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The Relation of Photosynthesis to Respiration

J.W. Weigl, P.M. Warrington, and M. Calvin

July 20, 1950

Berkeley, California

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ABSTRACT

The Relation of Photosynthesis to Respiration[1]

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ABSTRACT

July 20, 1950

The gas exchange by barley leaves of oxygen, carbon dioxide, and added radiocarbon dioxide has been measured in a closed system, with the following results:

1. Carbon dioxide follows different but not necessarily independent paths in photosynthesis and light respiration.

2. The carbon of newly formed photosynthetic intermediates is not available for respiration while the light is on, but becomes immediately respirable in the dark. The enhancement of dark respiration after a light period is largely due to built-up "photosynthates".

3. Photosynthesis proceeds at a measureable rate even at the lowest CO_2 pressures observed (0.03 mm. Hg). There is no evidence for a "threshold" concentration of carbon dioxide for the reaction; at the lowest concentrations reached, respiration exactly equals assimilation.

4. The mean rate of respiratory CO_2 evolution in strong light was found to be less than that in the dark. Internal re-photosynthesis of respiratory

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1 The work described in this paper was sponsored by the Atomic Energy Commission.

2 Present address: School of Chemistry, University of Minnesota, Minneapolis, Minnesota

3 Present address: Bechtel Corporation, San Francisco, California

carbon may have been sufficient to account for this effect.

5. The assimilation of $C^{14}O_2$ is about 17% slower than that of $C^{12}O_2$.

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The Relation of Photosynthesis to Respiration[1]

J.W. Weigl[2], P.M. Warrington[3] and M. Calvin

Radiation Laboratory and Department of Chemistry, University of California

Berkeley, California

July 20, 1950

The relation between photosynthesis and respiration in green plants is, to date, inadequately understood. Until recently, it was not even certain whether, in the light, there occurs any respiratory evolution of carbon dioxide simultaneously with the assimilation of carbon dioxide from the air, or whether, perhaps, the path of carbon in photosynthesis is merely the reverse of that in respiration. The reason for this uncertainty is that the net overall reactions which may be written for these two processes are opposite:

Photosynthesis: $CO_2 + H_2O \longrightarrow (CH_2O) + O_2$

Respiration: $O_2 + (CH_2O) \longrightarrow H_2O + CO_2$

where (CH_2O) represents carbohydrates, which are typical photosynthetic products and respiratory substrates.

Various attempts have been made by a number of investigators to distinguish between these simultaneous and opposite reactions, and to

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measure the rate of respiration in the light. These have involved the "selective" poisoning of one of the reactions (4,5,6), the inhibition of photosynthesis alone by removal of the carbon dioxide supply (7,8,9), the variation of temperature (10), of light intensity and color (11,12,13,14)

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4 Gaffron, H. Cold Spring Harbor Symp. Quant. Biol. 7, 377 (1939) Biochem. Z 292, 24 (1937)

5 Myers, J. and Burr, G.O., J.Gen.Physiol. 24, 45 (1940)

6 Föckler, H., Jahrb.wiss.Botan. 85, 267 (1938)

7 McAlister, E.D. and Myers, J., Smithson.Miscell.Coll. 99, No.6 (1940)

8 Gabrielsen, E.K., Nature 163, 359 (1949)

9 Warburg, O., Burk, D., Schocken, V., Korzenovsky, M. and Hendricks, S.B. Arch.Biochem. 23, 330 (1949)

10 Noddack, W. and Kopp, C., Z.physik.Chem. (A), 187, 79 (1940)

11 Emerson, R. and Lewis, C.M., Am.J.Bot. 30, 165 (1943)

12 Moore, W.E. and Duggar, B.M. in PHOTOSYNTHESIS IN PLANTS (ed. by Franck, J. and Loomis, W.E.), Iowa State Col. Press. Ames, 1949 (Chapter 11).

13 Kok, B. Enzymologia XIII, 1 (1947). Biochimica et Biophysica Acta, 3, 625 (1949)

14 van der Veen, R. Physiol. Plant. 2, 217 (1949)

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and finally, a study of respiration rates in the dark, subsequent to periods of illumination.

In general, the poisoning and low-carbon dioxide experiments yielded equal values for respiration in light and darkness. Kok (13) and van der Veen (14), on the other hand, observed a sharp decrease in the slope of the curve of gas exchange vs light intensity, not far above the compensation point; unfortunately, this could be interpreted either as a light-caused decrease in respiratory rate at low light intensities, or else as an enhancement at higher illuminations.

Extensive studies of dark respiration as a function of pre-treatment have revealed two effects, both of which often increase dark respiration after exposure of plants to the light: first, a direct stimulation, mainly due to violet and ultraviolet light absorbed by pigments other than chlorophyll (11,15,16,17); second, a mass action enhancement, due to the availability of recently assimilated products for respiration. The latter effect, first found by Borodin (18) was usually found to be strongest after active photosynthesis in plenty of carbon dioxide -- that is, under conditions where new respirable compounds have been rapidly synthesized (19,20,21).

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15 Parija, P. and Saran, A.B., Am.Bot. 48, 347 (1934)

16 Montfort, C. and Föckler, H., Planta 28, 515 (1938)

17 Gessner, F., Planta 29, 165 (1939)

18 Borodin, I., Mem.Acad.Imp.Sci.(St.Petersburg). VIII, 28, 1 (1881)

19 Weintraub, R.L., Botan.Rev. 10, 383 (1944)

20 Rabinowitch, E.I., PHOTOSYNTHESIS, Vol.1, Interscience Publ., New York, 1945 (Chapter 20).

21 Mothes, K., Baatz, I., and Sagronsky, H., Planta 30, 289 (1939)

None of these investigations have conclusively answered the following fundamental questions:

1. Are the enzymatic paths of carbon in photosynthesis and respiration closely linked? For instance, are recently photosynthesized compounds available for respiration while the light is on?

2. Assuming the processes to be independent, what is the rate of respiration during the photosynthesis of normal plants?

The availability of tracer carbon-14 has made it possible, at least in principle, to answer these questions. One way to do this is to allow leaves to photosynthesize in radioactive carbon dioxide in a closed system and to follow continuously, by means of non-destructive methods of analysis, the concentrations of radioactive and inactive carbon dioxide in the gas phase. If simultaneous photosynthesis and respiration are different reactions, at least the initial respiratory carbon dioxide will be inactive; the rate of reduction of the original specific activity of the radioactive carbon dioxide supplied should be a quantitative measure of the rate of respiration.

Since in these experiments we have been mainly concerned with the exchange of carbon dioxide in the gas phase, we have used the following terminology:

Photosynthesis: - Assimilation of carbon dioxide from the gas.

Respiration: - Evolution of carbon dioxide into the gas.

These definitions imply that even in strong light all respired carbon leaves the cells as carbon dioxide, is mixed with the entire gas

phase, and can only then be re-assimilated. Hence, the quantitative evaluation of the rate of respiration in the light will yield, not the total rate, but only that fraction of it which actually appears in the atmosphere as carbon dioxide.

EXPERIMENTAL

General. -- Fig. 1 shows the apparatus used. At the beginning of a run, the green leaves of one to two week old barley shoots (22) were cut, moistened well, and placed into a flat glass chamber measuring 46x13x1.5 cm. (23). This was closed and darkened, the entire system evacuated and filled with the desired gas mixture (containing radioactive carbon dioxide) to about 500 mm. pressure, the partial vacuum being necessary to hold the chamber together. A rubber tubing pump (24) took a continuous sample of the gas in the chamber and recycled it through a series of three instruments: an ionization chamber to measure radioactive carbon dioxide; an infrared carbon dioxide analyzer; and a paramagnetic - type oxygen analyzer. Within less than a minute the sample was returned to the plant chamber, the flow rate being over 500 cc/min. After two to five

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22 Variety Sacramento, kindly supplied by the Division of Plant Nutrition, University of California

23 Aronoff, S., Benson, A., Hassid, W.Z. and Calvin M., Science 105, 664 (1947)

24 Weigl, J.W. and Stallings, D.M. Rev.Scient.Inst., 21, 395 (1950

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minutes required for the initial mixing, all instrument readings became steady and meaningful. Time lags between the individual instruments were carefully checked and found to be less than half a minute; the results obtained were furthermore shown to be independent of the sequence of instruments in the circuit.

The components of the system were connected by about three meters of 5 mm. i.d. Tygon tubing. The rate of carbon dioxide diffusion through this and through the rubber tubing pump was measured and found to be about 0.38 ml. carbon dioxide (S.T.P.)/hr/meter of tubing/atmosphere of carbon dioxide. In our experiments this amounted to about 0.1% of a typical rate of photosynthesis and was hence negligible. A 25 cm. x 1 cm. tube filled with calcium chloride and calcium sulfate was used to dry the gas on its way to the instruments.

A plant chamber was immersed in a tank of cooling water, whole temperature remained constant within $\pm$ 1°; infrared filters were placed in the 5 cm. of water covering the chamber. A bank of spotlights provided between 7,000 and 14,000 f.c. from above; a sheet of aluminum foil reflected some of this light from underneath.

Determination of Radioactivity. -- Radioactive carbon dioxide was measured continuously by means of an ionization chamber and a Lindemann-Ryerson electrometer, the latter being used as a null instrument. The circuit of Janney and Moyer (25) was modified (26) so as to yield the

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25 Janney, C.D. and Moyer, B.J. Rev.Sci.Inst. 19, 667 (1948)

26 We are indebted to Dr. C.D. Janney for much valuable aid and advice.

instantaneous value of the ionization current as the product of a decade resistance (27) and of a current which could be recorded continuously on the chart of an Esterline-Angus milliammeter. Thus, radioactivity was measured in "millivolts". By its nature, the circuit was linear, and subject to the same per cent error over a range equal to ten thousand times the full scale of the ammeter. The sensitivity of the apparatus was limited by that of the electrometer (about 1000 div./volt), background being well below this level.

A large (100 cc.) and a small (10 cc.) ionization chamber were used in different experiments. Each was shown to respond linearly to increments of radioactive carbon dioxide at constant total pressure (25,28); the time required for 90% response to a sudden large change in radioactive carbon dioxide contents was about six seconds, respectively thirty seconds "Memory" effects due to the adsorption of radioactive carbon dioxide on the brass walls were shown to be negligible ($< .05\%$) after five minutes or less. Troublesome "leakage currents" (giving large apparent "background" readings) were eliminated by the use of Teflon insulators.

The linearity of both chambers and the electrometer, as well as "memory effects" were critically checked in a special experiment. A photosynthetic run was started, using the 100 cc. chamber on the Lindemann

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27 Leeds and Northrup decade resistance box, 1-999 ohms; for very large ionization currents, 10,000 ohm precision wire-wound resistors were added in series.

28 Weigl, J.W., Unpublished data

electrometer; after 99% of the radioactivity had been used by the plants, the small chamber, mounted on a sensitive vibrating reed electrometer (29,30) was cut into the circuit. After this, both ionization chambers and electrometers operated in tandem for three hours over a 20-fold range of radioactivity. Their readings were strictly proportional. Unless all four pieces of apparatus had compensating errors, this showed all to have linear response in the range used in our kinetic experiments and furthermore, demonstrated that the large chamber "remembered" less than 0.05% of its initial radioactivity.

Carbon Dioxide Analyzer. -- A selective-detector infrared gas analyzer of the general type developed by Luft (31) and modified by Eltenton, Pompeo and Smith (32) was used to measure carbon dioxide (33). Infrared radiation was passed through the sample cell into a detector chamber filled with pure carbon dioxide. Carbon dioxide in the "unknown" cell took out a certain amount of the radiation corresponding to its characteristic spectrum (mainly at 4.3 μ, some at 2.7 μ); whatever was left of the light of these wave lengths was completely absorbed by the carbon dioxide in the detector, which, as a result was warmed and expanded slightly. When the light was shut off, the gas cooled and contracted. The radiation was pulsed at 120 cycles

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29 Manufactured by the Applied Physics Corporation, Pasadena, California.

30 Palevsky, H., Swank, R.K. and Grenchik, R., Rev.Sci.Inst. 18, 298 (1947)

31 Luft, K.F., Z. für Physik, 24, 97 (1943)

32 Pompeo, D.J. and Smith V., Report at the Gordon Research Conference of the A.A.A.S, August 1949.

33 Drs. Otto Beeck and D.J. Pompeo of the Shell Development Co., Emeryville, California, were kind enough to let us copy an early model of their instrument; this was further modified to improve its sensitivity.

and set up a 120-cycle acoustical signal in the detector, whose amplitude was a measure of the amount of carbon dioxide in the sample cell, and which could be picked up by means of a condenser microphone. A similar optical and acoustical train, whose sample cell was filled with nitrogen, provided a standard signal. The two were balanced continuously on a Brown Electronik recording potentiometer.

The instrument was calibrated by means of previously analyzed mixtures of carbon dioxide and nitrogen. Two tanks were used: one of them contained nitrogen, the other a nitrogen-carbon dioxide mixture corresponding to the full-scale percentage of carbon dioxide desired (4.0% respectively 0.75% carbon dioxide). Parallel streams of these gases were measured by calibrated rotameters (34) and flushed through the sample cell. The gas stream was now partially exhausted to about 100 mm., 200 mm., and 300 mm. vacuum (measured to $\pm$ 1 mm. Hg by a differential manometer) and readings were taken at each pressure. The whole procedure was repeated for six or seven other gas mixtures. In order to eliminate possible systematic errors due to the flowmeter technique, a few mixtures were prepared by mixing gases to known partial pressures, in large, exhausted flasks.

Within experimental error, check points obtained in this way fell on the curves plotted from the flowmeter data. The response time of the instrument was about fifteen seconds, the time required to sweep out the 80 cc. volume with new gases. Readings were not affected by the rate of

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34 Manufactured by Fischer and Porter Co., Hatboro, Pa.; calibrations were re-checked periodically.

gas flow.

Fig. 2 shows calibration curves valid at atmospheric pressure and at 255 mm. (10 in.) Hg vacuum. The strong pressure broadening effect can be related to perturbations of carbon dioxide molecular vibrations by inert molecules (35). The curves were valid within ± 1 division below 2 mm. and, at most, ± 2 divisions above 2 mm. partial pressure. Calibrations were stable over periods of a week or more; they were rechecked before each major photosynthetic experiment.

Water vapor has an absorption band near 2.6 μ which overlaps the carbon dioxide spectrum by some 4%, and it is hence "seen" by the carbon dioxide in the detector cells to that extent. The calcium chloride-dried gas, however, contained only 0.3 mm. Hg of water, which was seen as a maximum of 0.01 mm. of carbon dioxide by the gas analyzer; this figure was just within experimental error in our most sensitive experiments. It is a curious fact (36) that the infrared spectra of $C^{12}O_2$ and $C^{14}O_2$ overlap only very slightly, if at all; as a result, the CO_2-analyzer measured only "inactive" and not "total" carbon dioxide. Drift and similar phenomena associated with the CO_2- analyzer were the main experimental difficulties encountered in this investigation.

Oxygen Analyzer. -- The Pauling Meter (37) consists of a small mag-

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35 Cross, P.C. and Daniels, F., J.Chem.Phys. 2, 6 (1934)

36 Sheline, R.K., and Weigl, J.W., J.Chem.Phys. 17, 747 (1949)

37 Pauling, L., Wood, R.E. and Sturdivant, J.H., J.Am.Chem.Soc. 68, 795 (1946)

netic torsion balance, whose position depends on the volume magnetic susceptibility of the gas in its sample cell. Since oxygen is the only common paramagnetic gas, the instrument can be calibrated directly in terms of partial pressure of oxygen. The particular instrument used in certain of our experiments had a fairly linear range from 0 to 100 mm. Hg and could be read to ± 0.2 mm. Hg (38).

Results. -- Experiments were started in the dark in order to have a minimum of change occurring during the initial mixing of gases. After this, light and dark periods were alternated as desired. Oxygen pressures were read directly; radioactive and inactive CO_2 readings were automatically recorded; they were read off the charts after each experiment and converted to "millivolts of radioactivity", respectively partial pressure of carbon dioxide. For convenience, the relative specific activity (39) at the start of the experiment was set arbitrarily equal to unity; this enabled us to calculate and plot "partial pressure of $C^{14}O_2$" to the same scale as the inactive $C^{12}O_2$. For certain calculations it was necessary to use the absolute specific activity of the CO_2; this was obtained by multiplying the initial fraction of isotope (1.1% in Barley 14, 4.75% in Barley 28) by the relative specific activity.

Although a number of similar experiments was performed, the behavior of the plants was most clearly shown in Barley 14 and 28. Others suffered

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38 Calibrated by the manufacturer, Arnold O. Beckman Co., Pasadena, Calif.

39 Relative specific activity $\equiv C^{14}O_2 / (C^{14}O_2 + C^{12}O_2)$ mm/mm.

TABLE I

Experiment	14-1	14-2	14-3	28-1	28-2
Barley age (days)	8	8	8	14	14
Fresh Weight	20	20	20	(15)	(15)
Pretreatment before light	L 24 h.	L 40 m.	L 15 m.	L 30 m.	L 135 m.
	D 40 m.	D 65 m.	D 70 m.	D 70 m.	D 80 m.
Initial P_{CO_2}, mm. Hg	17	3.6		4.2	1.16
P_{O_2}, mm. Hg	(10)	(23)		39.9	49.0
% $C^{14}O_2$ in CO_2	1.1	0.14		4.75	0.23
Vacuum, $\pm$ 5 mm. Hg	255	255		244	215 $\pm$ 13
Temperature, $\pm$ 1°C.	22	22		14.5	15.5
Illumination, f.c.	(7000)	(7000)		14,000	14,000

Experimental Conditions. -- Experiment 14-1, -2 and -3 refer to the three photosynthetic periods included in Barley 14; similarly for Barley 28-1 and -2. Under pretreatment, L = light, D = dark, h. = hours, m. = minutes.

TABLE II

Experiment	Time min.	P_{O_2}(av.) mm. Hg	Dark Respiration mm. CO_2/min. x 100	Pre-Treatment*
Barley 14	0-20	(10)	9.8	20 m. D
	60	(27)	10.6	40 m. L (15 m. SS)
	65		7.6	5 m. D
	70-85		6.6	10 m. D
	100	(24)	5.2	40 m. D
	110		4.4	50 m. D
	120		3.0	60 m. D
	140	(27)	9.6	15 m. L (5 m. SS)
	145		6.8	5 m. D
	150		6.4	10 m. D
	160-210	(26)	5.0	20 m. D
	225-310	(27)	6.0+	15 m. L (2m. SS)
Barley 28	0-20	40	1.45	50 m. D
	155-205	44-48	1.28	135 m. L (75 m. SS)
	230-235	49	1.91	75 m. D
	275-280	53	1.85	40 m. L (10 m. SS)
	280-300	53	1.68	5 m. D

Dark respiration. * L = light, D = dark, SS = steady state, at limiting CO_2 pressure. + obtained by sweeping out CO_2 and weighing barium carbonate.

TABLE III

Time	Event	CO_2 Analyzer (mm $C^{12}O_2$)	Ioniz. Chamber (mv. $C^{14}O_2$)	Specific Act. $C^{14}O_2/CO_2$ (mm/mm)
Barley 14				
0	Start of respiration before PS No. 1	17.0 ± 2%	1160 ± 1%	1.00 ± .03
20	Start of photosynthesis No. 1	18.8 ± 2%	1160 ± 1%	.90 ± .03
40	Specific activity peak No. 1	3.2 ± 3%	240 ± 1%	1.09 ± .04
48-60	Steady state No. 1	0.1 ± 50%	0.0 ± .05 mv	.00 ± .015
100	Middle of respiration after PS No. 1	2.7 ± 4%	20.4 ± 2%	.11 ± .006
125	Start of photosynthesis No. 2	3.6 ± 3%	30.0 ± 2%	.12 ± .006
133	Middle of photosynthesis No. 2	0.35 ± 20%	1.0 ± 20%	.042 ± .02
135-40	Steady state No. 2	0.1 ± 50%	0.0 ± .05 mv	.00± .015
185	Middle of respiration after PS No. 2	2.3 ± 4.5%	12.2 ± 5%	.078 ± .008
210	Start of photosynthesis No. 3	3.3 ± 3%	18.4 ± 3%	.082 ± .005
220	Middle of photosynthesis No. 3	0.45 ± 20%	1.4 ± 5%	.046 ± .01
224	Steady state No. 3	0.1 ± 50%	0.0 ± .05 mv	.00 ± .015
225-310	Average of respiration after PS No. 3	----	----	.066 ± .01+
Barley 28				
0	Start of respiration before PS No. 1	3.96 ± 2%	20,000 ± 1%	1.0 ± .03
20	Start of photosynthesis No. 1	4.20 ± 2%	20,000 ± 1%	.94 ± .03
37	Middle of photosynthesis No. 1	2.75 ± 2%	12,000 ± 1%	.865 ± .03
60	Specific activity peak No. 1	.27 ± 8%	1950 ± 1%	1.09 ± .10
63	Specific Activity peak No. 1	.22 ± 10%	1200 ± 1%	1.10 ± .11
80	Start of steady state No. 1	.03 ± 30%	47 ± 2%	32 ± .10
150	End of steady state No. 1	.03 ± 30%	9.5 ± 10%	.065 ± .025
200	Middle of respiration after PS No. 1	.60 ± 5%	148 ± 1%	.049 ± .003
235	Start of photosynthesis No. 2	1.16 ± 5%	248 ± 1%	.043 ± .003
250	Specific activity peak No. 2	.29 ± 7%	83 ± 1.5%	.056 ± .005
270	Steady state No. 2	.04 ± 25%	5.0 ± 10%	.025 ± .012
300	End of respiration after PS No. 2	.46 ± 4%	78.5 ± 1.5%	.034 ± .002

Precision of Measurements. +obtained by flushing out $C^{14}O_2$ with nitrogen, precipitating and counting as $BaC^{14}O_3$.

from various experimental difficulties; however, in no case did they contradict the conclusions drawn from these two runs. In view of the continuous data for $C^{14}O_2$ and $C^{12}O_2$ the only appreciable errors were systematic ones. In Table III maximum reasonable errors are estimated; these are approximately twice the "probable errors".

Discussion. -- Fig. 3 shows the changes in (CO_2) and $(C^{14}O_2)$ which took place in Barley 14. At time zero, a uniform gas mixture containing about 17 mm. CO_2 (1.1% C^{14}) and 10 mm. O_2 was present in the system. The plants respired inactive CO_2 in the dark, thus reducing the specific activity. When the light was turned on the specific activity first continued dropping due to induction effects, then rose to approximately 1.2 times its initial value, or 1.3 times the minimum which had occurred about time 25. The fact that this peak was some 10% higher even than the specific activity of the original $C^{14}O_2$ left no explanations other than an isotope effect (40,41). Finally, the continuous respiratory evolution of inactive CO_2 surpassed the photosynthetic isotope concentration and quickly reduced the specific activity to a very low value. Photosynthesis

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40 This isotope discrimination has been observed in six experiments of this type as well as in three sampling runs and one experiment in which algae were grown from $C^{12}O_2$ containing $C^{13}O_2$ and $C^{14}O_2$. After about 70% of this mixture had been assimilated, the algae were found to be about 75% depleted in C^{13} and 20% in C^{14}, as compared to the remaining CO_2. The effect has not been demonstrated, as yet, by direct sampling experiments using a mass spectrometer.

41 Weigl, J.W. and Calvin, M., J.Chem.Phys. 17, 210 (1949)

was CO_2-limited below about 1 mm. partial pressure and became exactly equal to respiration when the only carbon dioxide available for assimilation was provided by respiration. There was no evidence for a CO_2 "threshold" for photosynthesis (8,42).

After ten minutes, at this steady state, the lights were turned off. Both radioactive and inactive carbon dioxide were evolved immediately, and after five minutes, in a constant ratio; as a result, the specific activity of the gas rose, then remained constant over long periods of time. This means that in the dark recently assimilated radioactive compounds became immediately respirable in a constant ratio to the evolution of "dead" CO_2, whereas they were not respirable while the light was still on.

The light was now turned on again; at low CO_2 pressures the respiratory dilution overtook the isotope effect more quickly and the specific activity did not actually rise, but merely failed to drop immediately. Shortly after the steady state was reached, the lights were turned off once more and the specific activity found to rise again, to a slightly lower level than that observed after the first light period. As a check on the instruments, this procedure was repeated once more; this time, after the lights were turned off, the entire system was swept for 85 minutes with tank nitrogen (containing about 4 mm. of O_2) through a sodium hydroxide bubbler. The resultant carbonate was precipitated as barium carbonate and counted by means of a Geiger counter; when its specific activity was converted to ionization chamber units, it was found again to be slightly lower than the preceding

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42 Gabrielsen, E.K., Nature 161, 138 (1948)

level; the average rate of respiration was close to that of preceding dark periods (see Table II and III).

The steady level of specific activity in the dark was found to be roughly inversely proportional to the total light period from the time the first major assimilation of radio carbon dioxide took place. This merely signified that the photosynthetic intermediates were transformed into nonrespirable products much more quickly in the light than in the dark.

We have usually found that after a period of intense photosynthesis in the presence of plenty of CO_2 the dark respiration rate is enhanced by factors of two to three or more for periods varying from 10 - 200 minutes (for example, see Table II, Barley 14). On the other hand, in a couple of other experiments (e.g. Barley 28) this temporary rise did not appear; in these cases the plants had been kept in the light at the low, steady-state pressure of CO_2 for long periods of time.

One may interpret all these results in terms of the mass action effect first suggested by Borodin (18); the building-up of photosynthetic intermediates, which become respirable in the dark. If the plants are kept in the light with little CO_2 for long periods, these intermediates are transformed further into more stable structural and storage materials and are no longer readily available for the enhancement of respiration. This reasoning might lead one to expect no rise in the specific activity after such a long period of light and low carbon dioxide pressure. Barley 28 (Fig. 4) shows a striking case of this (43).

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43 Note, however, that in this experiment at time 275 the specific activity did rise as usual, after a short period at low CO_2 pressure.

Photosynthetic and respiratory quotients were computed in a number of experiments in which the Pauling meter was included among the instruments. Both quotients were quite variable, even within a given experiment; over long periods of time, they usually averaged about unity. A remarkably fast uptake of oxygen was usually observed for five to ten minutes after the light was turned off; this was about three to ten times as fast as the steady oxygen absorption rate later on and two to five times the initial enhancement of CO_2 evolution. The effect was increased by long periods at low CO_2 pressures; in view of this and of the high light intensities prevailing, this phenomenon may well have been evidence of photo-oxidation (20), which probably had been going on even faster in the light (44).

In Barley 28 alone, to date, has the precision of the data justified a detailed kinetic analysis to evaluate the rate of "light respiration". Unfortunately, the isotope effect introduces a third variable, in addition to the photosynthetic and respiratory rates (45). This makes an explicit solution impossible; however, one can choose a very sensitive function of these three parameters and try to fit it to the experimentally determined values. The function chosen was the time rate of change of specific activity, the expression for which is the same no matter how photosynthesis depends on CO_2-pressure (Michaelis type or mass action kinetics of any order). It could be

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44 This point might be worth checking by means of tracer oxygen.

45 This could be avoided by feeding inactive CO_2 to uniformly labeled plants.

derived from the following basic equations:

$$d/dt(C^{12}O_2) = R(1 - c) - k\,(C^{12}O_2)$$

$$d/dt(C^{14}O_2) = Rc - kU(C^{14}O_2)$$

where R is the rate of light respiration, k the rate constant for the assimilation of $C^{12}O_2$, U the isotope utilization factor (ratio of k for $C^{14}O_2$, to k for $C^{12}O_2$), c the specific activity of the respiratory carbon (very small), and s the isotope ratio in the gaseous CO_2. The resultant equation was:

$$ds/dt = \frac{1}{C^{12}O_2} \left[d/dt\,(C^{14}O_2)\,\frac{U-1}{U} - Rs(1-c) + \frac{Rc}{U} \right]$$

A family of curves of this function was plotted against time for various values of R and U (46); the ones which most closely matched the experimental curve corresponded to U = 0.83 ± .03 and R = 0.5 ± .1 times the preceding dark respiration. (These results were roughly confirmed by plotting the rate of gas exchange *vs.* CO_2 pressure and extrapolating to the ordinate.) However, the rate of respiration was by no means constant over the period of illumination; initially, at high CO_2 concentrations, it appeared to be faster even than dark respiration, whereas it dropped well below half the dark rate at subsequent low CO_2

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46 This treatment was justified by the fact that ds/dt was affected mainly by U at first and by R later on; the value of c was not critical.

pressures. In judging the significance of these figures one must remember that they were obtained by gas phase measurements alone; it is entirely possible that the observed depression of total respiration was merely due to a quick re-assimilation of respiratory carbon before it had a chance to leave the cells. This effect and similar diffusion limitations would be expected to reduce external gas exchange most drastically at low CO_2 pressures; our experimental evidence is in accord with this view. However, quite independent experiments of a different type (47) indicate that light inhibits the appearance of newly assimilated carbon in the respiratory intermediates. It would thus appear that at least some of the observed gas exchange effects are indeed the result of interference by the light in intracellular chemistry.

The authors are indebted to Prof. C. Ouellet for some valuable discussions and for his assistance in several experiments.

SUMMARY

The gas exchange by barley leaves of oxygen, carbon dioxide, and added radiocarbon dioxide has been measured in a closed system, with the following results:

1. Carbon dioxide follows different but not necessarily independent paths in photosynthesis and light respiration.

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47 Benson, A.A. and Calvin, M., J.Expe.Botany, Jan. 1950, 1, 63.

2. The carbon of newly formed photosynthetic intermediates is not available for respiration while the light is on, but becomes immediately respirable in the dark. The enhancement of dark respiration after a light period is largely due to built-up "photosynthates".

3. Photosynthesis proceeds at a measureable rate even at the lowest CO_2 pressures observed (0.03 mm. Hg). There is no evidence for a "threshold" concentration of carbon dioxide for the reaction; at the lowest concentrations reached, respiration exactly equals assimilation.

4. The mean rate of respiratory CO_2 evolution in strong light was found to be less than that in the dark. Internal re-photosynthesis of respiratory carbon may have been sufficient to account for this effect.

5. The assimilation of $C^{14}O_2$ is about 17% slower than that of $C^{12}O_2$.

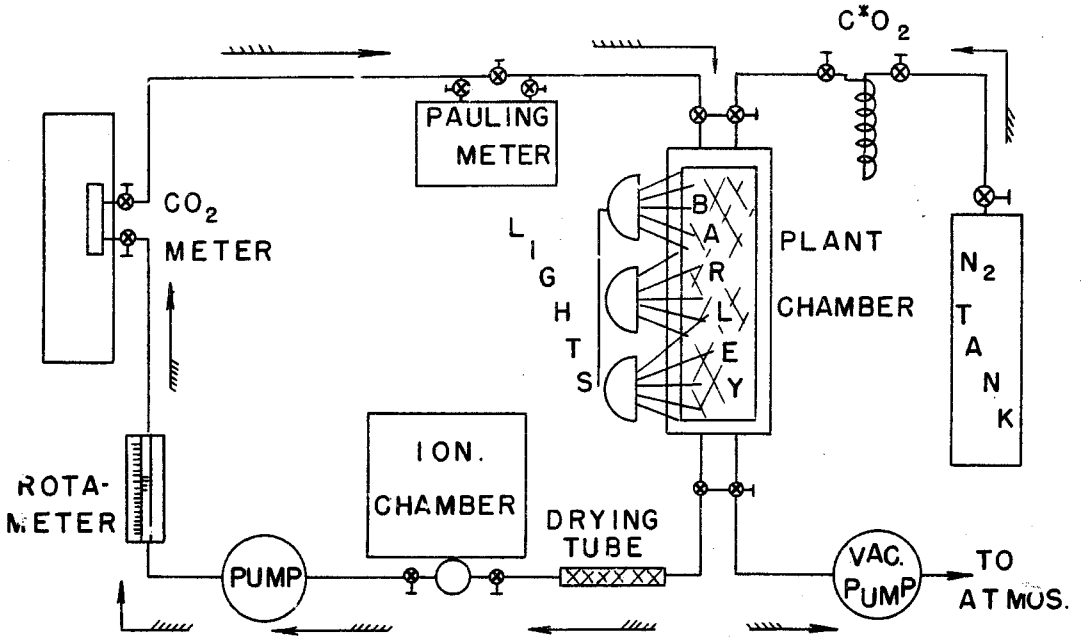

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FIG. 1

DIAGRAM OF APPARATUS

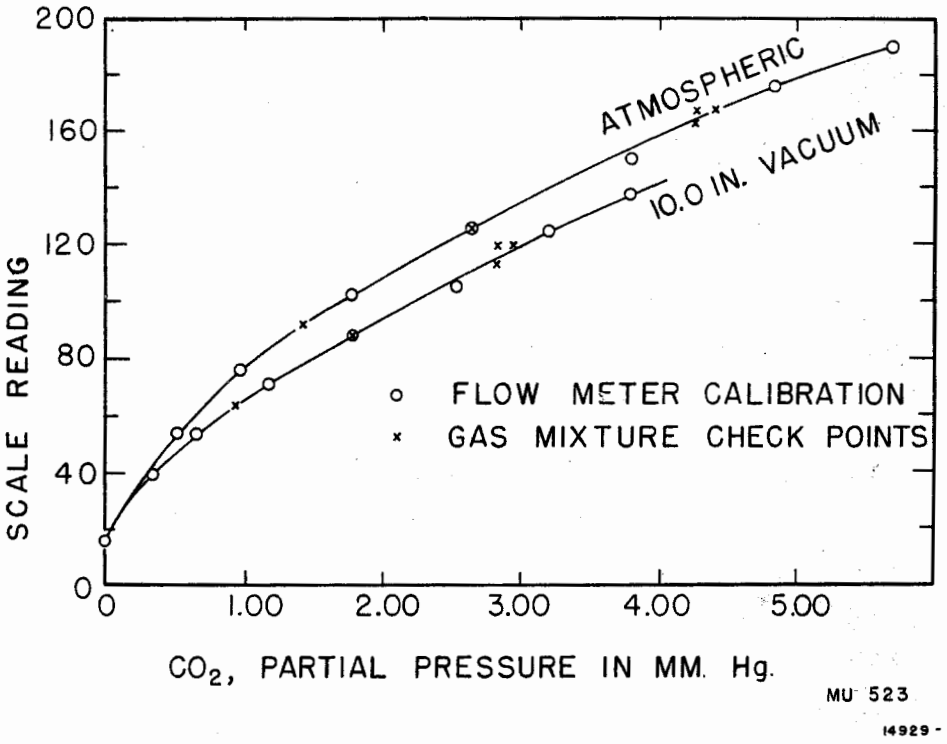

FIG. 2

CO_2 ANALYZER CALIBRATION CURVE, USED FOR BARLEY 28

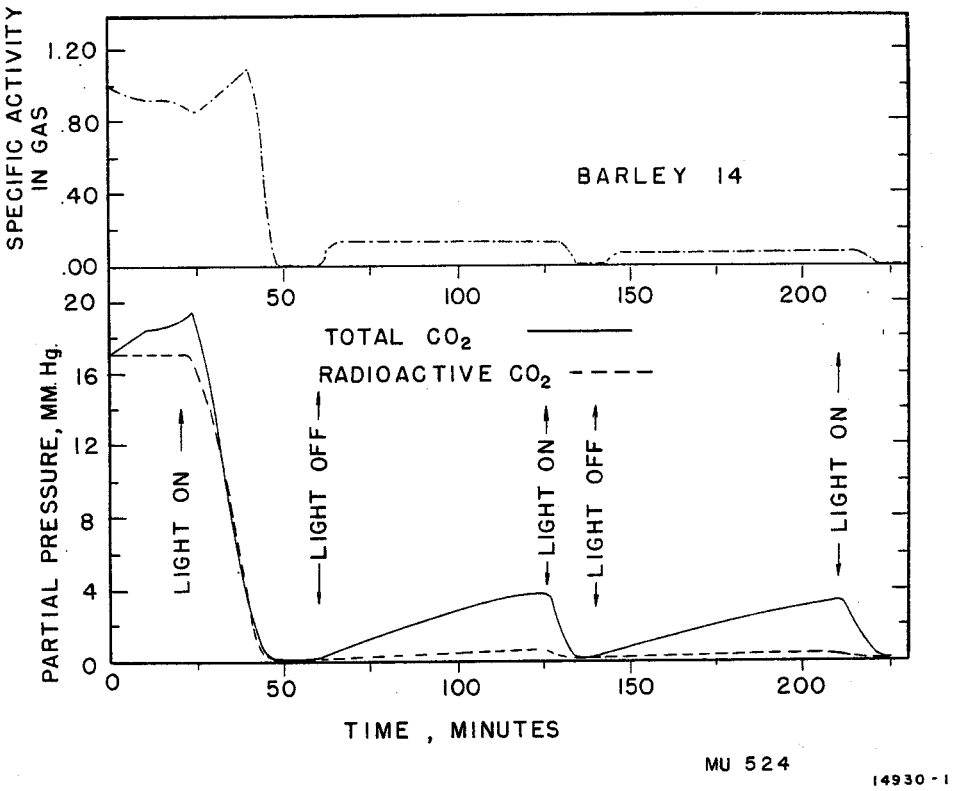

FIG. 3
EXPERIMENT 14

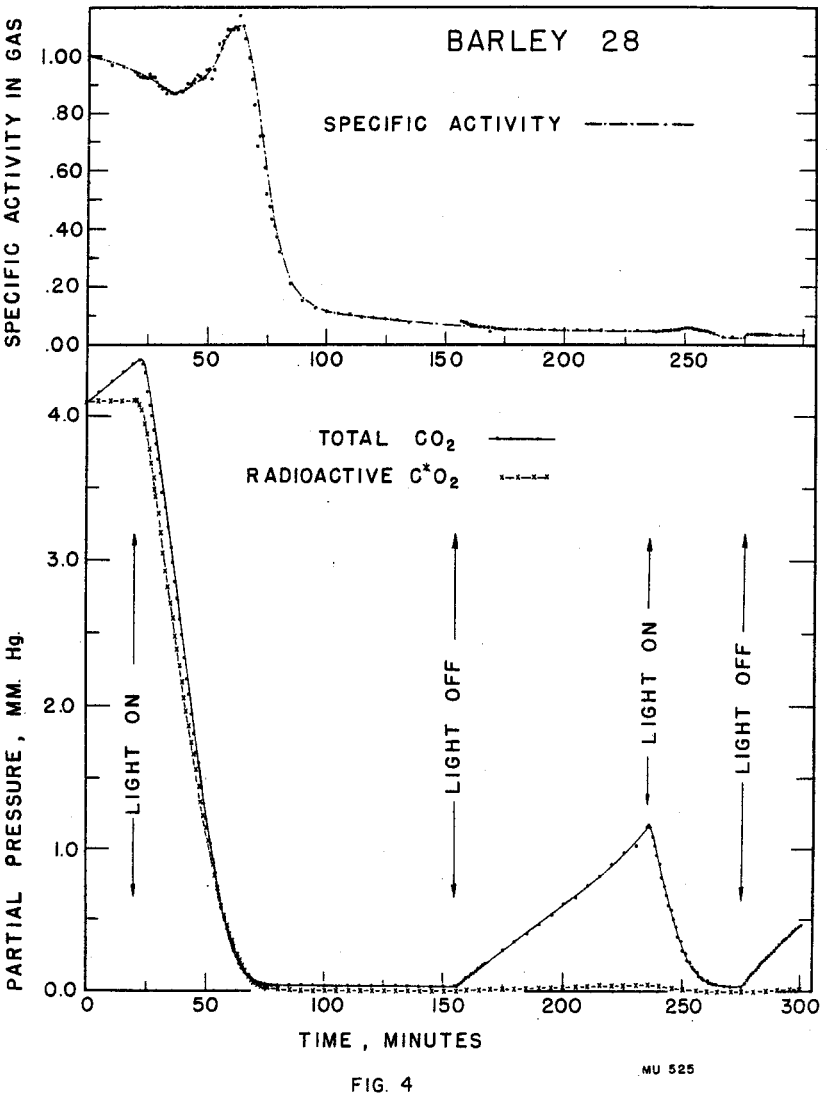

FIG. 4
EXPERIMENT 28