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ESTIMATION OF THE GASEOUS CO_2 CONCENTRATION IN INTERCELLULAR SPACES DURING PHOTOSYNTHESIS

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The equation which Browne and Escombe (1900) derived to estimate diffusive capacity of plant stomata contains an assumption of CO_2 gradients across the stomata which is quite unreasonable. A slightly modified form of the equation is reproduced below to demonstrate this unreasonable assumption:

$$Q = \frac{D\ A\ (C_o - C_s)}{L + F} \qquad (1)$$

In which,

Q is the CO_2 transport per cm.^{2} of leaf per second
D is the diffiusivity of CO_2 in air
A is the stomatal area in cm^2 per cm.^{2} of leaf
C_o is the CO_2 concentration in air outside the leaf
C_s is the CO_2 concentration in air inside the leaf
L is the length of the stoma tube
F is a correction introduced because the effective tube length is greater than the actual tube length. In tubes having a circular cross-section this correction is a function of tube radius, $F = \frac{\pi\ r.}{2}$

The unreasonable assumption made by Browne and Escombe was the assumtion that C_s equalled zero. The unreasonable nature of this assumption has been recognized (Spoehr, 1926, p. 77) but although a few investigators have tried to apply the equation to specific plant leaves (Maskell, 1928; Stalfelt, 1935) they retained this assumption and I have found no reported attempts to obtain more reasonable estimates of the value of CO_2 concentration within the intercellular spaces of leaves during photosynthesis.

An inspection of equation (1) shows that it can be used to estimate the concentration drop ($C_o - C_s$) across the stomata if the value of Q is specified, thus:

$$C_o - C_s = \frac{Q\ (L + F)}{D\ A} \qquad (2)$$

If we use the following values, all reasonable approximations for an average leaf under conditions favorable for photosynthesis:

$Q = 0.06 \times 10^{-6}$ g./cm.^{2}/sec. (20 mg./dm.^{2}/hr.)
$L = 15 \times 10^{-4}$ cm.
$F = 8.8 \times 10^{-4}$ cm.
D = 0.15 cm.^{2}/sec. (assuming a temperature of 20° C and a barometric pressure of 76 cm. Hg)
A = 0.01 cm.^{2}/cm.^{2} of leaf.

Then $(C_o - C_s)$ amounts to 0.095×10^{-6} g./cm.^{3}. This represents a concentration drop of about 16 percent, assuming a CO_2 concentration of 0.59 μg./cm^3. (0.03 vol. percent) in the air outside the leaf.

There are two questions concerning this application of the equation which must be raised: (1) what is the nature of the correction F, and (2) how does the influence of neighboring stomata affect the validity of the equation. To examine these questions I have made an analysis of gradients across a stoma tube based on the diagram of figure 1. The stomatal dimensions in this diagram are the same as in the above application of equation (2). The stoma tube has a length of 15 μ and a radius of 5.6 μ, giving it a cross-sectional area of 100 μ^2. On either side of the stoma hemispheres of influence are portrayed. The external hemisphere has an area of 10,000 μ^2, a limit set by the fact that stomata occupy 1 percent of the leaf area. The internal hemisphere has an area of 100,000 μ^2, a limit set by the fact that the surfaces of cells bordering on intercellular spaces present about 10 times as much area as the leaf exterior (Miller, 1938, p. 414). The regions of influence are assumed hemispherical to simplify mathematical treatment. The hemispherical shape of diffusion shells also suggests such a diagram.

Figure 1 can be used to analyze CO_2 concentration gradients across the stoma tube and its hemispheres of influence. Across the stoma tube the gradient is constant and can be expressed as:

$$\frac{dc}{dx} = \frac{C_a - C_b}{L} \tag{3}$$

where C_a is the CO_2 concentration at the outer end of the stoma tube, C_b is the concentration at the inner end of the tube, and L is the length of the tube. Between the ends of the stoma tube and the surfaces of the hemispheres of influence the gradients are variable, being inversely proportional to the square of the distance from the small hemispheres portrayed at the ends of the stoma tubes (cf. Verduin, 1949). The area of these small hemispheres is equal to the area of the stoma. The concentration gradient at any point within the external hemisphere is given by the equation:

$$\frac{dc}{dx} = \frac{k}{x^2} \tag{4}$$

where k is a proportionality constant. The concentration drop across the external hemisphere of influence can be estimated using the definite integral:

$$C_o - C_a = \int_{x=4\times10^{-4}}^{x=40\times10^{-4}} \frac{k}{x^2}\,dx = \left[-\frac{k}{x}\right]_{4\times10^{-4}}^{40\times10^{-4}} \tag{5}$$

The concentration gradient at any point in the internal hemisphere of influence is given by the equation:

$$\frac{dc}{dx} = -\frac{k}{x^2} \tag{6}$$

the minus sign indicating that the concentration decreases as x increases. The concentration drop across this hemisphere is given by:

$$C_b - C_s = -\int_{x=126\times10^{-4}}^{x=4\times10^{-4}} \frac{k}{x^2}\,dx = -\left[-\frac{k}{x}\right]_{126\times10^{-4}}^{4\times10^{-4}} \tag{7}$$

(The assault made by Romell (1927), on this portion of the gas diffusion path yielded results which do not differ significantly from that of the simple analysis presented here. Attempts to specify the magnitude of the invasion coefficient across the cell wall boundary, and of the diffusivity and length of diffusion path

in the cytoplasm, were premature then and remain so today.) We can evaluate k, for the rate of transport specified above, noting that the concentration gradient at the surface of the outer hemisphere of influence is valid for the entire leaf, hence, $\frac{dc}{dx} = \frac{Q}{D} = \frac{0.06 \times 10^{-6}}{0.15} = 0.4 \times 10^{-6}$g/cm^4., when $x = 40 \times 10^{-4}$cm. Substituting in (4) we obtain $k = 6.4 \times 10^{-12}$. The concentration drop across each hemisphere of influence can now be computed.

$$C_o - C_a = \left[\frac{6.4 \times 10^{-12}}{4 \times 10^{-4}} - \frac{6.4 \times 10^{-12}}{40 \times 10^{-4}} \right] = 1.44 \times 10^{-8} \text{g./cm.}^3$$

$$C_b - C_s = \left[\frac{6.4 \times 10^{-12}}{4 \times 10^{-4}} - \frac{6.4 \times 10^{-12}}{126 \times 10^{-4}} \right] = 1.55 \times 10^{-8} \text{g./cm.}^3$$

The concentration drop across the stoma tube can be computed noting that the gradient here is 100 times that computed for the entire leaf, or 0.4×10^{-4} g./cm.^{4} Substituting in (3) we obtain,

$C_a - C_b = 15 \times 10^{-4} \times 0.4 \times 10^{-4} = 6 \times 10^{-8}$ g./cm.^{3}

The total concentration drop $C_o - C_s$ then is the sum of these three components,

$(1.44 + 6.00 + 1.55) \times 10^{-8} = 9.0 \times 10^{-8}$, or 0.090×10^{-6}g./cm.^{3}

This estimate agrees closely with the value of 0.095×10^{-6} obtained from equation (2).

The above analysis clarifies the nature of the correction F in equations (1) and (2). The correction accounts for the gradients within the hemispheres of influence. In equation (2) therefore C_o represents the CO_2 concentration at the surface of the outer hemisphere of influence, and C_s represents the concentration at the surface of the cells bordering on the intercellular spaces. They do not represent concentrations at the immediate ends of the stoma tube.

So far nothing has been said about the effect of neighboring stomata on the validity of equation (2). In the analysis based on figure 1 the problem of the mutual influence of stomata is solved by designating the limits of the hemispheres of influence. Molecules from neighboring hemispheres will, of course, enter the hemisphere in question, but their effect will be cancelled by losses to neighboring hemispheres. The major influence of neighboring stomata is taken into account in equation (2) in essentially the same way as in the analysis based on figure 1. For example: in equation (2) if the area represented by the stomata (A) is doubled by doubling the number of stomata, the value of $C_o - C_s$ is reduced to 0.047×10^{-6} g./cm.^{3}. In the analysis based on figure 1 such a doubling of the stoma population would reduce the area of the hemispheres of influence by a factor of 2, the gradient at the stoma would be one-half as high as before, the upper limit in equation (5) would become 28.2×10^{-4}, the lower limit in (7) would become 89.2×10^{-4}, k would be 3.2×10^{-12} yielding a $C_o - C_s$ value of 0.0445×10^{-6} g./cm.^{3}. So it appears that the effect of neighboring stomata is adequately accounted for in equation (2) by the factor A, and that equation (2) provides a reliable estimate of the CO_2 concentration drop across the stoma tubes plus their hemispheres of influence. This statement is true for plant leaves because the stomatal areas represent so small a fraction of the leaf area. If stomata were so numerous that they represented a large fraction of the leaf area, then equation (2) and the analysis based on figure 1 yield more widely divergent estimates. For example: if $A = 0.5$ then $C_o - C_s$ from equation (2) becomes 0.0019×10^{-6} g./cm.^{3}, but in the analysis based on figure 1 it would amount to 0.0015×10^{-6}. These estimates differ by about 20 percent compared to only a 5 percent discrepancy when using stomatal areas similar to those observed in nature.

If one assumes that the CO_2 concentration at the surfaces of the external hemispheres of influence is equal to that in the outside air, then equation (2) can provide an estimate of the CO_2 concentration at the surfaces of the cells bordering on the intercellular spaces. Such an assumption requires high air turbulence near the leaf, but under outdoor conditions turbulence is high (Jeffreys, 1918) and the CO_2 concentration of the outside air is probably maintained to within one millimeter of the leaf. So under completely natural conditions a leaf exhibiting photosynthesis rates of 20 mg./dm.^{2}/hr. probably would have an internal CO_2 concentration of about 0.1 μg./cm.^{3} less than the CO_2 concentration of the outside air.

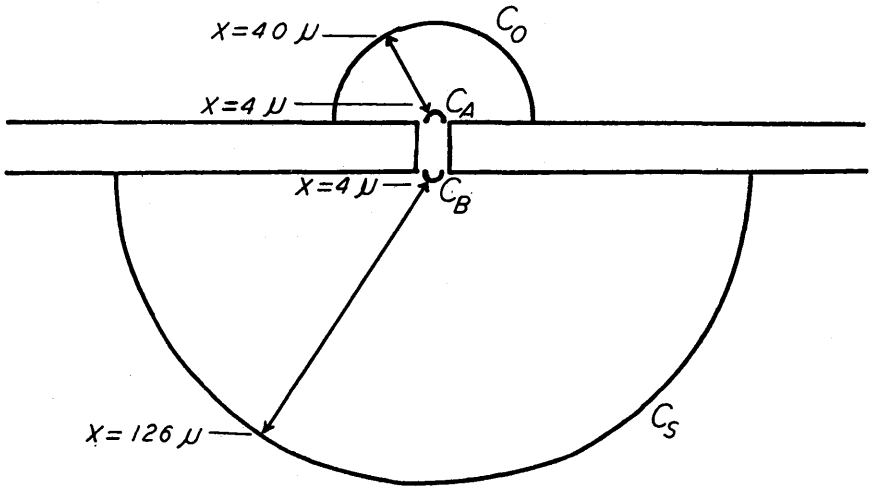

FIGURE 1. Diagram used to analyze concentration gradients across stoma. The outer hemisphere of influence represents an area of 10,000 μ^2 (radius, 40 μ) the inner hemisphere of influence represents an area of 100,000 μ^2 (radius, 126 μ) the stoma tube has a cross section of 100 μ^2 and is 15 μ in length. C_o represents the CO_2 concentration at the surface of the outer hemisphere of influence, C_a that at the outer end of the stoma tube, C_b at the inner end of the stoma tube, and C_s at the surfaces of the cells bordering on the intercellular spaces.

It was stated above that Browne and Escombe's assumption that $C_s = O$ was unreasonable. Measurements of the CO_2 compensation point (Rabinowitch II, 1, p. 898) suggest that C_s cannot be reduced much below 0.2 μg./cm.^{3}, so an estimate of the maximal photosynthesis rates possible under outdoor conditions with the stomatal characteristics specified above can be made by setting $C_s = 0.2 \times 10^{-6}$ and $C_o = 0.59 \times 10^{-6}$ in equation (1), thus:

$$Q = \frac{0.15 \times 0.01 \times (0.59 - 0.20) \times 10^{-6}}{23.8 \times 10^{-4}} = 0.25 \times 10^{-6} \text{ g./cm.}^2\text{/sec. or 90 mg./dm.}^2\text{/hr.}$$

It is probably a coincidence that this value is close to the highest maximal rates reported under near-natural conditions (Rabinowitch II, 1, p. 1001).

Most of our knowledge of photosynthesis under near-natural conditions is based on experiments in which the leaves were surrounded by transparent chambers through which air was drawn. The air flow through such chambers is not high enough to create turbulence similar to that in outdoor air. Heinicke and Hoffman (1933) for example, adopted a flow rate of about 2.5 l./cm.^{2} leaf/hr. (about 7 m./min. with their leaf cups) because this flow rate yielded CO_2 absorption by hydroxide solutions equalling the absorption rate by such solutions when exposed in a quiet room. Most investigators have used lower rates of air flow. In a

quiet room the air near an absorbing surface suffers significant CO_2 depletion. A fair quantitative estimate of this effect can be made by comparing the absorption rate for a hydroxide solution in quiet air with the rate in moving air, as Browne and Escombe did. They found that the maximum rate in moving air was 0.177 cm.^{3}/cm.^{2}/hr., while the quiet air rate was 0.120. If one assumes that the maximum rate represents a CO_2 concentration at the absorbing surface equal to 0.59 μg./cm.^{3}, then the CO_2 concentration at the absorbing surface under quiet conditions must have been about 0.40 μg./cm.^{3} A similar CO_2 concentration at the absorbing surface can be assumed for the experiments of Heinicke and Hoffman, and it is possible then to compute the effective length of the diffusion path over which a diffusivity of 0.15 cm.^{2}/sec. can be considered valid under their experimental conditions. Specifically, in their experiment with 0.2 N KOH a rate of 24.4 mg./dm.^{2}/hr. (0.068×10^{-6} g./cm.^{2}/sec.) was associated with a mean CO_2 concentration of 0.53 μg./cm.^{3} in the air stream passing over the solution. The effective length of the diffusion path can be estimated as:

$$\frac{0.15\times(0.53-0.40)\times10^{-6}}{0.68\times10^{-6}} = 0.29 \text{ cm.}$$

The drop in CO_2 concentration between the air in a leaf chamber (C_c) and the air at the surfaces of the external hemispheres of influence (C_o) can be approximated using the equation:

$$C_c - C_o = \frac{Q}{D}\times0.29 \tag{8}$$

Using the values of Q and D specified under equation (2) in equation (8) this drop in concentration amounts to

$$\frac{0.06\times10^{-6}}{0.15}\times0.29 = 0.12\times10^{-6} \text{ g./cm.}^3$$

In the photosynthesis studies of Heinicke and Hoffman photosynthesis rates of 20 mg./dm.^{2}/hr. were usually associated with mean CO_2 concentrations in the leaf chamber of about 0.50 μg./cm.^{3} The average CO_2 concentration at the surfaces of the cells bordering on the intercellular spaces (C_s) under such conditions would be estimated at $(0.50-0.12-0.09)=0.29$ μg/cm.^{3} This is approximately one-half the value for normal air, and the CO_2 concentration drop which occurs between the outer hemispheres of influence and the air in the chamber, is larger than the drop which occurs across the stomata.

The data of Stålfelt (1935) provide an opportunity to make similar computations for a wide range of stomatal areas, and the photosynthesis rates associated with them. Such computations are presented in table 1. The rate of air flow through Stålfelt's leaf chambers was about 4 m./min., so I have used equation (8) to provide estimates (conservative) of the concentration change ($C_c - C_o$) between the air in the leaf chamber and the surfaces of the external hemispheres of influence. The values of ($C_o - C_s$) were computed using equation (2). The values of A were approximated from Stålfelt's table 5 to correspond to the stomatal apertures listed in table 4. The values of C_c were computed from the data in table 4 knowing that the flasks used had a volume of 5.5 l., and that the CO_2 content of air outside the leaf chambers was approximately 0.59 μg./cm.^{3} The values of F were computed using the equation:

$$F = \frac{\pi}{2}\sqrt{\frac{A}{\pi\times8700}} \tag{9}$$

In this equation the stomata are assumed circular in cross-section. The number 8700 is based on Stålfelt's reported stomatal density of 87/mm^2 of leaf. The fact that *Avena* stomata are narrow slits makes the F values computed by this method somewhat smaller than they should be. It can be shown that the F value

for a narrow slit would be higher than that for a circular pore of the same area, but for the purpose of these computations equation (9) is regarded as a sufficiently close approximation. Stålfelt reported that the stoma tube length was about 10 μ. The reliability of this estimate was not indicated. It has been used to obtain the values of (L + F) in table 1.

Table 1 shows that the estimated values of ($C_c - C_o$) diminish, while the values of ($C_o - C_s$) increase as stomatal areas decrease. The net effect of these two concentration changes is such that the estimates of C_s are practically constant (0.37 μg./cm.^{3}) throughout the range of stomatal areas and CO_2 absorption rates listed there. The CO_2 concentration at the surface of the cells bordering on the intercellular spaces would seem to be considerably lower than that in normal air, but it was not proportional to stomatal area. The reduction in CO_2 absorption rate associated with reduced stomatal areas, therefore, was not caused by a reduction in CO_2 concentration at the boundary between gas and cell wall in the leaf, but reductions in CO_2 absorption rate occurred while this CO_2 concentration remained practically constant.

TABLE 1

Computation of C_s based on data from tables 4 and 5 of Stålfelt

Q μg./cm.^{2}/sec.	Stomatal width μ	Stomatal area cm.^{2}/cm.^{2} of leaf	Mean C_c μg./cm.^{3}	F μ	L + F μ	$C_c - C_o$ μg./cm.^{3}	$C_o - C_s$ μg./cm.^{3}	C_s μg./cm.^{3}
0.052	7.3	0.016	0.52	12.0	22.0	0.101	0.048	0.371
0.044	6.4	0.013	0.53	10.8	20.8	0.084	0.047	0.399
0.050	5.5	0.011	0.52	10.0	20.0	0.096	0.061	0.363
0.052	4.5	0.009	0.52	9.0	19.0	0.101	0.073	0.346
0.039	2.8	0.006	0.53	7.4	17.4	0.075	0.075	0.380
0.036	2.0	0.004	0.52	6.0	16.0	0.070	0.096	0.354
0.031	1.4	0.003†	0.54	5.2	15.2	0.061	0.105	0.374
0.017	0.5	0.001*	0.56	3.0	13.0	0.032	0.147	0.381

†Extrapolated by Stålfelt. *I extrapolated further to obtain the final value.

The concept of diameter proportionality of diffusion through small pores has frequently been invoked to explain the high diffusive capacity of stomata. An inspection of equation (1) shows that diffusive transport (Q) is proportional to pore area (A) divided by effective tube length (L + F). With circular pores A is proportional to diameter squared and F is proportional to diameter. If L is small compared to F then the quantity $\frac{A}{L + F}$ becomes approximately proportional to diameter. But in stomata L is not small compared to F, and table 1 shows that as stomatal areas diminish the magnitude of F also diminishes. The concept of diameter proportionality, therefore, has little bearing on stomatal diffusion. The high diffusive capacities of stomata are attributable to the increase in concentration gradients within their spheres of influence. Once this is taken into account it becomes evident that diffusion through stomata is a function of area just as it is in any other diffusion problem.

In my 1949 analysis of interference among stomata the effect of reduced interference as stomatal areas diminish was described. The effect appears in the present analysis in the diminishing values of F in table 1 as stomatal areas become smaller. Thus stomatal closure causes an increased concentration drop across the stomata and their hemispheres of influence, for a given rate of transport (Q) but the increase is less than would be predicted on the basis of stomatal area alone because the quantity (L + F) decreases.

The low values of C_s computed above imply that maximal CO_2 absorption rates occuring under completely natural conditions may be considerably higher than most of the maxima for near-natural conditions reported in the literature. Heinicke and Hoffman (1933) in their table 4 showed that increasing the air flow rate from 2.5 to 7.5 l./cm².hr. was associated with a photosynthesis increase from 22.1 to 30.8 mg./dm.²/hr. Most of this increase is probably attributable to an improvement in air turbulence in the leaf chamber, causing a shortening of the effective diffusion path between the CO_2 concentration in the chamber and the outer hemispheres of influence. There is no reason to believe that the turbulence in the latter case exceeded that of ordinary outdoor conditions. The importance of air turbulence within leaf chambers has not received adequate attention in studies of photosynthesis employing natural air supplies. It may be that this factor is responsible for the higher maximal rates reported by Russian investigators, and pointed out by Rabinowitch in his table 28.VI, p. 998–1001.

SUMMARY

An analysis of diffusion through stomata is presented which shows that the CO_2 concentration in air at the surface of the cells bordering on the intercellular spaces (C_s) of a leaf can be computed if the rate of CO_2 absorption and the stomatal dimensions are specified. Such computations indicate that in a leaf having average stomatal characteristics and absorbing 20 mg. of CO_2/dm.²/hr. under completely natural conditions C_s would be about 0.1 μg./cm.³ less than in the outside air. The maximal CO_2 absorption rate possible for such a leaf, computed by setting $C_s = 0.2$ μg./cm.³ (the CO_2 compensation point) would be about 90 mg./dm.²/hr. In experimental determinations of photosynthesis rates the air in leaf chambers is much less turbulent than outdoor air, and an important drop in CO_2 concentration occurs between the air in a chamber and the air near the leaf. In such experiments a CO_2 absorption of 20 mg./dm.²/hr. corresponds to a C_s of about 0.3 μg./cm.³, or approximately one-half the CO_2 concentration in normal air.

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