, rate of diffusion. No proof can be offered for the assumptions made in reaching this conclusion. Nevertheless, a constant rate of oxygen consumption with time at temperatures below 35° C was found for all root zones when the oxygen tension exceeded the critical pressure. This suggests that no progressive change in enzyme concentration occurred. Since this was not true at 35° C, this temperature was not used in any calculations shown in the log k *versus* 1/T curves in Figs 6, 7, 8, 9, and 10.

The changes in the μ values seen in Figs 8, 9, and 10 require some comment. The lower values to the left of the break in the curves become progressively larger as the pressure of oxygen increases (1600, 2500, 6600 calories per mole which should be compared with 4300 calories per mole in Fig. 7). Similarly the values to the right of the break change from 10300 to 9900 to 11200 calories per mole and these should be compared with the 13800 calories per mole in Fig. 6. There are three ways to interpret these changes: (1) the reaction giving rise to the μ value is different at each partial pressure of oxygen and possesses a different temperature characteristic; (2) the range of the different μ values represents the error in calculation; and (3) the probability of activation becomes progressively larger as the pressure of oxygen increases due to increased randomness and hence the entropy term, which appears in the equation relating the velocity constant to both the change of heat content and the change in entropy of the activated complex, becomes larger, thereby accounting for the changing μ values. The second interpretation is certainly the most obvious and probably the most likely even though the third cannot be entirely dismissed without consideration.

A temperature characteristic around 12000 calories per mole for respiration of the onion root is smaller by about 4000 than that reported by other workers for respiration of various biological materials of animal origin (see CROZIER[31]). The significance of this difference is obscure and it is doubtful whether it is worthy of much discussion. The proposal of CROZIER that this should represent the mastery of a different reaction when μ values varying by this amount appear has been vigorously attacked by BOOIJ AND WOLVEKAMP[22]. Their principal objection may be given in their own words as follows: "The main conclusion must be that as far as steady state processes are concerned the conception for the master process in physical chemistry as well as in biology is only of a limited value and that master reactions in the strict sense of the word do not occur at all ... If the complexity of processes like that of metabolic processes is great a mathematical treatment of the whole is very nearly useless." However, they admit that a biochemical process may be limited in borderline cases by such things as oxygen tension in the habitat of the biological system. MORALES[27] has recently answered these objections by showing the conditions under which a steady state enzymatic system of consecutive reactions permits kinetic analysis with some accuracy. This is permissible when it is assumed that the velocity of the irreversible splitting of the enzyme-substrate complex is slow and is dependent on the product of the velocity constant and total enzyme concentration, *i.e.*:

$$V = k\,[E]$$

He believes that the JOHNSON-EYRING[32] scheme developed for the luciferin-luciferase reaction fulfils these requirements. It should be noticed that similar assumptions were made above in considering the validity of the μ value for diffusion. The ultimate significance of these calculations therefore depends upon a proof of the accuracy of the assumptions.

SUMMARY

The rate of oxygen consumption and carbon dioxide production in different partial pressures of oxygen at 15°, 20°, 30° and 35° C were measured manometrically in segments of onion root tip 5, 10 and 15 mm in length. The rates for the regions 5–10 mm and 10–15 mm above the tip were determined by difference. Curves for the three zones of root tip relating oxygen uptake to pressure of oxygen were hyperbolic at all temperatures but the pressure just supporting the maximum rate (critical pressure) increased with temperature. R.Q.'s were essentially unity at pressures equal to or greater than the critical pressure and exceeded unity when the pressures were less than the critical pressure. Rate of carbon dioxide production paralleled that of oxygen consumption but showed a smaller proportionate reduction at low oxygen tensions. Log k *vs* 1/T curves for each root segment at different pressures of oxygen permitted calculations of activation energy. For the two basal zones, μ values of approximately 12000 calories were obtained at all pressures. For the apical 5 mm, a μ value of 4300 calories was found at 5% oxygen and one of about 13000 calories at 100% oxygen. The curves at pressures of 10%, 15%, and 21% oxygen for the same segment had breaks with the slopes of the two straight-line portions approaching these extremes. From these results, it is concluded that diffusion is the limiting factor in rate of respiration at the lower pressures of oxygen.

RÉSUMÉ

Nous avons mesuré avec un manomètre, sous diverses pressions partielles d'oxygène à des températures de 15°, 20°, 30° et 35° C, le taux de la consommation de l'oxygène et celui de la production du CO_2 dans des segments de l'extrémité d'une racine d'oignon ayant 5, 10, et 15 mm de longueur. Les taux pour les zones situées entre 5 et 10 mm et entre 10 et 15 mm au-dessus de l'extrémité ont été déterminés par différence. Les courbes qui montrent les rapports entre la consommation et la pression de l'oxygène dans les trois zones de la racine étaient hyperboliques à toutes les températures; mais la pression qui maintenait tout juste le taux maximum (la pression critique) augmentait avec la température. Les quotients de respiration (R. Q.'s) étaient essentiellement l'unité à des pressions qui égalaient ou qui dépassaient la pression critique, et ils excédaient l'unité quand les pressions étaient plus basses que la pression critique. Le taux de la génération du CO_2 était parallèle à celui de l'oxygène, mais il montrait une moindre diminution proportionnelle à de basses tensions d'oxygène. Les courbes log k *vs* 1/T pour chaque segment de racine à des pressions différentes d'oxygène ont permis les calculs de l'énergie d'activation. Pour les deux zones de base, nous avons obtenu des valeurs μ d'à peu près 12000 calories à toutes les températures. Pour les 5 millimètres apicaux, nous avons trouvé une valeur μ de 4300 calories à 5% d'oxygène, et une valeur μ d'à peu près 13000 calories à 100% d'oxygène. Les courbes pour le même segment aux pressions de 10, 15 et 21% d'oxygène n'étaient pas continues; l'inclinaison des deux segments rectilignes s'approchait de ces extrêmes. De ces résultats nous avons conclu que la diffusion est le facteur limitatif du taux de respiration aux pressions inférieures de l'oxygène.

ZUSAMMENFASSUNG

Die Geschwindigkeit der Sauerstoffaufnahme und der Kohlendioxydabgabe bei verschiedenen Partialdrucken von Sauerstoff bei 15, 20, 30 und 35° C wurde manometrisch in Zwiebelwurzelsegmenten von 5, 10 und 15 mm Länge gemessen. Für die 5–10 mm und 10–15 mm oberhalb der Spitze der Wurzel gelegenen Zonen wurde die Geschwindigkeit durch Substraktion bestimmt. Die Kurven, welche die Sauerstoffaufnahme in Abhängigkeit vom Sauerstoffdruck bringen, verlaufen für die drei Zonen der Wurzelspitze bei allen Temperaturen hyperbolisch, aber der Druck welcher gerade die grösste Geschwindigkeit aufrecht erhielt (der kritische Druck), nahm mit der Temperatur zu.

Die Atmungskoeffizienten (R.Q.) waren gleich 1 bei Drucken die grösser oder gleich dem kritischen Drucke waren. Die Abgabe von Kohlendioxyd verlief parallel zur Sauerstoffaufnahme, aber die proportionale Abnahme bei niedrigen Sauerstoffdrucken war geringer. Auf Grund der Kurven welche für jedes Wurzelsegment bei verschiedenen Sauerstoffdrucken den log k in Abhängigkeit von 1/T bringen, konnte die Aktivierungsenergie berechnet werden. Für die zwei Grundzonen wurden für μ bei allen Drucken Werte von ungefähr 12000 Kalorien gefunden. Für die ersten 5 mm von der Spitze haben wir bei 5% Sauerstoff μ = 4300 Kalorien und für 100% Sauerstoff ungefähr μ = 13000 Kalorien gefunden. Die Kurven für Drucke von 10%, 15% und 21% Sauerstoff für ein und dasselbe Segment bestanden aus zwei geradlinigen Stücken deren Neigung sich diesen beiden Grenzwerten näherte. Aus diesen Ergebnissen folgern wir, dass die Diffusion der Faktor ist, der die Atmungsgeschwindigkeit bei niedrigen Drucken begrenzt.

REFERENCES

[1] P. TANG, *Quart. Rev. Biol.*, 8 (1933) 260.
[2] W. KEMPNER, *Cold Spring Harbor Symposia Quant. Biol.*, 7 (1939) 269.
[3] O. WARBURG, *Biochem. Z.*, 142 (1923) 317.
[4] R. W. GERARD, *Biol. Bull.*, 60 (1931) 245.
[5] A. KROGH, *J. Physiol.*, 52 (1919) 409.
[6] W. B. MACK, *Plant Physiol.*, 5 (1930) 1.
[7] J. K. CHOUDHURY, *Proc. Roy. Soc.*, 127B (1939) 238.
[8] H. PLATENIUS, *Plant Physiol.*, 18 (1943) 671.
[9] J. B. BIALE AND R. E. YOUNG, *Am. J. Botany*, 34 (1947) 301.
[10] J. VLAMIS AND A. R. DAVIS, *Plant Physiol.*, 18 (1943) 685.
[11] D. L. TAYLOR, *Science*, 92 (1942) 129.
[12] H. WANNER, *Vierteljahrssch. naturforsch. Ges., Zürich*, 90 (1945) 98.
[13] L. JOE BERRY, *J. Cellular Comp. Physiol.*, 33 (1949) 41.
[14] H. WANNER, *Arkiv. Botanik*, 31 (1944) No. 9.
[15] L. MACHLIS, *Am. J. Botany*, 31 (1944) 281.
[16] L. JOE BERRY AND MARY JANE BROCK, *Plant Physiol.*, 21 (1946) 542.
[17] P. C. PREVOT, *Lejeuna*, 4 (1940) 37; *Biol. Abstracts*, 21 (1947) 10, 158.
[18] W. W. UMBREIT, R. H. BURRIS, AND J. F. STAUFER, *Manometric Techniques*, Burgess, Minneapolis (1945).
[19] E. A. MOELWYN-HUGHES, *The kinetics of reactions in solutions*, 2nd edition, Oxford at the Clarendon Press (1947).
[20] D. R. GODDARD, *The respiration of cells and tissues*. In R. HÖBER, *Physical chemistry of cells and tissues*, The Blakiston Co., Philadelphia (1945) p. 379.

[21] L. V. HEILBRUNN, *Science*, 62 (1925) 268.
[22] H. L. BOOIJ AND H. P. WOLVEKAMP, *Bibliotheca Biotheoretica*, 1 (1944) 145.
[23] I. W. SIZER, *Advances in Enzymol.*, 3 (1943) 35.
[24] S. GLASSTONE, K. J. LAIDLER, AND H. EYRING, *The theory of rate processes*, McGraw-Hill Book Co., New York (1941).
[25] L. JOE BERRY AND W. E. NORRIS Jr., *Biochim. Biophys. Acta*, 3 (1949) 607.
[26] A. C. BURTON, *J. Cellular Comp. Physiol.*, 14 (1939) 327.
[27] M. F. MORALES, *J. Cellular Comp. Physiol.*, 30 (1947) 303.
[28] H. F. ROSENE, *Bioelectric fields and growth*, edited by E. J. Lund, U. of Texas Press, Austin (1947).
[29] L. JOE BERRY AND ROSALIE C. HOYT, *Plant Physiol.*, 18 (1943) 372.
[30] E. BALDWIN, *Dynamic aspects of biochemistry*, The University Press, Cambridge (1947).
[31] W. J. CROZIER, *J. Gen. Physiol.*, 7 (1924) 189.
[32] F. H. JOHNSON AND H. EYRING, *Ann. N. Y. Acad. Sci.*, 49 (1948) 376.

Received January 20th, 1949