of giving such reproducible results on acid-binding capacity.[24]

Further studies are now under way in this Laboratory to investigate the feasibility of using measurements of permanently bound gas to assign acid- or base-binding capacity. Work on water sorption has shown[8] that equilibrium times in sorption may be reduced considerably by utilizing cells packed with metal wire (silver, platinum, aluminum, etc.) to improve thermal conductivity. Similar effects are being studied with other polar gases. The authors would like to suggest to other workers that apparatus be designed to give the maximum heat conductivity in the sample–thermostat system.

(24) In the case of BF_3, it should be noted that the length of the isobars in the sorption curve correspond to 0.93 mmole BF_3/g. protein which is very close to the figure cited for acid-binding capacity. The irreversibly bound BF_3 corresponds to about 2.5 mmoles BF_3/g. protein which does not as yet lend itself to simple interpretation.

Acknowledgment.—The authors wish to express their appreciation to the Research Corporation for a grant which made possible the present work.

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[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, OF THE UNIVERSITY OF CALIFORNIA]

The Relation of Photosynthesis to Respiration[1]

BY J. W. WEIGL,[2] P. M. WARRINGTON[3] AND M. CALVIN

The gas exchange of barley leaves has been studied in a closed system. Partial pressures of oxygen, carbon dioxide and added radiocarbon dioxide were measured simultaneously during periods of illumination and darkness. The following conclusions were reached: In strong light respiratory carbon dioxide originates primarily from endogenous sources and only to a very slight extent from recently assimilated carbon. In the dark recently photosynthesized compounds are actively oxidized in a fairly constant ratio to endogenous respiration. Photosynthesis proceeds at a measurable rate even at the lowest CO_2 pressures observed (0.03 mm.). There is no evidence for a "threshold" concentration of carbon dioxide for the reaction; at the lowest concentrations reached, respiration exactly equals assimilation. In the one experiment which could be evaluated, the mean rate of respiratory CO_2 evolution in strong light was found to be less than that in the dark. Internal rephotosynthesis of respiratory carbon may have been sufficient to account for this effect. Under the conditions of these experiments the assimilation of $C^{14}O_2$ was found to be about 17% slower than that of $C^{12}O_2$.

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 over-all reactions which may be written for these two processes are opposite

$$\text{Photosynthesis: } CO_2 + H_2O \longrightarrow (CH_2O) + O_2$$
$$\text{Respiration: } O_2 + (CH_2O) \longrightarrow H_2O + CO_2$$

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

Numerous investigators have attempted to distinguish between these simultaneous and opposite reactions, and to measure the rate of respiration in the light. The older literature has been reviewed by Weintraub[4] and Rabinowitch.[5] More recently, Kok,[6] van der Veen,[7] Gabrielsen[8] and Warburg, Burk, *et al.*,[9] have made contributions to the problem. The present investigation has attempted to further clarify the relation of photosynthesis to respiration, with emphasis on the role of carbon dioxide in these reactions.

Tracer carbon-14 has made possible a new approach which is, in principle, quite direct. One may place leaves in a closed system, allow them to photosynthesize in radioactive carbon dioxide, and 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 involve different chemical or physiological paths, 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 terminology

Photosynthesis: Assimilation of carbon dioxide from the gas
Respiration: Evolution of carbon dioxide into the gas

These definitions have certain shortcomings, resulting from the nature of the measurements. For example, non-photosynthetic carbon dioxide fixation is included with photosynthetic assimilation. However, tracer uptake in the dark was found to be very slow (less than 1% of the photosynthetic rates in runs 14 and 28 and never over 5% in other experiments); hence, non-photosynthetic fixation in the light was probably not very important.

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

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

(2) Department of Physics, The Ohio State University, Columbus, Ohio.

(3) Bechtel Corporation, San Francisco, California.

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

(5) E. I. Rabinowitch, "Photosynthesis," Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1945, Chapter 20.

(6) B. Kok, *Enzymologia*, **13**, 1 (1947); *Biochim. et Biophys. Acta*, **3**, 625 (1949).

(7) R. van der Veen, *Physiol. Plantarum*, **2**, 217 (1949).

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

(9) O. Warburg, D. Burk, V. Schocken, M. Korzenovsky and S. B. Hendricks, *Arch. Biochem.*, **23**, 330 (1949); D. Burk, S. B. Hendricks, M. Korzenovsky, V. Schocken and O. Warburg, *Science*, **110**, 225 (1949).

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.—Figure 1 shows the apparatus used. At the beginning of a run, about 20 g. of green leaves of one to two week-old barley shoots[10] were cut, moistened well, and placed in a flat one-liter glass chamber measuring 46 × 13 × 1.5 cm.[11] 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[12] 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 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. of 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. × 1 cm. tube filled with calcium chloride and calcium sulfate was used to dry the gas on its way to the instruments. Enough water was initially added to the chamber so that removal of this amount of moisture did not appear to affect the plants.

The plant chamber was immersed in a tank of cooling water, whose temperature remained constant within ±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 Ryerson–Lindemann electrometer, the latter being used as a null instrument. The circuit of Janney and Moyer[13] was modified[14] so as to yield the instantaneous value of the ionization current as the product of a decade resistance[15] 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 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[13,16]; the time required for a 90% response to a sudden large change in radioactive carbon dioxide contents was about 30 seconds for the larger chamber and only six seconds for the smaller one. "Memory" effects due to the adsorption of radioactive carbon dioxide on the brass walls were shown to be negligible (<0.05%) after five minutes or less. Troublesome "leakage currents" (giving large apparent "background" readings) were eliminated after experiment 12 by the use of Teflon insulators.[17] In a special experiment[16] in which a vibrating reed electrometer was employed[18] both chambers and the electrometer were found to be free of non-linearity, leakage current, "memory" and time lag effects.

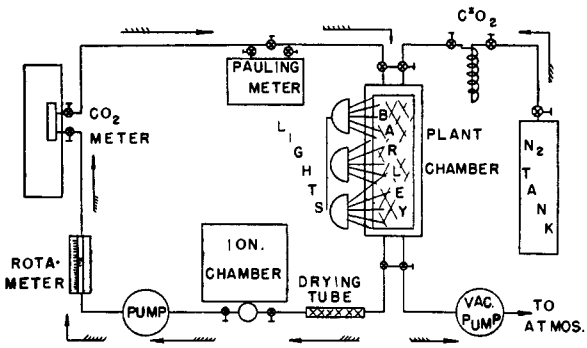

Fig. 1.—Diagram of apparatus.

Carbon Dioxide Analyzer.—A selective-detector infrared gas analyzer of the general type developed by Luft[19] and modified by Eltenton, Pompeo and Smith[20] was used to measure carbon dioxide.[21] 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); the remainder 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, setting 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 with previously analyzed mixtures of carbon dioxide and nitrogen, which were passed through calibrated rotameters,[22] mixed and flushed through the sample cell. Three additional readings were taken for each mixture, with the cell partially evacuated to allow compensation for pressure broadening effects.[23] In order to eliminate possible systematic errors, a few points were checked with gas mixtures prepared and measured manometrically (see Fig. 2).

Within experimental error, check points obtained in this way fell on the curves plotted from the flowmeter data. The response time of the carbon dioxide analyzer 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 gas flow.

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

(10) Variety *Sacramento*, kindly supplied by Messrs. T. C. Broyer and S. Gaede of the Division of Plant Nutrition, University of California.

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

(12) J. W. Weigl and D. M. Stallings, *Rev. Sci. Inst.*, **21**, 395 (1950).

(13) C. D. Janney and B. J. Moyer, *ibid.*, **19**, 667 (1948).

(14) We are indebted to Dr. C. D. Janney for much valuable aid and advice.

(15) 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.

(16) J. W. Weigl, unpublished data.

(17) The relatively large errors indicated for experiment 12 at low CO_2 pressures are mainly due to a small background current.

(18) Manufactured by the Applied Physics Corporation, Pasadena, Calif.

(19) K. F. Luft, *Z. tech. Physik*, **24**, 97 (1943).

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

(21) 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.

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

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

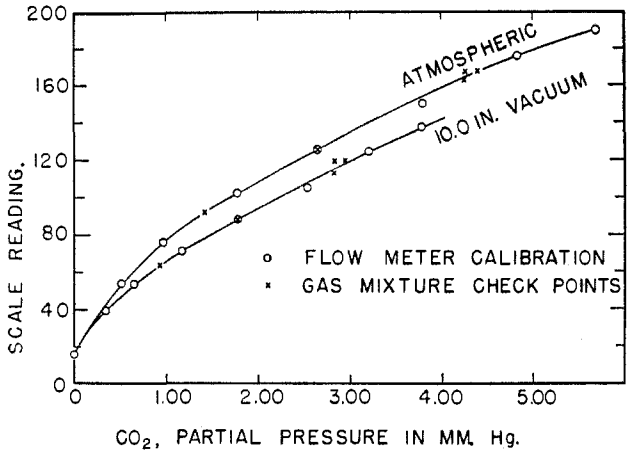

Fig. 2.—CO_2 analyzer calibration curve, used for Barley 28.

that extent. The calcium chloride-dried gas, however, contained only 0.3 mm. of water, which was recorded 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. The infrared spectra of $C^{12}O_2$ and $C^{14}O_2$ overlap only very slightly, if at all[24]; 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[25] consists of a small magnetic 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 instrument used in certain of our experiments had a fairly linear range from 0 to 100 mm. and could be read to ±0.2 mm.[26]

Experimental Procedure.—Experiments were started in the dark in order to minimize the changes during the initial mixing of the 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; after each experiment the former were converted to "millivolts of radioactivity," the latter to pressure of carbon dioxide. For convenience, the relative specific activity at the start of the experiment was arbitrarily set equal to unity; this enabled us to calculate and plot "partial pressure of $C^{14}O_2$" to the same scale as the inactive $C^{12}O_2$.[27]

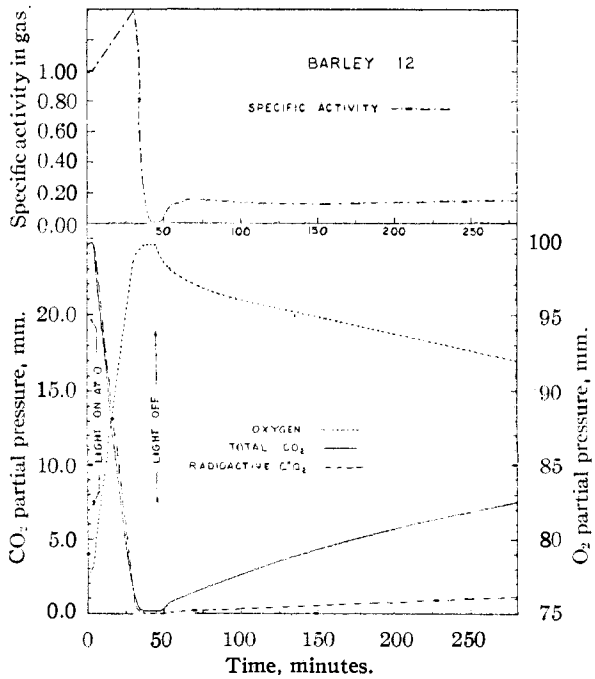

Fig. 3.—Experiment 12.

Results

Although a number of similar experiments was performed, the behavior of the plants was most clearly shown in experiments 12, 14 and 28. Others suffered from various experimental difficulties; however, in no case did they contradict the conclusions drawn from these runs. In view of the continuous data for $C^{14}O_2$ and $C^{12}O_2$ the only appreciable errors were systematic ones. Table I lists the experimental conditions. In Table II maximum reasonable errors are estimated; these are approximately twice the "probable errors."

Discussion

Figure 4 shows the changes in (CO_2) and ($C^{14}O_2$) which were observed in Barley 14. At time zero, a

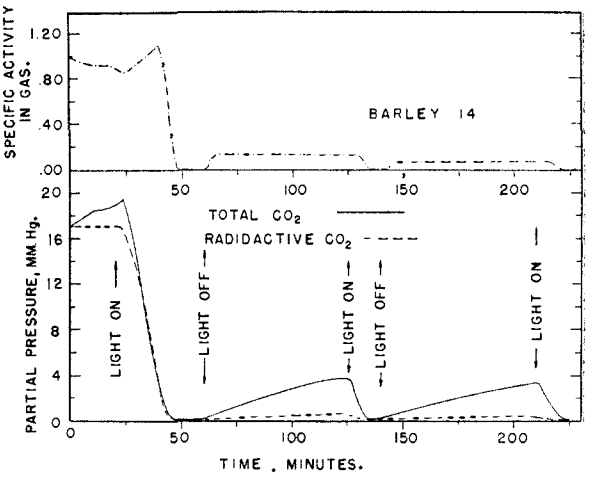

Fig. 4.—Experiment 14.

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 physiological induction effects, not instrumental time lags); it then rose to approximately 1.1 times its initial value, or 1.2 times the minimum which had occurred about time 25. The fact that this peak was some 10% higher than the specific activity of the original $C^{14}O_2$ seemed to allow no explanation other than a photosynthetic isotope discrimination.[28,29] Fi-

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

(25) L. Pauling, R. D. Wood and J. H. Sturdivant, THIS JOURNAL, **68**, 795 (1946).

(26) Calibrated by the manufacturer, Arnold O. Beckman, Co., Pasadena, Calif.

(27) Relative specific activity ≡ $C^{14}O_2/(C^{14}O_2 + C^{12}O_2)$ mm./mm. 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 experiments 12 and 14, 4.75% in experiment 28) by the relative specific activity.

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

(29) This isotope discrimination has been observed in six other experiments of this type, as well as in three sampling runs. It was further confirmed in a different experiment, in which algae were grown from a small inoculum in a large quantity of CO_2, containing the natural fraction of $C^{13}O_2$ and added $C^{14}O_2$. After about 70% of this mixture had been assimilated, the algae were found to contain about 5% less C^{13} and 20% less C^{14} than the remaining CO_2.

TABLE I

EXPERIMENTAL CONDITIONS

Experiment	12	14-1[a]	14-2	14-3	28-1	28-2
Initial p_{CO_2}, mm.	19	17	3.6	3.3	4.2	1.16
p_{O_2}, mm.	77	10	23	23	40	49
$C^{14}O_2$ in CO_2, %	1.1	1.1	0.14	0.082	4.75	0.23
Pressure, ±5 mm.	480	505	505	505	515	543 ± 13
Temperature, ±1°C.	16	22	22	22	14.5	15.5
Illumination, f.c.	7000	7000	7000	7000	14,000	14,000
Gas circulation rate, cc./mm.	100	500	500	500	500	500

[a] Designations -1, -2, etc. refer to the several photosynthetic periods in experiments 14 and 28.

TABLE II

PRECISION OF MEASUREMENTS

Time, min.	Event	CO_2 Analyzer, mm. $C^{12}O_2$	Ioniz. chamber, mv. $C^{14}O_2$	Specific activity, $C^{14}O_2/C^{12}O_2$ (mm./mm.)
Barley 12				
0	Start of photosynthesis	24.5 ± 3	1553 ± 1%	1.00 ± 0.04
30	Specific activity peak	1.4 ± 10	125 ± 8%	1.40 ± 0.25
45	End of steady state period	0.15 ± 70	0 ± 7 mv.	0.0 ± 1.0
280	End of respiration period	7.4 ± 6	72 ± 10%	0.15 ± .03
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%	0.90 ± .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 ± 0.05 mv.	0.00 ± .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
135–140	Steady state No. 2	0.1 ± 50	0.0 ± 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 ± 0.05 mv.	.00 ± .015
225–310	Average of respiration after PS No. 3	.....	.....	.066 ± .01[a]
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%	0.94 ± .03
37	Middle of photosynthesis No. 1	2.75 ± 2	12,000 ± 1%	0.865 ± .03
60	Specific activity peak No. 1	0.27 ± 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%	0.32 ± .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	0.29 ± 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

[a] Obtained by flushing out $C^{14}O_2$ with nitrogen, precipitating and counting as $BaC^{14}O_3$.

nally 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 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.[8,30]

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 endogenous carbon of the plant, whereas they were not respirable while the light was still on (or the CO_2 from their respiration did not escape).

The light was now turned on again; the specific activity dropped to near zero as before. 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

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

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 level. The average rate of respiration was close to that of preceding dark periods.

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 radiocarbon dioxide took place. This merely signified that the photosynthetic intermediates were transformed into non-respirable products much more quickly in the light than in the dark.

Analogous changes in the concentration of normal and isotopic carbon dioxide were observed in exp. 12 (Fig. 3), which also illustrates changes in the oxygen tension during photosynthesis and respiration. When averaged over periods of the order of 30–60 minutes, changes in oxygen and carbon dioxide tension were usually roughly equivalent; for example, in experiment 12, the integrated quotient $\Delta(O_2)/\Delta(CO_2)$ was equal to 0.92 for photosynthesis and to 1.04 for dark respiration. On the other hand, there were large temporary deviations from unity. Of these, the most interesting was a remarkably fast uptake of oxygen, usually observed for five or 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. This phenomenon was most noticeable after long periods at low CO_2 pressures; in view of this and of the high light intensities prevailing, it may have been an after effect of photooxidation.[5]

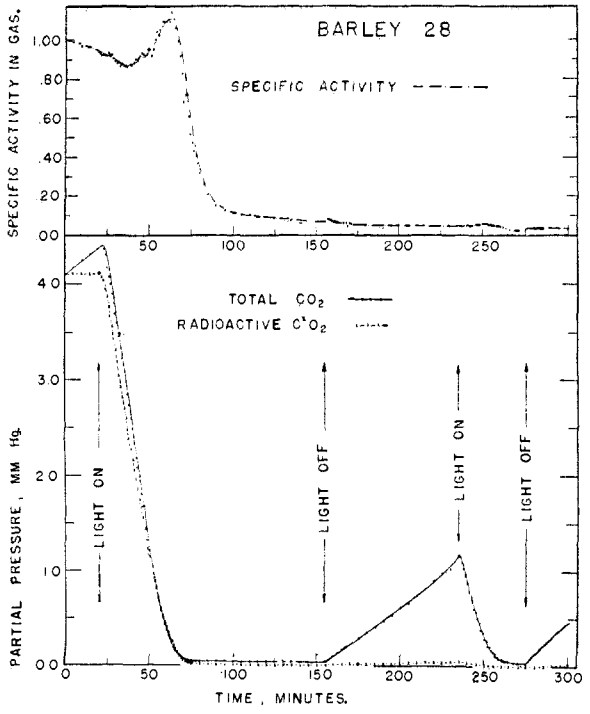

Fig. 5.—Experiment 28.

We have usually found that after a period of intense photosynthesis in the presence of excess CO_2 the dark respiration rate is increased by factors of two to three or more for periods varying from 10–200 minutes (for example, see Barley 12, Barley 14). On the other hand, in two 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[31]: a 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 would lead one to expect *no* rise in the specific activity after a long period of light and low carbon dioxide pressure. Barley 28 (Fig. 5) shows a striking case of this.[32]

In Barley 28 alone 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. 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 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 derived from the 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 and s the isotope ratio in the gaseous CO_2. Parentheses indicate actual concentrations of $C^{12}O_2$ and $C^{14}O_2$ in moles/liter. Since there was no way to evaluate independently the contributions of non-photosynthetic fixation of CO_2 and of a possible respiratory isotope discrimination, these factors were not explicitly included. The derivation follows

$$s = \left(\frac{C^{14}O_2}{C^{12}O_2}\right)$$

$$\frac{ds}{dt} = \frac{(C^{12}O_2)\, d/dt(C^{14}O_2) - (C^{14}O_2)\, d/dt\,(C^{12}O_2)}{(C^{12}O_2)^2}$$

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

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

$$k = \frac{-d/dt(C^{14}O_2) + Rc}{U(C^{14}O_2)}$$

(31) I. Borodin, *Mem. Acad. Imp. Sci.*, VIII, **28**, 1 (1881).

(32) 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.

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

A family of curves of this function was plotted against time for various values of R and U between times 20 and 150 minutes[33]; the ones which most closely matched the experimental curve corresponded to $U = 0.83 \pm 0.03$ and to respiration in the light, $R = 0.5 \pm 0.1$ times as fast as in the preceding dark period. (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 even faster than dark respiration, whereas it dropped well below half the dark rate at subsequent low CO_2 pressures. In judging the significance of these figures one must remember that they were obtained by gas phase measurements alone; it is possible that the observed depression of total respiration was merely due to 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.

The reason why little, if any, radioactive carbon dioxide was evolved in the light, and why it did appear in a subsequent dark period may be closely connected with the structure of the cells. Strong light may have inhibited the respiration of newly formed compounds located near the chloroplasts, while in the dark these sources were able to contribute a fairly constant share of total respiration. Independent experiments of a different type[34] also indicate that light inhibits the appearance of newly assimilated carbon in respiratory intermediates. It would thus appear that at least some of the observed gas exchange effects are the result of interference of light in intracellular chemistry.

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(33) 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.

(34) A. A. Benson and M. Calvin, *J. Exp. Botany*, **1**, 63 (1950).

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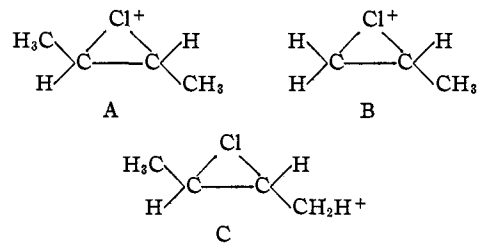
The Configuration of Optically Active 1,2-Dichloropropane

BY W. FICKETT, H. K. GARNER[1] AND H. J. LUCAS

An optically active 1,2-dichloropropane of known configuration, important from the standpoint of absolute configuration, has been obtained from L(+)-*erythro*-3-chloro-2-butanol by reactions not affecting the C–Cl bond. The product, L(−)-1,2-dichloropropane, is believed to have an optical purity exceeding 95%.

Three substances that lend themselves to the problem of absolute configuration by the theoretical method of Professor J. G. Kirkwood[2] and coworkers are active propylene oxide, 2,3-epoxybutane, and propylene chloride (or bromide) of known configuration. The configurations of propylene oxide[3] and 2,3-epoxybutane[4] are known. The possibility of obtaining propylene bromide of known configuration seems quite remote, in view of the well-known tendency of an adjacent bromine atom to participate in displacement reactions.[5] Moreover, there are fewer physical data available for making the calculations[2c] on the dibromide. The probability that an adjacent chlorine atom might participate in a displacement reaction, as for example, when a propylene chlorohydrin is converted into propylene chloride, is believed to be less than in a similar reaction to form 2,3-dichlorobutane from 3-chloro-2-butanol, because the cyclic chloronium ion, A, is stabilized by resonance more than is B, as a result of hyperconjugation, C, involving two methyl groups in the former and only one in the latter. Thus, B would be less likely to form. It is known that A is not a factor in the reaction of *erythro*-3 chloro-2-butanol with thionyl



chloride in the presence of pyridine,[6] although it is a factor in other reactions. The resonance picture that accounts for the relative stabilities of the cyclic intermediates applies also to the activated complexes that precede them, and justifies the conclusions that the activated complex leading to B also is less probable than the activated complex leading to A, and that active 1,2-dichloropropane

(1) Arthur A. Noyes Research Fellow in Chemistry, 1948–1949.

(2) (a) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937); (b) W. W. Wood, J. G. Kirkwood and W. Fickett, *J. Chem. Phys.*, in press; (c) W. W. Wood, Thesis, California Institute of Technology, 1951.

(3) P. A. Levene and A. Walti, *J. Biol. Chem.*, **68**, 415 (1926).

(4) H. Lucas and H. K. Garner, THIS JOURNAL, **70**, 990 (1948). The oxide was shown to be related to L(+)-lactic acid through L(−)-α-chloropropionic acid, and the configuration of the latter was established unequivocally. This result is in agreement with the conclusions of a number of other workers (references given) including those of Fregda, who obtained evidence on the basis of the melting points of quasi-racemic compounds; A. Fregda, "The Svedberg," Almquist and Wiksells, Upsala, 1944, p. 261.

(5) (a) S. Winstein and H. J. Lucas, THIS JOURNAL, **61**, 1576, 2845 (1939); (b) S. Winstein, *ibid.*, **64**, 2791, 2792 (1942).

(6) H. J. Lucas and C. W. Gould, Jr., *ibid.*, **63**, 2541 (1941).