INHIBITORY EFFECT OF WATER ON OXYGEN CONSUMPTION BY PLANT MATERIALS [1, 2, 3]

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As early as 1891 Devaux (4) showed that wetting the surface of potato tubers markedly decreased the diffusion rate of oxygen through the tissue. If the oxygen uptake rate was sufficiently high, wetting significantly decreased the rate of oxygen consumption.

Ruhland and Ramshorn (10), Kandler (8), James (7), and Allerup (1) have subsequently observed the same or similar effects of water on respiration of various plant tissues. The significance of the water inhibition, however, has apparently drawn little attention, since most of the data on respiration in current literature were obtained with tissues either immersed in or floating on aqueous solutions.

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In this report some characteristics of the inhibitory effect of water on oxygen consumption of corn scutellum and other plant tissues are described and their significance is discussed.

MATERIALS AND METHODS

Freshly harvested maturing soybeans (*Glycine max* (L.) Merr., var. Roanoke); germinating soybeans (var. Clark); corn (*Zea mays* L., single-cross hybrid WF9 × M14); barley (*Hordeum vulgare* L., var. Black Hull-less); oats (*Avena sativa* L., var. California Red); potato tubers (*Solanum tuberosum* L.), and carrot roots (*Daucus carota* L., var. sativa, DC.) were used. Seeds were germinated and allowed to grow four to seven days in the dark at 22° C, after which various tissues were excised for use.

Tissues were prepared as follows: individual scutella were cut into four longitudinal pieces, sliced to thicknesses of 0.25, 0.50, and 1.00 mm, or used intact. All pieces in a group were pooled, and eight to ten pieces (about 100 mg, fresh weight) were used

TABLE I

EFFECT OF WATER ON OXYGEN UPTAKE OF VARIOUS PLANT TISSUES

MATERIALS	OXYGEN UPTAKE ($\mu l\ O_2$/HR/G)		
	WITHOUT WATER AIR	WITH WATER AIR	WITH WATER O_2
Immature soybean seeds			
Intact	340	120	370
2 mm Slice	625	250	625
Germinating soybeans (*4 days*)			
Intact cotyledon	900	600	850
Root	460	330	470
Hypocotyl	400	385	430
Cotyledon*	127	113	190
Germinating corn			
Whole scutellum	1,900	680	2,000
Shoot	1,000	630	930
Root	570	360	580
Corn coleoptile	290	250	310
Barley coleoptile	280	160	255
Oat coleoptile	420	350	375
Carrot root	190	160	170
Carrot root previously washed with water	115	140	160
Potato tubers	51	41	46

* Seeds were soaked in water for 14 hrs at 4° C.

for each measurement. Immature soybean seeds of about 75 mg dry weight (about half the total dry weight at maturity) were used intact immediately after harvesting or were sliced to 2 mm thickness; intact seeds were also used after a storage period of one month at 4° C. Roots, hypocotyls, shoots, and coleoptiles were cut to 3 mm lengths, pooled, and weighed into the vessels. Carrot and potato tissues were cut to discs 7 mm in diameter by 1 mm thick. A quantity of tissue sufficient to absorb more than 100 μl of oxygen per hour was used in each vessel except that in experiments with potato slices 500 mg were used. Materials were not washed. Oxygen uptake measurements were started as rapidly as possible.

Respiratory gas exchange was measured in 15 ml Warburg flasks. Temperature was 29° C except as noted otherwise. Vessels were shaken horizontally with an amplitude of about 4 cm at a rate of 115 strokes per minute. For oxygen measurements, the center well contained 2 × 2 cm pieces of filter paper wetted with 0.2 ml of 4 N KOH. Carbon dioxide output was measured by the indirect method (12), correcting for retention of CO_2 as follows: two vessels without KOH each contained 0.5 ml of 5 % trichloracetic acid in the side arms; the acid of one vessel was tipped into the main chamber at the start of the measurement and that of the other at the end. A third vessel containing KOH was used for measurement of oxygen uptake. Except as stated otherwise, 2.0 ml of water was used in the main chamber for experiments with water. For without water experiments, 0.3 ml of water was placed in the side arm to maintain high humidity. The gas phase was air except when replaced with oxygen as indicated. The oxygen environment was established by flushing each vessel with 0.5 l tank oxygen. Since the duration of respiration measurements was less than 60 min, influence of contaminating bacteria was considered negligible.

Results and Discussion

The results of survey experiments on the inhibitory effect of water on tissues of several species are summarized in table I. A decrease of oxygen uptake upon adding water was of common occurrence. The magnitude of the inhibition differed between plant species and also between tissues of a given species. In all cases, however, the decreased activity due to addition of water was fully or partially recovered upon replacing air with oxygen.

Because of the magnitude of water effect on corn scutellum, this tissue was selected for subsequent experiments.

Table II

Effect of Thickness of Corn Scutellum on Oxygen Uptake*

Thickness (mm)	Oxygen uptake (ml O_2/hr/g)			
	With water		Without water	
	Air	Oxygen	Air	Oxygen
0.25	1.22	1.38	1.20	1.26
0.50	1.26	1.98	1.86	1.82
1.00	1.06	1.88	1.90	1.78
Quartered longitudinally	0.98	1.81	1.98	1.86

* Scutella from 6-day-old corn seedlings were used.

The effect of tissue thickness on water inhibition is shown by the data of table II. As thickness was increased, so was the magnitude of the inhibition. Except for the 0.25 mm slices, oxygen uptake in the absence of water was virtually independent of tissue thickness. Substitution of oxygen for air in the gas phase did not accelerate oxygen uptake in the absence of water at any tissue thickness. This indicated that in the absence of water oxygen, uptake was not limited by oxygen supply to respiratory sites.

Respiration of 0.25 mm slices was always less than that of thicker material. Evidently it is limited by some factor other than oxygen diffusion. For instance, in slices of this thickness there would be a greater ratio of damaged to intact cells than in thicker slices.

The temperature coefficient of oxygen consumption over the range 15 to 36° C is shown in table III.

Table III

Temperature Coefficient of Oxygen Uptake by Corn Scutella*

Temperature °C	With water				Without water			
	Air		O_2		Air		O_2	
	O_2 Uptake	Q_{10}	O_2 Uptake	Q_{10}	O_2 Uptake	Q_{10}	O_2 Uptake	Q_{10}
15	0.60		0.76		0.68		0.70	
		1.52		2.30		2.57		2.44
22	0.80		1.36		1.32		1.30	
		1.25		1.86		1.84		1.65
29	0.94		2.09		2.02		1.85	
		1.29		1.68		1.62		1.80
36	1.12		3.00		2.82		2.80	

* 5-day-old corn scutella, quartered longitudinally. Oxygen uptake is expressed as ml/hr/g fresh weight.

At 15° C the oxygen uptake rates under all four conditions were similar. As the temperature was increased, the rate in the presence of water in air increased more slowly than those under the other three conditions. Thus, in water at 29° C the rate was about half that in air (0.94 vs 2.02). The temperature coefficient of oxygen uptake of scutellum tissue in the presence of water in air (1.3 to 1.5, table III) is similar to that of oxygen diffusion in water (7), and is less than those typical of enzymatically catalyzed reactions (6). Thus, in the presence of water in air, the rate-limiting process in oxygen uptake is probably the diffusion of oxygen. Under the other three experimental conditions of table III, temperature coefficients were sufficiently high to indicate that the rate-determining steps were enzymatically catalyzed reactions.

The results presented in tables I, II, and III suggest that water inhibition is caused by a decrease in the oxygen diffusion rate. This may be due to the water surrounding the scutellum tissue or to internal water. The shaking rate was sufficient to provide oxygen up to 400 μl/hr without restriction due to the diffusion of oxygen through the external water (12). It is improbable, therefore, that the inhibition is due to external water, and the results shown in table IV exclude this possibility. The addition of even minute amounts of water decreased the oxygen uptake markedly. Nearly 50 % inhibition was obtained from addition to each vessel of 0.3 ml of water, an amount far less than that required to cover the tissue. The decreased rate of oxygen diffusion is therefore attributed mainly to conditions within the tissue itself. When scutellum tissue was soaked in water and then blotted dry, the rate of oxygen uptake was diminished.

TABLE IV

EFFECT OF ADDITION OF WATER AND PREVIOUS SOAKING ON RESPIRATION OF CORN SCUTELLA*

TREATMENT	OXYGEN UPTAKE-%
No water added	98 %
0.1 ml Water added	68
0.3 ml Water added	56
0.6 ml Water added	54
1.3 ml Water added	40
3.0 ml Water added	37
10 Min soaking period	80
30 Min soaking period	84
180 Min soaking period	65

* Each Warburg vessel contained about 100 mg of 4-day-old corn scutella quartered longitudinally. Oxygen uptake in the absence of water in air, previously measured for 30 minutes, was 2.26 ± 0.14 ml O_2/hr/g. This value was taken as 100 %. Subsequently, water indicated in the table was added and oxygen uptake was again measured. Scutella in other flasks were removed and soaked in 50 ml of distilled water per 100 mg of tissue for indicated periods. Oxygen uptake in the absence of water in air was measured again after blotting to remove surface water. Because of the gradual increase of rate with time, readings for only the first 30 minutes were taken.

TABLE V

EFFECT OF OXYGEN SUPPLY ON RESPIRATORY QUOTIENT OF CORN SCUTELLUM

EXPT NO.		WITH WATER		WITHOUT WATER	
		AIR	O_2	AIR	O_2
1*	O_2 uptake (ml/hr/g)	1.04	1.91	1.99	1.98
	CO_2 output (ml/hr/g)	0.97	1.24	1.18	1.00
	Respiratory quotient	0.93	0.65	0.65	0.58
2*	O_2 uptake (ml/hr/g)	1.20	2.23	2.49	2.45
	CO_2 output (ml/hr/g)	1.48	1.51	1.56	1.51
	Respiratory quotient	1.23	0.68	0.63	0.60

* 6- and 5-day-old corn scutella quartered longitudinally were used in expts. 1 and 2, respectively.

However, the activity of such tissue gradually increased to the original level so that even during the first 30 min, there is less inhibition than occurs in the presence of small amounts of water.

Table V shows the marked effect of water addition on the respiratory quotient of scutellum tissue. CO_2 production varied little under the four conditions, in contrast to the decrease in oxygen uptake in the presence of water. Consequently, the respiratory quotient was increased by adding water. This result is similar to those of Ruhland and Ramshorn (10), and Kandler (8), but does not confirm that of Steward, et al (11).

In a preliminary experiment no accelerating effect of 2,4-dinitrophenol (DNP) on the oxygen uptake of intact or sliced corn scutellum in the presence of water in air was observed. The data in table VI, however, indicate that oxygen uptake by slices or longitudinal quarters is accelerated in an oxygen atmosphere by DNP concentrations up to 3.5×10^{-5} M. This result also supports the view that in the presence of water, oxygen diffusion through the tissue is the process determining the rate of oxygen uptake. The fact that greater acceleration was ob-

TABLE VI

EFFECT OF 2,4-DINITROPHENOL (DNP) ON OXYGEN UPTAKE BY CORN SCUTELLUM

DNP FINAL CONCENTRATION	RELATIVE OXYGEN UPTAKE BY SCUTELLA CUT IN		
	LONGITUDINAL QUARTERS	1.00 MM SLICES	0.50 MM SLICES
0	100 %	100 %	100 %
10^{-6} M	104	99	110
3.5×10^{-6} M	...	...	112
10^{-5} M	114	124	129
3.5×10^{-5} M	123	145	142

Each reaction mixture contained 0.03 M KH_2PO_4, pH 5.0. Gas phase was oxygen. Scutella were from 6- and 7-day-old seedlings. Data are expressed as the percentages of activities in the absence of DNP.

tained with 1.00 and 0.50 mm slices than with longitudinal quarters suggests that, even in an oxygen atmosphere, the oxygen diffusion rate was not sufficient to fulfill the elevated requirement for oxygen in the presence of DNP.

The present study shows that the inhibitory action of added water is caused by a decrease in the oxygen diffusion rate within the tissue itself. The diffusion rate is much higher in the absence of water than in its presence. The presence of an intercellular gas phase in many plant tissues (5) and its contribution to the high oxygen diffusion coefficients of potato tubers (2) and carrot roots (3) has already been demonstrated. It is therefore assumed that water lodged along the diffusion path decreased the oxygen diffusion rate in these tissues. That the water inhibition is not simply a surface phenomenon is indicated by the decrease in inhibition which accompanied a decrease in tissue thickness. If inhibition resulted from a surface film of water, it should be largely independent of tissue thickness.

The magnitude of the water inhibition may also vary with age of the tissue. Results in figure 1 show how the oxygen uptake of soybean cotyledons changes during germination.

In a preliminary experiment it was found that 0.25, 0.50, and 1.00 mm cotyledon slices had similar oxygen uptake rates in water with oxygen as the gas phase. It was therefore assumed that under the conditions of curve II (fig 1) oxygen supply was sufficient to support maximum oxygen uptake. Respiration may have been limited by some other factor such as phosphate acceptor supply (9). The values of curve I were therefore considered to approximate more nearly the real oxygen uptake capacity of the tissue. The optimal concentration of DNP for maximum activation was somewhat different for tissues of different ages. For 1- to 3-day-old tissue, the optimal concentration of DNP was 10^{-4} M, whereas for 4-day-old and older tissue it was 3.5×10^{-5} M. Data in figure 1 were obtained with concentrations giving maximum activation at each age.

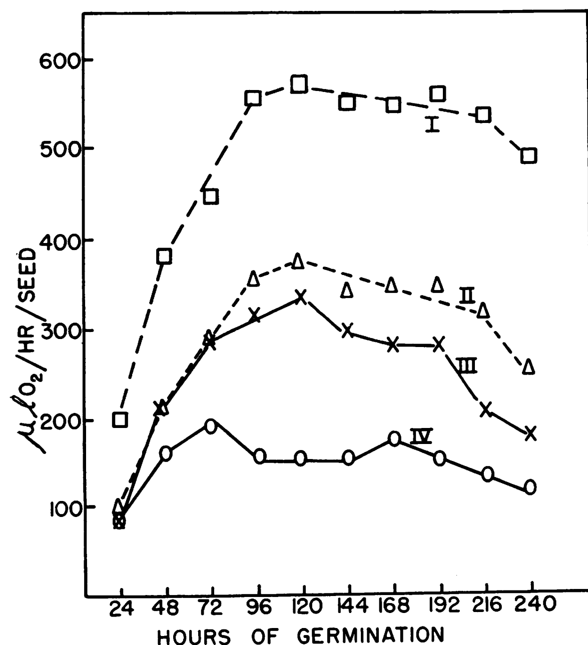

FIG. 1. Oxygen uptake of soybean cotyledons under various conditions. I. 0.50 mm slices in DNP (1st 3 days 10^{-4} M, 4th day and subsequently 3.5×10^{-5} M), gas phase oxygen; II. 0.50 mm slices in water, gas phase oxygen; III. whole cotyledons in absence of water, gas phase air; IV. whole cotyledons in water, gas phase air.

The oxygen uptake of whole cotyledons in air and in the absence of water (curve III) is considered to approximate the actual rate of oxygen uptake of the intact tissue. However, the temperature difference between measurements (29° C) and germination (22° C) and injuries caused by removal of hypocotyls may make observed values somewhat different from the actual ones. Conditions for curve IV were the same as for curve III except for the presence of water. The difference between curve IV and curve II shows the magnitude of diffusion inhibition in the presence of water.

The respiratory capacity of the cotyledon (curve I) increases rapidly following germination and continues at a high level at least through the tenth day. On the other hand, respiration in air and in the absence of DNP and water (curve III) reaches its maximum on the fifth day. It then declines faster than under the conditions of curve I. During the first three days, diffusion through the tissue can supply oxygen just sufficient to fulfill the decreased requirement for oxygen which exists in the absence of DNP (curves II and III vs. curve I). After the third day, diffusion is too slow to fulfill even this requirement. At least 35 % of the respiratory capacity is not in use throughout the whole ten day period (curve I vs. curve III) because of an insufficient supply of phosphate acceptors and the lack of oxygen after the third day.

SUMMARY

Adding water to corn, soybean, and barley tissues resulted in marked decreases of oxygen uptake. Carrot, oat, and potato tissues were affected similarly but to a smaller degree. Substitution of oxygen for air in the gas phase partially or completely reversed the effect of added water. The inhibitory action of water on oxygen uptake of corn scutellum was attributed to the decreased rate of oxygen diffusion through the tissues for the following reasons: I. the inhibition was removed by replacing air with oxygen; II. the inhibitory effect decreased with decreasing thickness down to a tissue thickness of 0.50 mm; III. in the presence of water the uptake of oxygen had a low temperature coefficient similar to that of oxygen diffusion through water; IV. minute amounts of water and previous soakings had inhibitory effects.

The oxygen uptake of intact soybean cotyledons during germination is rate-limited by the lack of

sufficient phosphate acceptors and by the rate of oxygen diffusion.

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PHOTOSYNTHESIS BY ISOLATED CHLOROPLASTS IX. PHOTOSYNTHETIC PHOSPHORYLATION AND CO_2 ASSIMILATION IN DIFFERENT SPECIES [1]

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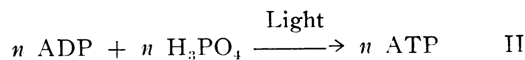
Understanding the role of chloroplasts in photosynthesis has been greatly increased during the past five years by experiments with chloroplasts isolated from the leaves of one species, spinach (*Spinacia oleracea* Linn.). Previously, the only experimentally documented photochemical activity of chloroplasts isolated from several species, including spinach, was the Hill reaction (18) in which illuminated chloroplasts evolve oxygen in accordance with equation I, where

$$A + H_2O \xrightarrow{\text{Light}} AH_2 + 1/2\ O_2 \qquad \text{I}$$

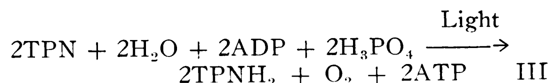
A represents a non-physiological electron or hydrogen acceptor such as ferricyanide or benzoquinone (27).

The new work with spinach chloroplasts has provided direct experimental evidence for the capacity of isolated chloroplasts to assimilate CO_2 photosynthetically to the level of starch and sugars (4, 5, 1, 17, 25). Previously, the view that photosynthetic CO_2 assimilation in green plants is, like oxygen evolution, localized in chloroplasts, was at first asserted without the support of critical experimental evidence (22, 23) and was later abandoned because of evidence to the contrary (18, 15, 14, 21, 6).

By fractionating spinach chloroplasts, CO_2 assimilation proper was shown to be a dark process (24), but one dependent on assimilatory power, i.e., $TPNH_2$ and ATP[4], formed by two light reactions (11, 13), cyclic (equation II) and non-cyclic photophosphorylation (equation III).



$$n\ ADP + n\ H_3PO_4 \xrightarrow{\text{Light}} n\ ATP \qquad \text{II}$$



$$2TPN + 2H_2O + 2ADP + 2H_3PO_4 \xrightarrow{\text{Light}} 2TPNH_2 + O_2 + 2ATP \qquad \text{III}$$

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[4] The following abbreviations will be used: TPN, $TPNH_2$, oxidized and reduced forms of triphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; P, *ortho*phosphate; FMN, riboflavin phosphate; Tris, Tris(hydroxymethyl)-aminomethane buffer, neutralized with HCl.