

CARBON DIOXIDE IN THE INTERCELLULAR SPACES OF LEAVES DURING PHOTOSYNTHESIS

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IT was recently found by Gabrielsen¹ that leaves of elder (*Sambucus nigra*), enclosed either in ordinary air of 0.03 per cent carbon dioxide content or air poor in carbon dioxide (0.0024 per cent) and illuminated with light of 1,100 foot-candles intensity, brought the carbon dioxide content to a constant value of about 0.009 per cent. He has suggested^{1,2} that this 0.009 per cent represents a threshold value below which no photosynthesis can take place. Audus³ found that even at high light intensities leafy shoots of cherry laurel gave out carbon dioxide into a slow stream of carbon dioxide-free air, although E. F. Blackman⁴ and Maskell⁵ had found no detectable quantity of carbon dioxide emitted by illuminated leaves under such conditions.

In 1938-39, as a part of an investigation of the relation between carbon dioxide assimilation and stomatal movement, I carried out experiments with *Pelargonium zonale* and *Begonia sanguineum* in which air of known carbon dioxide content was drawn through the intercellular spaces of the leaf at predetermined rates of flow by balanced push and pull; the leaf was illuminated with light at 2,450 foot-candles and the carbon dioxide content of the air emerging was estimated by a conductivity method. In this way, stomatal control of assimilation was entirely eliminated and the air of known carbon dioxide content was brought into direct contact with the walls of the assimilating cells. For this type of experiment, first suggested in principle by Prof. F. G. Gregory, somewhat complex apparatus was necessary and the technique has already been described in detail⁶. These experiments were terminated by the War, and it is only recently that an opportunity for detailed analysis of the results has occurred. The purpose of this preliminary communication is to record the approximate agreement, in the results so far calculated, of the carbon dioxide content of the air emerging from the leaf with the equilibrium value found by Gabrielsen for air surrounding a leaf in a closed system; also to indicate an interesting connexion between this value and stomatal response to carbon dioxide.

Air was passed into the leaf over an area of 9 sq. cm. and emerged from another 9 sq. cm.; the total area involved including the washer separating these two areas was some 23 sq. cm. Over a wide range of rates of flow (0.4-2.5 litres/hr.) and with either 0.03 per cent or 0.14-0.18 per cent carbon dioxide content in the air on entry, the air emerging from leaves of either species had a mean carbon dioxide content approximating to 0.01 per cent. Some representative results are presented in Table 1. The determinations were made over successive short

periods, ranging from 1 m. 40 s. to 4 m. 40 s. for different experiments, so that considerable fluctuations in the individual values are to be expected, and these are indicated by the ranges in Table 1. During these 'through' experiments, stomatal apertures as indicated by the resistance porometer^{7,8} varied over a wide range without apparently affecting the assimilation-rates. In one experiment (No. 19) with high carbon dioxide content (0.18 per cent) and high rates of flow (1.5 and 2.1 litres/hr.) the air emerging from a *Pelargonium* leaf showed a carbon dioxide content markedly in excess of 0.01 per cent (italics in Table 1), suggesting that the supply had been increased to a level at which carbon dioxide was no longer so severely 'limiting'. On the other hand, in one of the *Begonia* experiments (No. 13), similar high values were given at the lower but not at the higher rates of flow (italics in Table 1), and in general there is little correlation to be seen in the results between rate of carbon dioxide supply and carbon dioxide content of the air emerging. In any event, it clearly must be possible to increase the rate of carbon dioxide supply until the light intensity used or some other factor appreciably limits the increase in uptake; the most important result for the present purpose is the finding that even with a normal initial carbon dioxide content (0.03 per cent) and a low rate of flow through the leaf the mean carbon dioxide content of air emerging from the intercellular spaces is never reduced below 0.005 per cent.

It seems that the value of approximately 0.01 per cent represents the minimum carbon dioxide concentration at the surface of the assimilating mesophyll cell. There are, in effect, two sources of carbon dioxide supply: that in the external air, either outside the leaf or, in the present experiments, close to the cell

Table 1. 'Through' experiments

Expt. No.	Date	Rate of flow in litres (at N.T.P.) per hr.	Observations		Initial	% CO ₂ content	
			Period (sec.)	No. (N)		Mean (of N)	Range
<i>(a) Pelargonium zonale</i>							
7A	27. 9.38	2.47	280	8	0.0298	0.0059	0.0048-0.0079
8A	5.10.38	2.08	"	15	"	0.0051	0.0034-0.0078
23	25. 8.39	1.01	100	11	"	0.0075	0.0037-0.0122
"	"	1.87	"	3	"	0.0096	0.0075-0.0120
"	"	1.50	"	7	"	0.0083	0.0055-0.0117
9A	11.10.38	1.03	120	11	0.143	0.0091	0.0038-0.0155
"	"	0.69	"	10	"	0.0099	0.0077-0.0122
"	"	0.51	"	9	"	0.0082	0.0032-0.0150
19	24. 7.39	1.01	100	5	0.184	0.0115	0.0085-0.0151
"	"	2.05	"	4	"	0.0165	0.0162-0.0169
"	"	1.01	"	8	"	0.0083	0.0036-0.0131
"	"	1.50	"	3	"	0.0197	0.0171-0.0225
"	"	0.95	"	3	"	0.0119	0.0059-0.0153
"	"	0.69	"	3	"	0.0110	0.0081-0.0145
					Mean =	0.0102	
<i>(b) Begonia sanguineum</i>							
11	10.11.38	0.78	110	4	0.143	0.0073	0.0033-0.0126
"	"	0.59	"	4	"	0.0050	0.0020-0.0090
"	"	0.43	"	6	"	0.0057	0.0040-0.0083
13	17.11.38	1.04	"	7	"	0.0120	0.0034-0.0144
"	"	0.79	"	3	"	0.0105	0.0096-0.0111
"	"	0.61	"	4	"	0.0160	0.0140-0.0176
"	"	0.43	"	6	"	0.0142	0.0098-0.0198
					Mean =	0.0101	

Table 2. 'Over' experiments

Expt. No.	Date	Rate of flow in litres (at N.T.P.) per hr.	Observations		Log R	Initial	% CO ₂ content Final	
			Period (sec.)	No. (N)			Mean (of N)	Range
(a) <i>Pelargonium zonale</i>								
23	25. 8.39	1.89	100	4	\bar{I} -297	0.0298	0.0174	0.0119-0.0238
"	"	"	"	4	\bar{I} -134	"	0.0091	0.0076-0.0110
"	"	1.01	"	4	\bar{I} -029	"	0.0054	0.0016-0.0105
"	"	1.89	"	4	\bar{I} -872	"	0.0074	0.0068-0.0085
17	17. 7.39	1.00	"	4	\bar{I} -671	0.184	0.0574	0.0431-0.0782
"	"	"	"	9	\bar{I} -398	"	0.0229	0.0080-0.0319
20	26. 7.39	1.02	"	4	\bar{I} -994	"	0.1021	0.0861-0.1203
"	"	"	"	8	\bar{I} -950	"	0.0223	0.0132-0.0282
"	"	"	"	4	\bar{I} -929	"	0.0278	0.0266-0.0297
(b) <i>Begonia sanguineum</i>								
14	18.11.38	1.92	100	4	\bar{I} -989	0.0298	0.0169	0.0152-0.0199
"	"	"	"	8	\bar{I} -854	"	0.0132	0.0109-0.0163
15	22.11.38	1.88	"	4	0.121	"	0.0173	0.0146-0.0215
"	"	"	"	9	0.033	"	0.0163	0.0117-0.0233
11	10.11.38	1.02	120	10	0.074	0.143	0.0578	0.0409-0.0686
12	15.11.38	1.03	110	4	0.288	"	0.0717	0.0591-0.0951
"	"	"	100	6	0.122	"	0.0422	0.0335-0.0493

surface, and the carbon dioxide of respiration produced in the cytoplasm. Owing to the latter, the carbon dioxide tension at the cell surface could never fall to zero even if carbon dioxide-free air were drawn through the leaf. (Unfortunately this experiment was not carried out owing to the outbreak of war.) That the carbon dioxide content of the air emerging should fluctuate so closely about 0.01 per cent over a wide range of rates of supply indicates that a similar value might be expected with a very slow stream of carbon dioxide-free air passed through the leaf, and this suggestion is supported by Gabrielsen's closed-system experiments. The results, in fact, appear consistent with his hypothesis of a threshold concentration for photosynthesis, though it appears improbable that a true threshold value should exist.

It is clear that in the present experiments, over the range of rates of carbon dioxide supply used, carbon dioxide was very severely 'limiting'. It appears that once inside the leaf all the carbon dioxide except the 0.01 per cent (approx.) is taken up with great efficiency. Some results from experiments in which air was passed over the leaf surface instead of through the intercellular spaces are presented in Table 2 for comparison with those in Table 1. Here the carbon dioxide had to diffuse into the leaf through the stomata. In the case of *Pelargonium*, when the stomata were relatively widely open (low 'log R') and the normal 0.03 per cent initial carbon dioxide concentration was used, the air leaving the leaf chambers showed the same low concentration as in the 'through' experiments, although the leaf area concerned was now only 9 sq. cm. This result was doubtless due to the design of the leaf chambers⁶, which reduced the diffusion path to the leaf surface to at most 2 mm.; it is of importance as indicating that in similar 'over' assimilation experiments, carried out with non-turbulent flow of normal air but in the more usual and deeper types of leaf chamber, the carbon dioxide content near a part of the leaf surface may, in fact, be down to the 'threshold concentration', although the emerging air may show a much higher average value. This would render doubtful the effective area of leaf; it also stresses the need for caution in

estimating the mean carbon dioxide content to which the leaf is exposed and using such an estimate to correct assimilation data to a constant carbon dioxide content. With relatively closed stomata (high log R) or with higher initial carbon dioxide contents, the *Pelargonium* leaves failed to reduce the concentration to the 'threshold value'; but even with 0.184 per cent carbon dioxide the depletion was severe and amounted to about 88 per cent of the total content in some cases.

The *Begonia* results are of some interest, for whereas in 'through' experiments (Table 1) the uptake of carbon dioxide was almost the same as for *Pelargonium*, in 'over' experiments (Table 2) the latter proved appreciably the more efficient. This might be expected from the small and localized areas of stomata and the long internal diffusion paths to the chlorenchyma in the *Begonia* leaf⁷.

The value of approximately 0.01 per cent indicated for the carbon dioxide content of the air in the intercellular space system of a brightly illuminated leaf is of especial interest in view of a recent extension of the discovery of the sensitivity of the stomata to small changes of carbon dioxide tension⁸. In the further investigation of the response of wheat stomata to carbon dioxide, experiments carried out by Dr. F. L. Milthorpe in this Institute have shown that although there is very marked stomatal opening with reduction of carbon dioxide in the air supply from 0.03 per cent to 0.01 per cent, from 0.01 per cent to zero there is no evidence of any further opening. It appears likely, therefore, that the stomata respond only down to the minimum carbon dioxide content that normally occurs in the sub-stomatal cavities. Any response below this level would, of course, be of no biological advantage to the plant.

Detailed publication of the investigations mentioned will follow in due course, and further work along these lines is projected.

¹ Gabrielsen, E. K., *Nature*, **161**, 138 (1948).

² Gabrielsen, E. K., *Nature*, **163**, 359 (1949).

³ Audus, L. J., *Ann. Bot.*, **11**, 165 (1947).

⁴ Blackman, F. F., *Phil. Trans. Roy. Soc.*, B **186**, 503 (1895).

⁵ Maskell, E. J., *Proc. Roy. Soc.*, B, **102**, 467 (1928).

⁶ Heath, O. V. S., *Ann. Bot.*, **3**, 469 (1939).

⁷ Gregory, F. G., and Pearse, H. L., *Proc. Roy. Soc.*, B, **114**, 477 (1934).

⁸ Heath, O. V. S., *Ann. Bot.*, **5**, 455 (1941).

⁹ Heath, O. V. S., *Nature*, **161**, 178 (1948).

AMINO-ACIDS AND PROTEIN HYDROLYSATES

THERE was a large and attentive audience, including participants from the Continent of Europe, at each session of the recent symposium upon 'Amino-Acids and Protein Hydrolysates' organised by the Food Group of the Society of Chemical Industry. The symposium consisted of six paper-reading sessions held in the William Beveridge Hall at the Senate House, University of London, and an exhibition of relevant equipment and techniques presented in the Physical Chemical Laboratory,