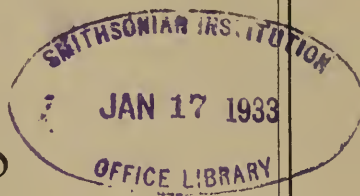


SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 87, NUMBER 16



Roebli \ddot{u} ng Fund

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A HIGHER PLANT

(WITH TWO PLATES)

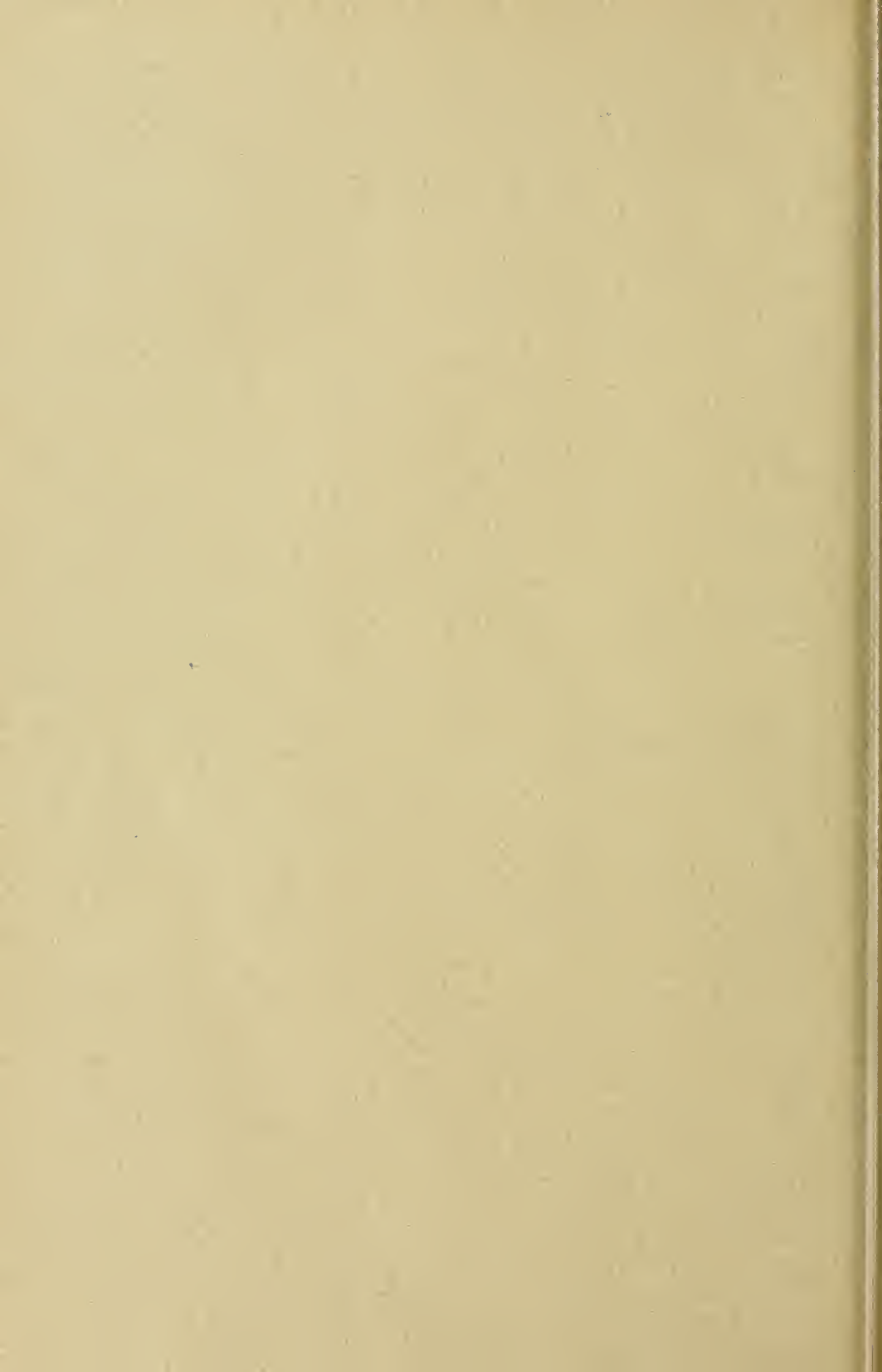
BY

W. H. HOOVER, EARL S. JOHNSTON, AND F. S. BRACKETT
Division of Radiation and Organisms, Smithsonian Institution



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(WITH TWO PLATES)

INTRODUCTION

Carbon dioxide assimilation of young wheat plants grown under controlled environmental conditions has been determined for a wide range of radiation intensities and carbon dioxide concentrations. Special growth chamber and control equipment have been developed for determinations using the entire plant. The purpose of this investigation is to determine the possibilities and limitations inherent in such technique for the investigation of photosynthesis.

The use of excised leaves common in such investigations, while offering the advantage of more ready isolation and control, and lending itself to better radiation distribution, raises many questions regarding the possible influence of accumulated end products and abnormal growth conditions. Work with algae has many advantages, chief among them being (1) avoidance of shielding, (2) the maintenance of definite temperature through immediate contact with water (owing to the high heat capacity of water, the actual temperature of the algae structure cannot differ perceptibly from the observed temperature of the surroundings), (3) because of their small size, unicellular algae offer the possibility of greater simplification and wider latitude in methods of illumination. Disadvantages common to the algae work however are (1) problems of diffusion of gas through the nutrient solution, and (2) the difficulties of obtaining suitable buffer solutions which can maintain algae continuously in a healthy condition over a long period. A great deal of work in this field has been done in such solutions that algae can exist only for a few hours. This again raises questions as to the influence of physiological factors upon the reactions. These difficulties common to most of the earlier work have been to a considerable extent overcome by Van den Honert.¹ Our observations

¹ Van den Honert, T. H., Carbon dioxide assimilation and limiting factors. *Rec. trav. bot. neerl.*, vol. 27, pp. 149-286, 1930.

offer an interesting basis for the comparison of photosynthesis in higher plants with his observations on algae. A discussion of this comparison will be taken up in connection with our experimental data.

GROWTH CHAMBER AND CONTROL EQUIPMENT

The problem of controlling the various factors affecting the rate of photosynthesis is somewhat simplified by construction of a growth chamber designed for tall slender plants such as wheat, which can be

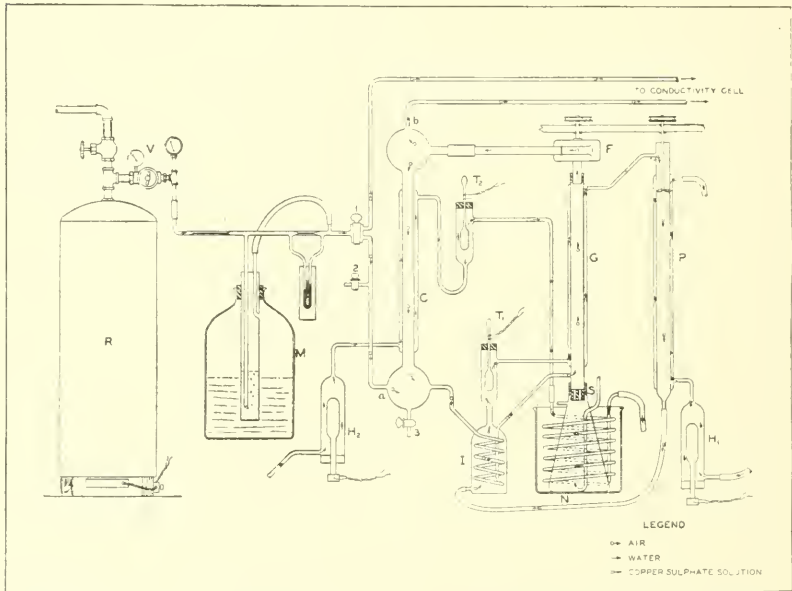


FIG. 1.—Diagram of plant growth chamber (G) with auxiliary control systems.

confined in a narrow tubular space. The simple form of the apparatus depends to some extent on the fact that the plants are grown in nutrient solution. A general view of the apparatus is shown in Plate I.

Figure 1 is a diagrammatic drawing of the growth chamber and the necessary auxiliary apparatus for controlling the air-carbon dioxide supply, temperature, and humidity. The light intensity was controlled by the number, size, and distance of the incandescent lights placed symmetrically around the growth chamber.

The growth chamber, G, consists of a double-walled glass tube about 90 cm long and 2.6 cm inside diameter with the inner tube extending about 5 cm below the seal with the outer tube. The inside diameter of

the neck of the flask, N, which holds the nutrient solution, is somewhat greater than the diameter of the inner tube of the growth chamber. The two are connected by a rubber annulus, S. The plants are supported by inserting them through small holes in a paraffined cork stopper which fits into the lower end of the growth chamber. The cork, in addition to supporting the plants, separates the flask of solution containing the roots from the space occupied by the tops of the plants. The roots are aerated by passing air through a tube sealed in the side of the flask and extending to the bottom, the air escaping through a side tube near the top of the flask. Air for the plant stems and leaves is admitted through a glass tube entering the growth chamber just above the stopper.

The supply of air containing any desired concentration of carbon dioxide is derived from a 50-gallon galvanized iron tank, R. Air in this tank under a pressure of 75 pounds per square inch is sufficient for the plants for a period of 14 to 16 hours. An electric heater is placed on the bottom of the tank to insure rapid mixing of the air and carbon dioxide.

The rate of flow of the air is regulated by means of the flowmeter, M, reducing valve, V, and capillary tube. The capillary tube is inserted in the air line between the reducing valve and the flowmeter to produce a back pressure, as the reducing valve does not regulate well unless the back pressure is 8 pounds per square inch or more. This precaution insures that a sufficient quantity of air is delivered to the flowmeter for all pressures in the tank between 10 and 75 pounds per square inch. The flowmeter used is designed to maintain a constant pressure difference between the intake and the discharge, for small changes of pressure on the discharge side. To prevent leakage of carbon dioxide the entire air system is made of glass, with the exception of the recirculating air pump, F, and three short rubber connections. The rubber connections are coated with paraffin. To test for a possible leakage of carbon dioxide the entire apparatus was assembled, without plants, and the concentration of carbon dioxide in the air measured before and after passing through the apparatus. No change in the concentration was detected.

A two-way stopcock is placed in the air line at 1, one branch leading directly to the carbon dioxide measuring apparatus for determining the original concentration of carbon dioxide, and the other leading to the growth chamber. During a determination of the original concentration, air is supplied to the plants through stopcock 2.

The flowmeter is adjusted to give 600 cc of air per minute. Under extreme conditions the plants absorbed as much as one half the avail-

able carbon dioxide. It is necessary, therefore, to recirculate the air in order to reduce the change in concentration of carbon dioxide along the plants. The air is recirculated by pump F, the direction of circulation, as indicated by the arrows, being down the double-walled tube, C, through the coil in I, and up through the growth chamber. Air enters the circulating system at *a* and is discharged at *b* for analysis. Pump F delivers about 21 liters of air per minute. Thus the effective quantity of air passing over the plants is increased by a factor of 35. If we assume the air entering at *a* contains .04 per cent carbon dioxide and the air discharged at *b* .02 per cent, the difference between the two or .02 per cent represents the amount absorbed by the plants. If the air is not recirculated the change in concentration of carbon dioxide along the plants amounts to 50 per cent. Since the effective flow has been increased by a factor of 35, the actual change in carbon dioxide concentration along the plants is $.02 \div 35$, or .00057 per cent of the total gas supply. Then if .02 per cent represents the concentration of the discharged air, .02057 per cent represents the concentration of carbon dioxide in the air entering the growth chamber. This amounts to a change in concentration along the plants of 2.77 per cent of the initial concentration. This represents about the maximum change in the course of the experiment.

Temperature control.—Water is recirculated through the space between the walls of tube G by a small propeller in the inner tube of P, through the cylinder, I, and the small bottle containing the thermostat, T₁. A continuous stream of water flows past the electric heater, H₁, and through the space between the walls of P. A resistance in series with the heater is adjusted to give about the desired temperature and the final regulation obtained by the thermostat, T₁, which changes the resistance in series with the heater. There is some lag in this method of controlling the temperature, but thermometers at the two ends of the growth chamber showed differences of only one half of a degree or less.

Humidity.—The tube, C, is maintained at a temperature of about 3° C. below the temperature of the growth chamber by a continuous flow of water past the heater, H₂, the tube, C, and past the thermostat, T₂. In order to hold the humidity constant it is necessary to have the humidity of the air supply high enough so that the addition of moisture due to transpiration will produce saturation at the temperature of C; any excess will be condensed on the walls of tube C. Due to the rapid recirculation of air we have air saturated at the temperature of C raised to the temperature of the growth chamber in I. From these data the relative humidity may be calculated. This calculated

value is not exactly the humidity in the growth chamber, since moisture is added by transpiration of the plants and the recirculated air is mixed with the air supply at the bottom of tube C. The error, however, is small since the temperature of tube C may be raised to within half a degree Centigrade of the temperature of G without moisture collecting in tube G.

The roots of the plants are kept at a fairly constant temperature by passing the water from tube C through a coil immersed in water surrounding the flask of nutrient solution.

Illumination.—The plants were illuminated by means of eight 500-watt Mazda lamps arranged in two planes perpendicular to the axis of the growth chamber. The two planes were 30 cm apart, and the lights in each plane were equally spaced around the growth chamber. The light intensity was varied by varying the distance of the lamps from the plants. A thermocouple with a cylindrical receiver, constructed in this laboratory, was used to measure the intensity. Intensity measurements were made by a thermocouple placed within the growth chamber. For each position of the lights determinations were made at several points along that portion of the tube which was occupied by the plants during observation. A mean of these values was taken, and these mean values were assumed to be proportional to the average intensities on the surface of the leaves. During an experiment the thermocouple was kept at a fixed position in the tube in order to control the illumination.

To express the intensity of radiation in watts per cm^2 the thermocouple was calibrated against a standard radiation lamp yielding a calibration factor, 3.56×10^{-4} . Thus, reducing the light intensities given in Table 2 to absolute units we get for the maximum and minimum .0689 and .0058 watt per cm^2 respectively, or in terms of calories, .985 and .083 calorie per cm^2 per minute. The intensity as determined by the thermocouple is not the true intensity at the surface of a single leaf, for a single leaf does not receive energy from all the lights. Since the lights are equally spaced around the plants a leaf with its surface perpendicular to the incident beam from one of the lights would receive energy from only one light in each plane. For any other positions of the leaf it would receive energy from two lights in each plane, but since the radiation impinges on the surface at an angle the energy received from the two lights is practically equal to that which would be received from one at normal incidence. Thus we may take $\frac{1}{4}$ of the above values, or .246 and .021 calorie per cm^2 per minute as the approximate limits of the radiation intensity on the surface of the leaves.

Measurements of the light intensity were also made with a photronic cell in order to express the results in foot-candles. The photronic cell, with a dark shade heat-absorbing filter, giving a resulting sensitivity curve approximating the visibility curve, was calibrated against a standard of illumination. Readings of the photronic cell, at any particular light intensity, were taken at various vertical positions along the growth chamber and at various orientations. A mean of these readings is taken as the mean intensity on the leaf surface. In order to convert the value given in arbitrary units to value in foot-candles, multiply by 4.96. The above limits of intensities expressed in foot-candles are 947 and 80, respectively.

It is interesting to compare the light intensities used in this experiment with the total solar intensity. The daily values of the total solar radiation received on a flat surface, expressed in calories per cm^2 , are given in the *Monthly Weather Review*. On cloudless days in Washington during May and June the total solar radiation may be as high as 600 or 700 calories per cm^2 . On the basis of a 10-hour day we may conclude that the average solar intensity for cloudless days during these months is approximately one calorie per cm^2 per minute. The intensities used in this experiment varied from 0.021 to 0.246 calorie per cm^2 per minute. Thus the intensities used varied from $1/48$ to $1/4$ that of sunlight.

APPARATUS FOR MEASURING CARBON DIOXIDE

The apparatus² for determining the carbon dioxide content of the air, shown in Figure 2, is one developed and built at the Fixed Nitrogen Research Laboratory, United States Department of Agriculture, and loaned to us for this work. The principle is to scrub a definite volume of the air with a definite volume of potassium hydroxide solution and to determine the titer of the resulting solution by means of its electrical conductivity.

The flow of the KOH solution (in this case .02 N) from the large reservoir, R, is maintained fairly constant by means of the constant level device, S, so that the liquid falls from the tip of the capillary tube, A, in drops of uniform volume at intervals of about eight seconds. Each drop falls through the bulb, B, upon the opening, C, trapping the air in the tube, CD, and forcing it along to the constriction in the tube at E. The constriction in the tube is of such size and shape as to arrest the drop at this point until displaced by the impact

² White, Ernest C., An apparatus for continuous gas analysis. *Journ. Amer. Chem. Soc.*, vol. 50, pp. 2148-2154, 1928.

of the next drop at C. The air to be analyzed enters through the tube, N, and is brought to the temperature of the bath before entering the bulb, B, by passing through the coil, O. Since only a small portion of the air is used for analysis the excess escapes through the side

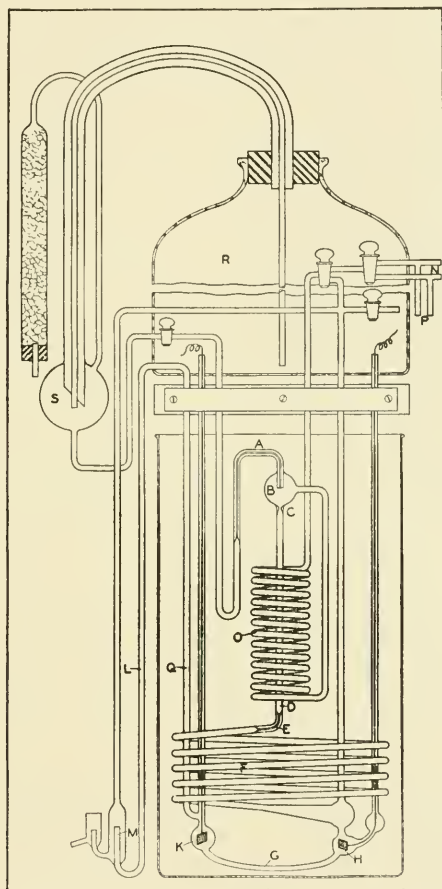


FIG. 2.—Diagram of apparatus for carbon dioxide determination.

tube, P. After the air and KOH solution have been brought together in definite proportion by volume they traverse the coiled tube, F, sufficiently long to insure practically complete absorption of the carbon dioxide. They are separated at the bottom end of the coil, F, which is widened at this point to disrupt the liquid film, the liquid flowing into the cell, HGK, and the air escaping through the tube. The overflow from the cell passes out through the siphon, L, the outer portion

being bent up so that the end, M, is slightly higher than the electrode, H. The cell is a few centimeters of small-bore glass tubing bent upward at the two ends to prevent bubbles of gas collecting on the walls. The electrodes, H and K, are made of platinized platinum.

As the sensitivity required for this work was greater than that for which the apparatus was originally developed it was necessary to improve the temperature control and eliminate to a greater degree the effects of polarization. For temperature control a special thermostat was developed, whose bulb was a cylinder of annular section. This has the advantage of securing a maximum of exposed surface with a minimum of heat capacity. This thermostat was of the liquid expansion type, using carbon tetrachloride as the working material. The temperature fluctuation was limited to $\pm 0.005^\circ$ C. Accurate control of the temperature is necessary, since the conductivity of the cell changes rapidly with temperature.

The conductivity of the solution was determined by making the cell one arm of a Wheatstone's bridge. To reduce the amount of polarization when using direct current on the bridge the direction of the current through the cell was reversed several times a second by a special commutator running in oil. There were also contacts on the commutator to short-circuit the galvanometer during the time of reversal. Very consistent results were obtained in this manner when the speed of the commutator was held constant.

For obtaining a zero point means were provided to recirculate the air inside the apparatus by connecting the exhaust with the intake. Thus in a short time the carbon dioxide was completely absorbed from the recirculated air and the conductivity cell filled with standard KOH solution.

Since small concentrations of carbon dioxide were used, a calibration of the apparatus was determined from the available conductance data and a measurement of the volume of air associated with each drop of solution. At a pressure of 760 mm and a temperature of 25° C. the volume of air is 3.125 cc and the volume of each drop is .0991 cc. The volume of carbon dioxide under the above conditions, necessary to neutralize .0991 cc of .02N KOH, is .02444 cc. Thus $.02444 \div 3.125$ gives .782 per cent, the concentration of carbon dioxide in the air necessary to neutralize the KOH solution and fill the cell with $\frac{1}{2}K_2CO_3$. From the conductance data we find the equivalent conductance of .02N KOH and $\frac{1}{2}K_2CO_3$ to be 225 and 109.2 respectively. The resistance of the cell full of KOH is 26450 ohms, then the resistance of the cell full of $\frac{1}{2}K_2CO_3$ is $225/109.2 \times 26450$ or 54513 ohms. For zero concentration of carbon dioxide the conduc-

tance of the cell is given by $1/26450$, and for a concentration of .782 per cent the conductance is $1/54513$. Intermediate concentrations will be proportional to the change in the reciprocal of the resistance.

EXPERIMENTAL PROCEDURE

Wheat³ of the Marquis variety was selected for this work. It grows well in nutrient solutions, and by the use of such a medium a more accurate control of the nutritional phases of the work can be obtained. The nutrient solution used and the general cultural methods have been described in a previous publication.⁴ The wheat was germinated between layers of moist filter paper in a covered glass dish at a temperature of 25° C. When the roots had grown to a length of 2 to 4 cm the young plants were transferred to a germination net stretched over a glass dish through which tap water flowed. The plants were illuminated by a 200-watt Mazda lamp placed 30 cm above the netting. When the seedlings were approximately 4 to 5 cm in length, four individuals selected for uniformity of size were transferred to the growth chamber. The plants were supported by means of cotton in the four small holes in a paraffined flat cork stopper which fitted into the lower end of the tubular growth chamber. The growth chamber was then placed over the Erlenmeyer flask containing the nutrient solution and held in place by means of a rubber annulus.

From the work of Johnston⁵ with tomato plants grown under Mazda lamps it appears that the large proportion of infra-red radiation found in this type of illumination is somewhat injurious. More nearly normal growth and physiological response were obtained where heat absorbing filters were used. Nearly normal wheat plants were produced when the excessive infra-red radiation from the lamps was absorbed by a solution of copper sulphate. This was easily accomplished by using a solution of copper sulphate (1.046 sp. gr.) in place of water in the circulating system for controlling the temperature of the growth chamber. Plate 2 shows four wheat plants grown in the growth chamber for a period of 20 days.

In the following tables the light intensities are expressed in arbitrary units, and the carbon dioxide concentrations and assimilation rates expressed as changes in the reciprocal of the resistance of the conductivity cell. To express the concentration in terms of volume

³ The wheat used in these experiments was obtained through the courtesy of H. H. McKinney, Jr., of the United States Department of Agriculture.

⁴ Johnston, Earl S., The functions of radiation in the physiology of plants. II. Some effects of near infra-red radiation on plants. *Smithsonian Misc. Coll.*, vol. 87, no. 14, pp. 1-15, 1932.

⁵ *Idem.*

percentage the results should be multiplied by .000041, the calibration constant of the instrument. To express the assimilation rate in cubic centimeters of carbon dioxide per minute the results should be multiplied by 0.025, *i. e.*, 0.000041×600 .

Two experiments were performed. In the preliminary one certain difficulties were encountered which suggested the procedure followed in the second experiment. A number of points of interest, however,

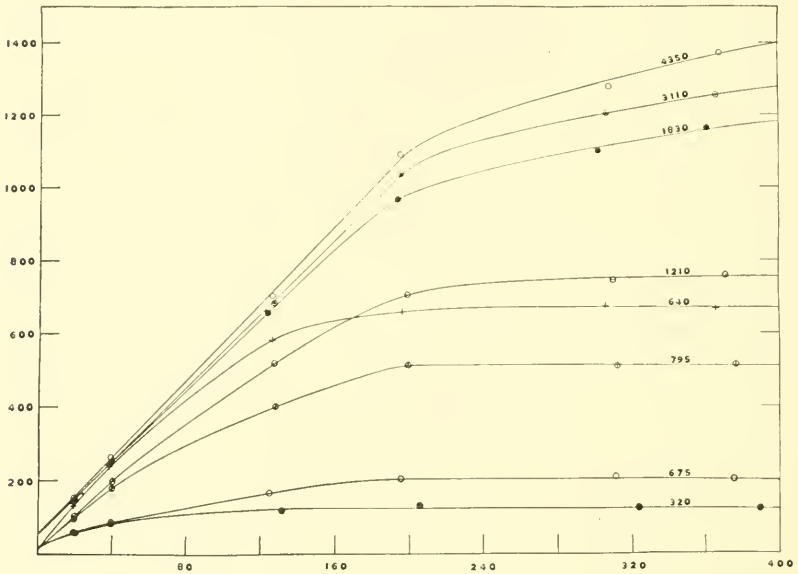


FIG. 3.—Light-assimilation curves from first experiment uncorrected for growth.

Ordinates, carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.

Abscissas, light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm².
Multiply by 4.06 to obtain foot-candles.

Parameters, carbon dioxide concentrations. Multiply by 0.000041 to obtain volume per cent.

brought out in the first experiment make it worth while to present both experiments.

PRELIMINARY EXPERIMENT

On a given day the plants were supplied with air containing a given concentration of carbon dioxide and the rate of photosynthesis determined for six light intensities. This procedure was repeated on other days for different concentrations of carbon dioxide. The results of this series of experiments for several concentrations are given numerically in Table 1 and graphically in Figure 3.

TABLE 1. *Assimilation data from first experiment*^a

Light intensity variable; carbon dioxide concentration the parameter; temperature 19° C., relative humidity 74 per cent.

CO ₂ concentration of air supply	Light intensity	Assimilation	Corrected assimilation	Growth factor	Corrected assimilation divided by growth factor
882	19.0	57	42	1.00	42
	39.8	85	70	1.00	70
	124.7	164	149	1.00	149
	195.0	202	187	1.00	187
	311.4	206	191	1.00	191
	375.1	206	191	1.00	191
444	20.5	58	43	1.25	34
	41.3	83	68	1.25	54
	131.1	118	103	1.25	82
	205.8	131	116	1.25	93
	323.5	125	110	1.25	88
	389.2	125	110	1.25	88
1308	19.3	96	81	2.20	37
	39.2	175	160	2.20	73
	127.1	397	382	2.20	174
	199.2	510	495	2.20	225
	312.1	508	493	2.20	224
	376.2	512	497	2.20	226
1970	19.5	102	87	2.23	39
	39.8	199	184	2.23	85
	126.6	515	500	2.23	224
	198.6	702	687	2.23	308
	310.4	742	727	2.23	326
	371.0	757	742	2.23	333
2993	19.5	147	97	2.41	40
	39.1	247	197	2.41	82
	123.0	653	603	2.41	251
	193.2	967	917	2.41	380
	302.1	1101	1051	2.41	437
	361.5	1165	1115	2.41	463
4364	19.9	145	95	2.49	39
	39.7	251	201	2.49	81
	126.1	681	631	2.49	253
	196.7	1036	986	2.49	396
	306.6	1205	1155	2.49	463
	366.1	1256	1206	2.49	483
5722	19.1	151	101	2.64	39
	38.3	260	210	2.64	80
	125.9	700	650	2.64	248
	196.6	1089	1039	2.64	397
	307.9	1278	1228	2.64	468
	368.0	1371	1321	2.64	504
1307	19.0	130	115	3.61	32
	38.1	235	220	3.61	61
	125.2	570	565	3.61	156
	195.5	657	642	3.61	178
	306.2	671	656	3.61	182
	366.0	667	652	3.61	180

^a Conversion factors:

Carbon dioxide concentration. Multiply by 0.000041 to obtain volume per cent.

Light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm². Multiply by 4.96 to obtain foot-candles.

Carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.

The data, without correction for growth, are plotted in Figure 3. An extrapolation of the curves in this figure indicate a small apparent assimilation rate for zero light intensity. This may be explained by a leakage of carbon dioxide into the apparatus through the rubber tubing which constituted part of the air line in this first experiment. This trouble was eliminated in later experiments by making a practically all-vitreous air system.

We had hoped to determine the growth curve of the plants from respiration measurements, but the changes in concentration of carbon dioxide in the air due to respiration proved to be too small to give accurate results. An approximate curve was obtained from the daily measurements of leaf lengths. The interpretation of these curves will be discussed in connection with the second experiment.

SECOND EXPERIMENT

In this experiment the order of procedure was reversed. The light intensity was held constant and the assimilation rate on a given day determined for several different carbon dioxide concentrations. This was repeated for five other light intensities. The data for this series of experiments are given in Table 2 and Figures 7 and 8. Thus we obtain immediately curves showing the assimilation rate as a function of the carbon dioxide concentration for six light intensities (fig. 7). The data for Figure 8 are derived from Figure 7. The curves in this figure show the assimilation rate as a function of the light intensity for several concentrations of carbon dioxide.

In the second experiment the rubber connections were eliminated by construction of a practically all-vitreous system. The growth curve for this experiment was determined in a manner somewhat similar to that employed by Van den Honert. The assimilation rate of the plants was measured under the same conditions, twice each day, and a linear growth relation assumed for the intervening time.

DISCUSSION OF RESULTS

In order to compare the curves of the first experiment with those of the second experiment growth correction must be applied to the data of Figure 3. Applying arbitrary growth factors which will bring the two sets of curves into harmony after making the zero correction for leakage, we obtain Figure 4. These arbitrary growth factors are plotted in Figure 5 and are shown by the solid curve. It is interesting to compare this curve with the curve for total leaf length indicated by the dotted line and those obtained from the respiration measurements shown by the broken line. It must be borne in mind that the

TABLE 2. Assimilation data from second experiment ^a

Carbon dioxide concentration variable; light intensity the parameter; temperature 22° C., relative humidity 79 per cent.

Light intensity	CO ₂ concentration	Assimilation	Growth factor	Assimilation divided by growth factor	
175	2902	621	1.00	621	
	1793	580	1.05	552	
	957	504	1.32	382	
	745	433	1.35	321	
	622	355	1.38	257	
	283	169	1.40	121	
	7525	881	1.42	621	
	7031	945	1.51	625	
	5191	957	1.54	620	
	4064	984	1.60	615	
	3078	1009	1.62	622	
	191	7826	1293	1.85	699
		4823	1273	1.87	682
		5277	1313	1.88	700
4371		1314	1.89	697	
7812		1485	2.12	700	
3394		1493	2.14	698	
2425		1383	2.16	640	
1866		1287	2.18	590	
1010		1015	2.45	415	
512		549	2.47	236	
127		6390	1175	2.49	472
		9657	1358	2.88	472
		6977	1326	2.90	457
		5552	1329	2.92	455
	4013	1328	2.93	453	
	2772	1330	2.93	452	
	7412	1479	3.24	457	
	4146	1497	3.25	461	
	2102	1519	3.26	465	
	981	948	3.27	290	
	223	358	3.28	109	
	1706	1370	3.29	415	
	500	726	3.30	220	
	80.4	10418	1010	3.32	304
9655		1062	3.50	304	
7079		1057	3.52	301	
5173		1046	3.53	296	
3599		1077	3.54	304	
2505		1061	3.55	299	
10984		1123	3.70	304	
2004		1099	3.72	296	
1506		1017	3.73	273	
1030		860	3.74	230	
687		650	3.75	173	
350		508	3.76	135	
200		308	3.77	82	
31.5		9876	455	3.95	115
	5094	460	3.96	116	
	2558	450	3.97	113	
	1267	439	3.98	110	
	855	319	3.99	80	
	300	240	4.00	60	
	150	161	4.01	40	
16.2	10569	252	4.20	60	
	3020	260	4.21	62	
	1223	250	4.22	59	
	808	214	4.23	50	
	400	148	4.24	35	
	150	85	4.25	20	

^a Conversion factors:

Light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm². Multiply by 4.96 to obtain foot-candles.

Carbon dioxide concentration. Multiply by 0.00041 to obtain volume per cent.

Carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.

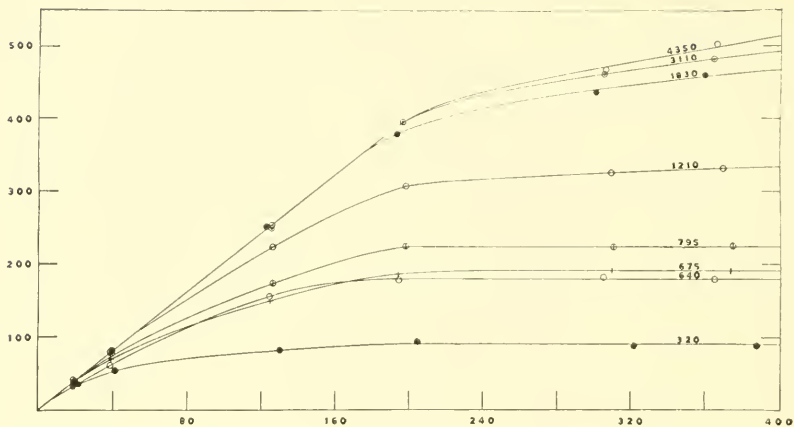


FIG. 4.—Light-assimilation curves from first experiment corrected by arbitrary growth factors.

Ordinates, carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.

Abscissas, light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm².
Multiply by 4.96 to obtain foot-candles.

Parameters, carbon dioxide concentrations. Multiply by 0.000041 to obtain volume per cent.

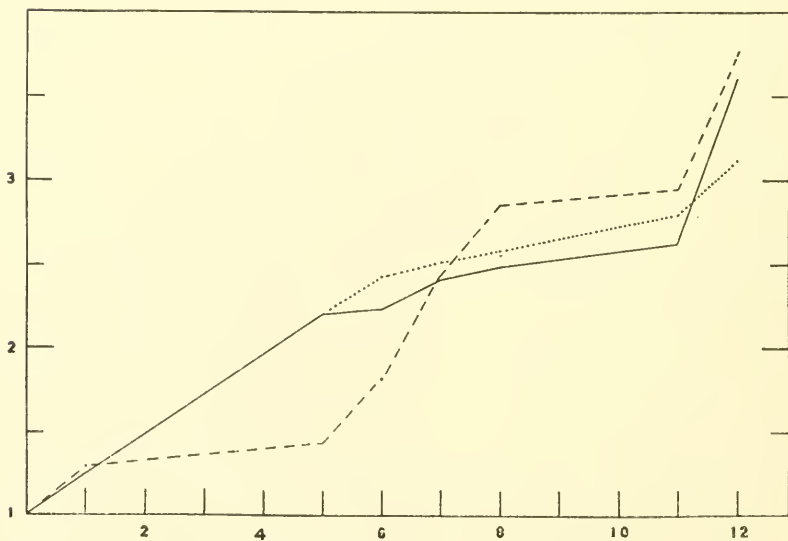


FIG. 5.—Growth factors from first experiment.

— Arbitrary factors applied to Figure 3.

.... Factors obtained from leaf measurements.

--- Factors obtained from respiration measurements.

Ordinates, numerical factors.

Abscissas, duration of experiment in days.

respiration measurements involve changes in concentration which are small compared to those of assimilation measurements. This accounts for the fact that whereas our error of observation is sufficient to account for the difference between these growth curves, it is still small when considered on the scale of the assimilation curves. The method of growth correction adopted in the second experiment being based upon assimilation under identical conditions has an accuracy of the same order as the assimilation measurements themselves. Thus the arbitrary factor involved in the interpretation of the first experiment is wholly eliminated in the second.

The first set of curves are interesting, however, first, because they show observations made over a wider range of light intensities, and, second, because although made in a completely different order of sequences in time, they are in complete agreement. If any marked effects of day and night or age were present, such agreement would not be possible even through arbitrary growth correction.

In order to further compare the results of the first experiment with those of the second, curves have been derived from the corrected data (fig. 4) showing carbon dioxide assimilation as a function of carbon dioxide concentration with light intensities as parameters. These are shown in Figure 6.

Directly from the second experiment we obtain assimilation rate as a function of carbon dioxide concentration for six light intensities as given in Figure 7. In the main points the two experiments are in reasonably good agreement, remembering of course that different plants have been used so that the assimilation values are not directly comparable. For the highest light intensity, 191 (fig. 7), we see that the assimilation rate is proportional to the carbon dioxide concentration from 0 to about 850. The maximum rate is not reached until the concentration has been increased to about 3,500. A further increase in the concentration produces no change in the assimilation rate. As the light intensity is decreased the departure from the linear relation occurs at a lower and lower carbon dioxide concentration. The maximum assimilation rate is less and the maximum is reached at a lower carbon dioxide concentration. In Figure 8 we see, for a carbon dioxide concentration of 3,500 or more, that the assimilation rate is proportional to the light intensity from 0 to the highest intensity used. As the concentration is decreased the departure from the linear relation occurs at a lower and lower light intensity. For small carbon dioxide concentrations a maximum rate of photosynthesis is reached for the light intensities used in this experiment. A further increase in intensity produces no change in the assimilation

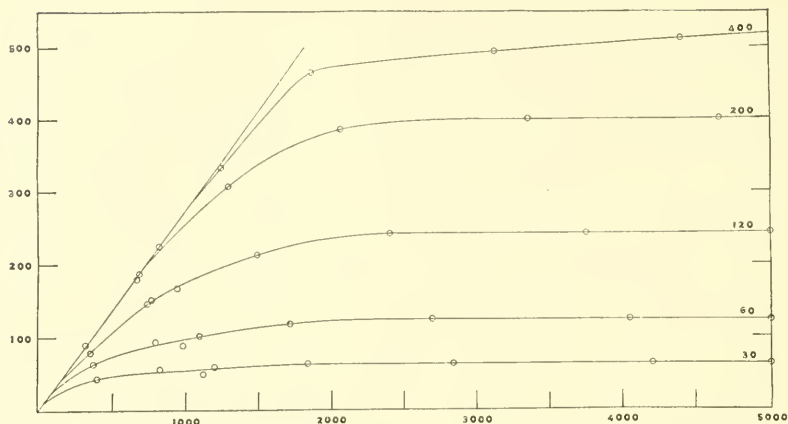


FIG. 6.—Carbon dioxide-assimilation curves derived from Figure 4.
 Ordinates, carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.
 Abscissas, carbon dioxide concentration. Multiply by 0.000041 to obtain volume per cent.
 Parameters, light intensities. Multiply by 3.56×10^{-4} to obtain watts/cm². Multiply by 4.96 to obtain foot-candles.

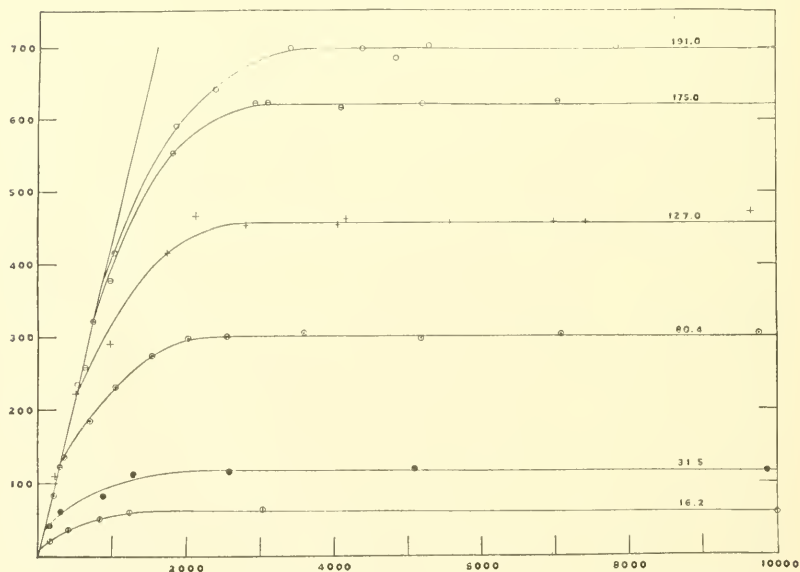


FIG. 7.—Carbon dioxide-assimilation curves from second experiment.
 Ordinates, carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.
 Abscissas, carbon dioxide concentration. Multiply by 0.000041 to obtain volume per cent.
 Parameters, light intensities. Multiply by 3.56×10^{-4} to obtain watts/cm². Multiply by 4.96 to obtain foot-candles.

rate. It thus appears, for the condition of this experiment, that carbon dioxide may be the limiting factor for the high light intensities, assimilation being proportional to the carbon dioxide concentration over a considerable range. For the high carbon dioxide concentrations, the light intensity may be the limiting factor, assimilation being proportional to the light intensity. There exist well-defined regions over which the assimilation seems to depend upon both factors.

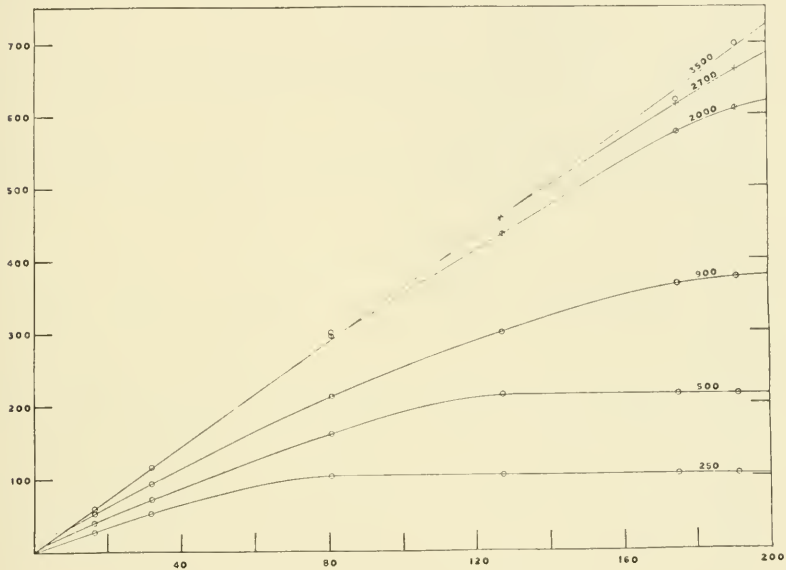


FIG. 8.—Light-assimilation curves derived from Figure 7.

Ordinates, carbon dioxide assimilated. Multiply by 0.025 to obtain cubic centimeters per minute.

Abscissas, light intensity. Multiply by 3.56×10^{-4} to obtain watts/cm².
Multiply by 4.96 to obtain foot-candles.

Parameters, carbon dioxide concentrations. Multiply by 0.000041 to obtain volume per cent.

Assuming the simplest type of Blackman reaction involving only linear segments, some transition range is to be expected, since ideal conditions cannot be obtained. Not all the chloroplasts can be maintained in the same light intensity, nor can all the surfaces of the leaves be brought in contact with exactly the same concentration of carbon dioxide. The fact that the lights are symmetrically placed around the plants not only reduces the fluctuation of intensity over the surfaces of the leaves, but, owing to the fact that the leaves are exposed to radiation from both sides, reduces to a minimum the

variations of intensity through the leaf. Rapid recirculation of the air insures a small variation in concentration of carbon dioxide over the plants.

In view of these precautions, is it possible to explain the whole of the transition range as being due to variations in the environmental conditions? The actual variation of intensity over the surface of the leaves, as determined by thermocouple and photronic cell measurements, may be as much as 10 or 12 per cent from the mean values given. The chloroplasts are distributed more or less uniformly throughout the entire body of the wheat leaf; thus, there must be a considerable variation in intensity when we consider chloroplasts at different distances below the surface of the leaf. In order to get some idea of the absorption of the light in a leaf, the transmission of a wheat leaf was measured with a photronic cell, a 500-watt lamp being used as the source of light. The transmission of the leaf for the whole range to which the cell is sensitive was 18 per cent. When the radiation from the lamp was filtered through a heat-absorbing glass the transmission was reduced to 14 per cent. This is due, of course, to the regions of selective absorption. Even with the leaf illuminated from both sides it must be concluded that there is a large variation in the intensity of radiation at the surfaces of the various chloroplasts throughout the leaf. If one assumes that the whole transition range may be explained by a variation in light intensity then the variation in the intensity may be determined from Figure 8. For a concentration of carbon dioxide of about 900 we see that light is the limiting factor for a very small range. At an intensity of 10, carbon dioxide has already become the limiting factor for some of the chloroplasts. It is necessary, however, to increase the intensity to 200 or more before carbon dioxide is the limiting factor for all the chloroplasts. This would indicate that some of the chloroplasts were receiving only 5 per cent or less of the effective radiation intensity of others. It does not seem likely that the variation of intensity is so great.

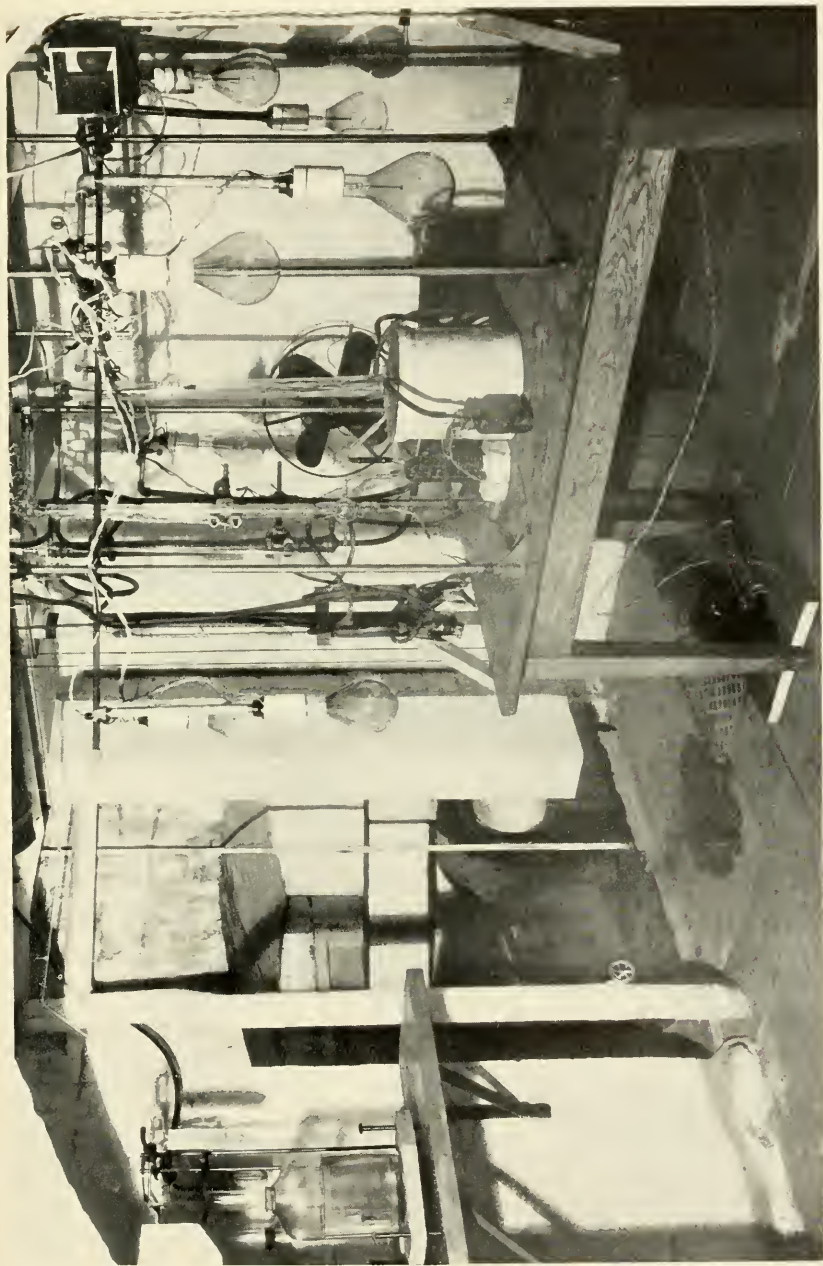
Comparing these families of curves obtained from young wheat plants with those of Van den Honert obtained from algae one observes a close agreement as to general form. Both show well-defined linear variations over restricted ranges; both show transition from one linear range to the other in which the two variables affect the photosynthesis. Higher plants show a wider range of transition, as is to be expected from the fact that both light intensity and carbon dioxide concentration vary over a considerable range for different chloroplasts. Whereas light intensity has been measured with considerable

accuracy in absolute terms the lack of accurate data as to leaf area and chlorophyll concentration prevents one from attaching particular significance to the slope of the linear variation.

It is evident from this discussion that such an experiment does not lend itself to an analysis of the transition range in terms of chemical kinetics. The difficulty lies entirely in the nature of the organism examined, so that further efforts in the direction of obtaining more ideal conditions and further analysis of the energy distribution, absorption characteristics, etc., would seem relatively fruitless. It remains possible, however, to make a critical attack upon many interesting problems of photosynthesis in cases where one is chiefly concerned with the range in which one or the other of the variables acts as a limiting factor. The method has the advantage of eliminating many of the objectionable factors involved in experiments where organisms are placed in abnormal growth conditions.

CONCLUSIONS .

A set of families of curves has been obtained showing assimilation of carbon dioxide by young wheat plants over a wide range of carbon dioxide concentrations and light intensities. Linear variation of assimilation with carbon dioxide concentration in the presence of excess light has been observed over a limited range. Linear variation of carbon dioxide assimilation as a function of light intensity for excess carbon dioxide concentration has also been observed over a limited range. The transition range between the two regions of limiting factors is more extensive in higher plants than in algae. This may be expected from the lack of homogeneity of light intensity and carbon dioxide concentration throughout the leaf. These experiments indicate that a wide range of critical experiments upon photosynthesis may be carried out with higher plants, using the technique developed, so long as one is not particularly concerned with problems such as constants of dissociation and others particularly relating to the transition range.



Growth chamber and control equipment



Wheat plants after 20 days of growth