# STUDIES OF ONION ROOT RESPIRATION

## II. THE EFFECT OF TEMPERATURE ON THE APPARENT DIFFUSION COEFFICIENT IN DIFFERENT SEGMENTS OF THE ROOT TIP*

by

L. JOE BERRY AND W. E. NORRIS JR

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

The exchange of many materials between cells or tissues and their immediate environment is dependent upon the process of diffusion. This fact has been recognized for years and numerous investigations have been devoted to the subject. The review by JACOBS[1] gives a comprehensive treatment of diffusion under various conditions applicable to the living system. In spite of the volume of work on the general subject, little has been done specifically with diffusion of oxygen into higher plant tissues. Only WANNER[2] and BERRY[3] have calculated coefficients of diffusion, and in both cases the experimental material was the onion root. The values obtained differed by a factor of from 3–5, which may have been due at least in part to the techniques. WANNER used a polarographic method and BERRY the Warburg constant volume manometer.

The diffusion of oxygen into animal cells and tissues has been more thoroughly studied. KROGH[4] calculated coefficients of diffusion for sheets of muscle and connective tissue from data on the rate of transfer of oxygen across these materials serving as membranes. The coefficients obtained have been used extensively as characteristic of most living systems. Subsequently, additional determinations have shown a considerable range of values to exist[5]. This is particularly important in connection with the widespread use of manometric techniques for measuring oxygen consumption, since the rate of diffusion sets the limit of thickness of the experimental material under investigation[6]. Diffusion also may be capable of acting as the limiting factor in the kinetics of metabolic processes. This was strongly suggested as the case for the apical 5 mm of onion root at 5% oxygen[7]. Since no reference can be found to a complete analysis of diffusion in tissues of different age at several temperatures, the present investigation was undertaken as a natural sequel to the results presented in the previous papers[3, 7]. It is believed that for the first time evidence is given that clearly shows the limits imposed on a root system by diffusion. This is of interest especially because masterprocesses are being subject to question[8].

### METHODS

In order to calculate the diffusion coefficient of oxygen for the onion root, the equation inde-

---

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

pendently derived by FENN[9] and GERARD[10] for diffusion into a cylinder is used. The equation states that

$$U = C - \frac{a}{4D}(R^2 - r^2) \qquad (1)$$

where U is the pressure of oxygen at the desired point in the tissue and C is the external pressure of oxygen, both in g per ml; a is the volume of oxygen consumed in g per ml of tissue per second; R is the radius of the cylinder in centimeters; r is the radial distance in centimeters from the cylinder axis where U is wanted; and D is the coefficient of diffusion in units of cm² per second. When conditions are such that the pressure of oxygen (U) is zero just at the axis of the cylinder then $r = 0$ and the equation reduces to:

$$D = \frac{a\,R^2}{4\,C} \qquad (2)$$

It is possible, therefore, to solve for D when the pressure of oxygen in the environment (C) is just great enough to support the maximum rate of respiration. This pressure, the critical pressure, was determined for three segments of onion root; namely, the region 0–5 mm above the tip which is essentially a zone of mitotically active cells; the region 5–10 mm above the tip which is essentially a zone of elongation; and the region 10–15 mm above the tip which is a zone of differentiation. The Warburg constant volume manometric technique was employed. The procedure followed is described by BERRY[3], and the data have been given in the previous paper by BERRY AND NORRIS[7]. The value of R is taken from BERRY.

The necessary assumptions implied in the calculation of D and the limitations of the method are considered in an earlier paper[3] and will not be repeated here.

## RESULTS

The data necessary for the calculation of the coefficients of diffusion for each of the three zones of onion root at temperatures of 15°, 20°, 30° and 35° C are given in Table I along with the values of the coefficients. The volume of each root segment which was used in converting the rate of oxygen consumption as $\mu$l per root per hour into g per ml of root tissue per second was determined as the volume of a cylinder having a diameter of 0.073 cm and a height of 0.5 cm. In the case of the apical root zone where the tip is tapered, the volume is less than that actually used in the calculation. If it is assumed that the first millimeter of the root tip is a cone, the values of D for the 0–5 mm segment would be about 15% larger than those given in the table. This does not take into consideration the undetermined reduction in the value of R² which would be necessary if the conical shape of the very tip is used. It appears therefore that the error is less than 15% because of this simplification.

The coefficient of diffusion for the apical zone increases with temperature from 15° to 30° but declines from 30° to 35° C. The latter is due to the decrease in "a" at the higher temperature which appears to be the result of heat inactivation of enzymes (see Fig. 5 of previous paper[7]). If the rate of oxygen uptake per hour is calculated from the rate during the first 15 minutes of the run a value of 3.06 $\mu$l/root/hour is obtained and when this is used in solving for D, the value shown in parenthesis in Table IA, column 6, is obtained. For both the 5–10 mm and the 10–15 mm segments, the diffusion coefficients at 30° seem to be out of line with the values for other temperatures. It would appear that the critical pressures are actually higher than the measurements indicated. The coefficients for the other temperatures have the same trend as the apical segment in showing a progressive increase with temperature.

In Fig. 1, plots are made of log D for each root zone *versus* the reciprocal of the absolute temperature. This permits the calculation of the Arrhenius $\mu$ (or critical increment) from the slope of the curve, the numerical values of which are shown. This

### TABLE I

#### DATA USED IN SOLVING FOR DIFFUSION COEFFICIENTS

A

0–5 mm Segment

| Temperature °C | a* | | C* | | D |
|---|---|---|---|---|---|
| | µl/root | g/µl/sec | $\%O_2$ | g/µl | cm²/sec |
| 1 | 2 | 3 | 4 | 5 | 6 |
| 15 | 0.85 | $1.529 \cdot 10^{-7}$ | 15 | $0.720 \cdot 10^{-5}$ | $7.077 \cdot 10^{-6}$ |
| 20 | 1.24 | $2.192 \cdot 10^{-7}$ | 21 | $0.910 \cdot 10^{-5}$ | $8.028 \cdot 10^{-6}$ |
| 30 | 2.78 | $4.751 \cdot 10^{-7}$ | 45 | $1.606 \cdot 10^{-5}$ | $9.843 \cdot 10^{-6}$ |
| 35 | 2.68 | $4.509 \cdot 10^{-7}$ | 50 | $1.648 \cdot 10^{-5}$ | $9.114 \cdot 10^{-6}$<br>$(10.40 \cdot 10^{-6})$ |

B

5–10 mm Segment

| | | | | | |
|---|---|---|---|---|---|
| 15 | 0.40 | $0.719 \cdot 10^{-7}$ | 10 | $0.480 \cdot 10^{-5}$ | $4.992 \cdot 10^{-6}$ |
| 20 | 0.56 | $0.990 \cdot 10^{-7}$ | 15 | $0.650 \cdot 10^{-5}$ | $5.065 \cdot 10^{-6}$ |
| 30 | 1.45 | $2.478 \cdot 10^{-7}$ | 21 | $0.750 \cdot 10^{-5}$ | $11.04 \cdot 10^{-6}$ |
| 35 | 1.87 | $2.144 \cdot 10^{-7}$ | 40 | $1.318 \cdot 10^{-5}$ | $7.961 \cdot 10^{-6}$ |

C

10–15 mm Segment

| | | | | | |
|---|---|---|---|---|---|
| 15 | 0.33 | $0.593 \cdot 10^{-7}$ | 10 | $0.480 \cdot 10^{-5}$ | $4.12 \cdot 10^{-6}$ |
| 20 | 0.45 | $0.795 \cdot 10^{-7}$ | 10 | $0.434 \cdot 10^{-5}$ | $6.117 \cdot 10^{-6}$ |
| 30 | 0.75 | $1.282 \cdot 10^{-7}$ | 10 | $0.357 \cdot 10^{-5}$ | $11.04 \cdot 10^{-6}$ |
| 35 | 1.30 | $2.186 \cdot 10^{-7}$ | 30 | $0.988 \cdot 10^{-5}$ | $7.368 \cdot 10^{-6}$ |

* The values shown under columns 2 and 4 taken from BERRY AND NORRIS[7].

The radius of the onion root used in calculating the volume of a given segment was 0.0365 cm taken from BERRY[3].

*References p. 614.*

type of graphical representation is permissible only if D is a rate constant equivalent to the specific reaction rate constant for a chemical process*. MOELWYN-HUGHES[11] states that under conditions where the rate of a heterogeneous reaction is determined by a diffusion process, dk/dT should equal dD/dT and the critical increment should thus be about 4500 calories at 25° C. Since D was obtained under conditions when diffusion appears to be just limiting, the graphical procedure seemed justified.

The curve for the 0–5 mm segment of root in Fig. 1 falls exactly on the points for all temperatures except 35° C (see above). The critical increment was calculated as 3900 calories per mole. The 4100 calories per mole for the 5–10 mm curve checks closely with the apical zone. There is more scatter of the points in this case and the 30° C value is omitted entirely. However, there is included a value for 25° C which was taken from BERRY[3]. The graph for the results obtained with the 10–15 mm root zone gives a straight line for all points (including one for 25° taken from the earlier work[3]), except for 35° C. The critical increment in this case, 12500 calories per mole, is identical to the value found for rate of oxygen consumption by the same zone (see Fig. 6 of BERRY AND NORRIS[7]). The critical increments for the zones 0–5 mm and 5–10 mm above the tip of the root are in good agreement with the value predicted by MOELWYN-HUGHES[11] and with those reported for diffusion of simple solutes in water[12, 13]. They are also in excellent agreement with the critical increment of rate of oxygen consumption by the apical 5 mm of the root at 5% oxygen (see Fig. 7 of BERRY AND NORRIS[7]), when diffusion of oxygen was clearly the limiting factor. The results with the 10–15 mm segment must, as previously suggested, be in error and serve as a good example of diffusion coefficients calculated under conditions when the critical pressures for diffusion have not been precisely determined. On the evidence presented in the first paper of this series[7] along

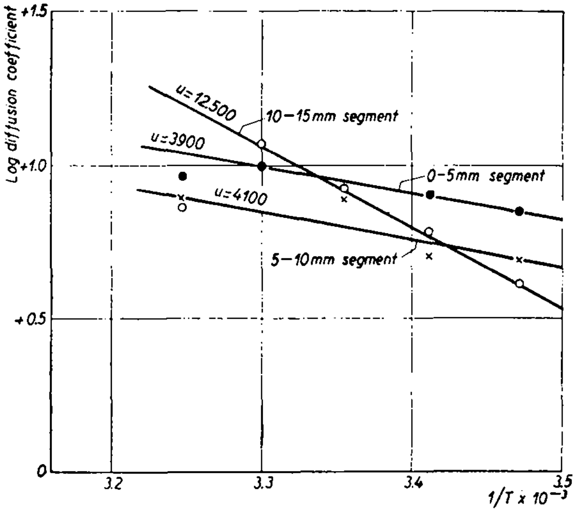

Fig. 1. Logarithm of the diffusion coefficient for different root zones plotted against the reciprocal of the absolute temperature

---

* The reaction rate constant (k) has dimensions of number (mol, mass, ml, etc.) per unit time and the diffusion coefficient (D), which is actually a constant for certain concentration ranges, also has dimensions of number per unit time but with the addition of per unit area. Even k has implied in it a volume term.

with that shown in the first two curves of Fig. 1, it would seem conclusive that metabolism and not diffusion was limiting in the 10–15 mm zone. It would also appear that a dD/dT plot, as MOELWYN-HUGHES[11] states, offers an excellent criterion for a heterogeneous reaction limited by diffusion when the critical increment is found to be approximately 4500 calories. Fig. 1 gives examples therefore of results for and against diffusion as a limiting process.

One of the most important assumptions that must be made in calculating the diffusion coefficient for a cylinder is a uniform rate of respiration from the surface to the axis. The validity of this assumption can be tested with the data for rate of oxygen consumption by the different segments at pressures of oxygen lower than the critical pressure, the pressure at which D was calculated and the lowest pressure supporting the maximum metabolic rate (see Figs 2, 3, and 4 of previous paper). If equation (1) is used to solve for r, when U is zero at a distance r from the axis, with C and "a" known

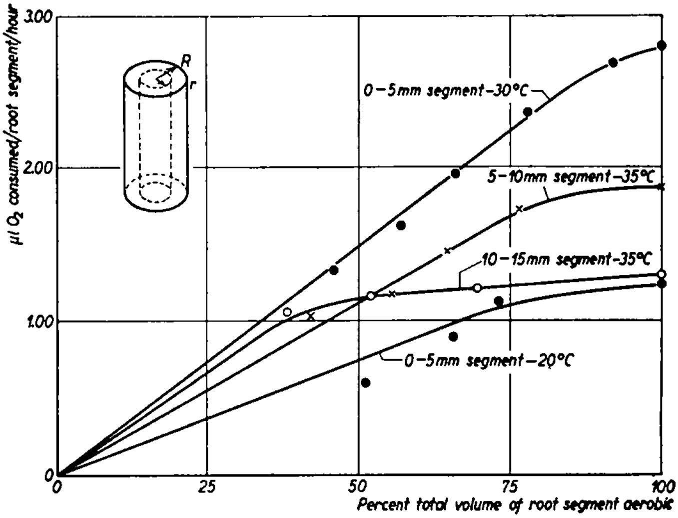

Fig. 2. μl of oxygen consumed/root/segment/h as a function of the percent of the total volume of the root segment that is aerobic. Curves for different root zones at different temperatures are shown.

from the experiments and D calculated above, then the volume of the anaerobic cylinder (the dotted zone in the inset in Fig. 2) can be found. When this volume is compared to the total root volume, the percent of the root that is aerobic is then known. This can be plotted against the measured rate of oxygen consumption (in μl of oxygen per root per hour) as shown by the four typical curves in Fig. 2. The original assumption would necessitate a linear relationship in these graphs but it can be seen that this does not hold. In every case, the curve levels off as the center of the root is approached. This was true for every root zone at every temperature in which the curves in Fig. 1 suggest that the values for D are reliable. In three of the four curves the break falls between 75% and 100% of the root aerobic and only curve 3 has the break at 50%. The value for D which was used in obtaining curve 3 is the least reliable of the four and an inspection of equation (1) shows that when D is smaller than it should be, all points will be moved to the left. Also, if C is actually smaller than the value used, an error of 1% in the pressure of oxygen would shift the points to the left four units (percent). There is reason to doubt the accuracy of curve 3 but there is no reason to believe that a linear relationship holds in any case. If we assume the break falls between 75% and 100%, the former value corresponds to a distance where $r = R/2$. Histologically, this falls in the cortex and no anatomical structure is there to suggest such a change in metabolism. The stele in a segment of root 10–15 mm above the tip occupies only 3.5–4% of the volume. A much lower metabolic rate in this zone, however, might very well account for the break observed in these curves.

## DISCUSSION

A diffusion coefficient calculated for a simple solute diffusing through a pure solvent states the quantity of material that passes unit cross-sectional area in unit time under unit concentration gradient. Since the region across which diffusion occurs in the onion root is not homogeneous, the numerical significance of the coefficient is problematical. It seems logical to presume that in reality a number of coefficients actually exist and these vary with the specific locus of the root being traversed. The single values arrived at in this report by substituting appropriate quantities in the equation that specifically applies only to a simple physical system may be considered approximate means of the many individual coefficients which it is impossible to determine. One way in which the data so obtained can be tested is to determine the activation energy as shown in Fig. 1. The fact that agreement with the theoretical value is found in two of the three cases is merely presumptive evidence for reliable results since, as MOELWYN-HUGHES[11] states, it is possible for reactions to possess a real energy of activation of the same value. There is no indication for this in onion root respiration, however, since in every case where diffusion seems to be completely eliminated as the rate limiting factor, the activation energy is about 12500 (as shown in Figs 6, 7, 8, 9, and 10 of the previous paper[7]). If we assume therefore, that diffusion coefficients having activation energies of about 4500 calories are reliable, then the values for the 0–5 mm segment and the 5–10 mm segment of onion root are acceptable and those for the 10–15 mm segment are not. Furthermore, the greater scatter of the points on the log D *vs* 1/T curve for the 5–10 mm segment compared to the 0–5 mm segment suggests that the coefficients for the latter zone of root are more precise than those for the former.

The above reasoning tells us nothing about the accuracy of the absolute value of the diffusion coefficient since only the rate of change in D with temperature is involved. From the curve for the 0–5 mm segment in Fig. 1 it is possible to find D for any temperature. When this is known, as well as the rate of respiration in pure oxygen at the same temperature, equation (2) can be used to calculate the critical pressure of oxygen at that temperature. If this should check with the experimentally determined critical pressure, the absolute values for D, previously found, would appear to be more reliable. In the experiments of BERRY[3], air was taken as the critical pressure for the 0-5 mm segment of root at 25° C and the diffusion coefficient which was calculated was greater than the curve in Fig. 1 indicates. When the coefficient was taken from the curve, the critical pressure was found to be 30% oxygen. Subsequent experiments have verified this value. It is therefore possible to calculate the entire curve for rate of respiration *versus* pressure of oxygen from the value of D and a single experimental determination of the maximum rate of respiration; *i.e.*, the rate in pure oxygen.

Since the diffusion coefficient was found to decrease as the cells in the root become progressively older, the values reported by WANNER[2] come into very close agreement with the ones shown in this report. The segment of root investigated by WANNER was basal to those used in the present study and the fact that the coefficients he found are one-half to one-third the values shown here may be due in part to the difference in age of the tissue. The coefficients of diffusion reported by KROGH[4] ($6.2$ to $7.5 \cdot 10^{-6}$) are practically identical to certain ones given in Table I. It is also interesting to note that when the coefficients of diffusion obtained by WANNER[2] are plotted as log D *vs* 1/T, a critical increment equal to the ones found for the apical two segments is obtained between 15° and 20° C, but between 20° and 25° C, an increment like that found for the 10–15 mm segment results. The fact that this latter value (about 12500 calories per mol) has been found repeatedly for respiratory processes in the onion root may be interpreted as strongly suggesting that a reaction, characterized by a critical increment of this magnitude, is limiting the rate of respiration.

Inasmuch as the curves of Fig. 2 indicate that the basic assumption of linear rate of respiration across the radius of the root, used in calculating the diffusion coefficient, is in error, it is important to examine the possible causes of the error and their effect on D. The neglect of the diffusion of oxygen into the ends of the root, which assumes its greatest importance when the 0–5 mm segment is used (the 5–10 mm zone and the 10–15 mm zone were studied by using 10 and 15 mm lengths of root respectively), would increase the area of diffusion by about 7%; this would give an apparent diffusion coefficient larger than the true value. This is in the opposite direction from the change in D which would result if the metabolic rate were linear across the entire radius of the root. Thus if each curve in Fig. 2 were extended as a straight line to the 100% aerobic volume, D would be increased. The sum of these effects on D are probably not equal and opposite and the most likely result would seem to be a diffusion coefficient somewhat larger than the true value. However, the small departure from linearity in three of the curves of Fig. 2 suggests that no gross error results from the assumption when the experimental measurements are precise. Therefore, when diffusion coefficients calculated in the manner described in this paper withstand these tests of reliability, it is believed that they closely approximate the true apparent rate of penetration of oxygen into the living tissue and very strongly suggest that diffusion is the limiting factor in respiratory rate under certain conditions.

## SUMMARY

The Gerard-Fenn equation for diffusion into a cylinder was used to calculate the apparent diffusion coefficient of oxygen in each of three segments of onion root tip at each of the four temperatures described in the first paper of this series.

These coefficients were then used to determine the activation energy of diffusion (log D *vs* 1/T graphs) with the result that those for the two most apical zones of root agreed closely with the theoretical value. Certain coefficients were also used to calculate the distance to which oxygen penetrates the root when the external pressure of oxygen is limiting. From these values it was shown that the rate of oxygen consumption across the radius of the root is not perfectly linear (as the Gerard-Fenn equation demands) but the departure from linearity is not sufficient to completely invalidate the results.

## RÉSUMÉ

Nous avons employé l'équation de Gerard-Fenn pour la diffusion dans un cylindre pour calculer le coefficient apparent de diffusion de l'oxygène dans chacun des trois segments de l'extrémité d'une racine d'oignon aux quatre températures décrites dans la première note de cette série.

Nous avons ensuite employé ces coefficients pour déterminer l'énergie d'activation de la diffusion (v. les graphiques: log D *vs* 1/T); il en a résulté que les valeurs pour les deux zones les plus apicales de la racine ont répondu assez exactement aux valeurs théoriques. Certains coefficients ont été employés aussi pour calculer à quelle profondeur l'oxygène pénètre la racine à des pressions extérieures limites d'oxygène. Ces valeurs nous ont permis de constater que le taux de consommation de l'oxygène le long du rayon de la racine n'est pas tout à fait linéaire (comme l'exige l'équation de Gerard-Fenn); mais ce défaut de linéarité ne suffit pas à infirmer complètement les résultats.

## ZUSAMMENFASSUNG

Auf Grund der Gleichung von Gerard-Fenn für die Diffusion in einem Zylinder wurde der scheinbare Diffusionskoeffizient für Sauerstoff in jedem der drei Segmente einer Zwiebelwurzelspitze bei den vier in der ersten Abhandlung dieser Reihe beschriebenen Temperaturen berechnet.

Mit Hilfe dieser Koeffizienten wurde hierauf die Aktivierungsenergie der Diffusion bestimmt (vgl. die Diagramme log D : 1/T); für die beiden der Spitze am nächsten gelegenen Segmente stimmten die gefundenen Werte mit den theoretischen gut überein. Auf Grund einiger Koeffizienten wurde auch berechnet, wie weit der Sauerstoff in die Wurzel eindringt, wenn der äussere Sauerstoffdruck verschiedene Grenzwerte hat. Diese Werte liessen erkennen, dass der Sauerstoffverbrauch dem Wurzelradius entlang nicht ganz linear verläuft (wie es die Gleichung von Gerard-Fenn voraussetzt), aber diese Abweichung ist nicht gross genug um die Ergebnisse vollkommen zu entkräften.

## REFERENCES

[1] M. H. JACOBS, *Ergeb. Biol.*, 12 (1935) 1.
[2] H. WANNER, *Vierteljahrsschr. naturforsch. Ges. Zürich*, 90 (1945) 98.
[3] L. JOE BERRY, *J. Cellular Comp. Physiol.*, 33 (1949) 41.
[4] A. KROGH, *J. Physiol.*, 52 (1919) 391.
[5] N. RASHEVSKY, *Advances and applications of mathematical biology*, U. of Chicago Press, Chicago, Ill. (1940).
[6] O. WARBURG, *Biochem. Z.*, 142 (1923) 317.
[7] L. JOE BERRY AND W. E. NORRIS JR, *Biochim. Biophys. Acta*, 3 (1949) 593.
[8] H. L. BOOIJ AND H. P. WOLVEKAMP, *Bibliotheca Biotheoretica*, 1 (1944) 145.
[9] W. O. FENN, *J. Gen. Physiol.*, 10 (1927) 767.
[10] R. W. GERARD, *Am. J. Physiol.*, 82 (1927) 381.
[11] E. A. MOELWYN-HUGHES, *The kinetics of reactions in solution*, Oxford Press (1947).
[12] S. GLASSTONE, K. J. LAIDLER, AND H. EYRING, *The theory of rate processes*, McGraw-Hill Book Co., New York (1941).
[13] I. W. SIZER, *Advances in Enzymol.*, 3 (1943) 35.

Received January 20th, 1949