SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 104, NUMBER 4

THE INFLUENCE OF LIGHT AND OF CARBON DIOXIDE ON THE RESPIRATION OF ETIOLATED BARLEY SEEDLINGS

(With Two Plates)

BY
ROBERT L. WEINTRAUB
AND
EARL S. JOHNSTON
Division of Radiation and Organisms
Smithsonian Institution

(Publication 3769)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
JUNE 28, 1944

SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 104, NUMBER 4

THE INFLUENCE OF LIGHT AND OF CARBON DIOXIDE ON THE RESPIRATION OF ETIOLATED BARLEY SEEDLINGS

(With Two Plates)

BY
ROBERT L. WEINTRAUB
AND
EARL S. JOHNSTON
Division of Radiation and Organisms
Smithsonian Institution

(Publication 3769)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
JUNE 28, 1944

The Lord Baltimore Press
BALTIMORE, MD., U. S. A.

THE INFLUENCE OF LIGHT AND OF CARBON DIOXIDE ON THE RESPIRATION OF ETIOLATED BARLEY SEEDLINGS

By ROBERT L. WEINTRAUB and EARL S. JOHNSTON
Division of Radiation and Organisms, Smithsonian Institution

(With Two Plates)

During the course of a study of the carbon dioxide production by etiolated barley seedlings, there was obtained evidence that this process is appreciably influenced by a number of factors of the plant's environment, both during and prior to the actual measurements of respiration. The work has been interrupted indefinitely, but we believe sufficient data have been accumulated to justify a preliminary report. The results may also serve to emphasize the importance, in studies of gaseous exchange, of close control of various conditions which frequently have been disregarded.

In the present paper the term "respiration" will be used as synonymous with "excretion of carbon dioxide." No measurements of oxygen absorption were made.

EXPERIMENTATION

Determination of carbon dioxide.—The spectrographic method for measurement of carbon dioxide developed in this laboratory (McAlister, 1936, 1937 *a, b*) has been used, with some modifications of the apparatus and technique originally described. The optical system is indicated in figure 1, and the apparatus is illustrated in plates 1 and 2. By means of a photographic method, an automatic record of the carbon dioxide content of the air surrounding the plants is obtained at regular intervals—5 minutes in the present study (see figs. 2 and 3). At a carbon dioxide concentration comparable with that of the atmosphere (about 0.03 percent) the sensitivity of the present apparatus is approximately 1 part in 2 million by volume. The uncertainty of a determination, arising chiefly from the error in measuring the galvanometer deflection and from slight fluctuations of the filament current, is 1 to 2 parts per thousand; with the present apparatus this

corresponds to about 8 cmm. CO_2 at a concentration equal to normal air and to about 2 cmm. CO_2 at zero CO_2 concentration.

Control and measurement of air temperature.—Unless otherwise specified, the air temperature was maintained at approximately 26.5° C. The temperature of the air in the plant chamber was measured to 0.01° C. by means of a thermocouple with one junction (shielded by a housing of bright metal foil) situated adjacent to the leaves and the other junction in a bath of ice and water. Readings were taken at 10-minute intervals during the respiration runs.

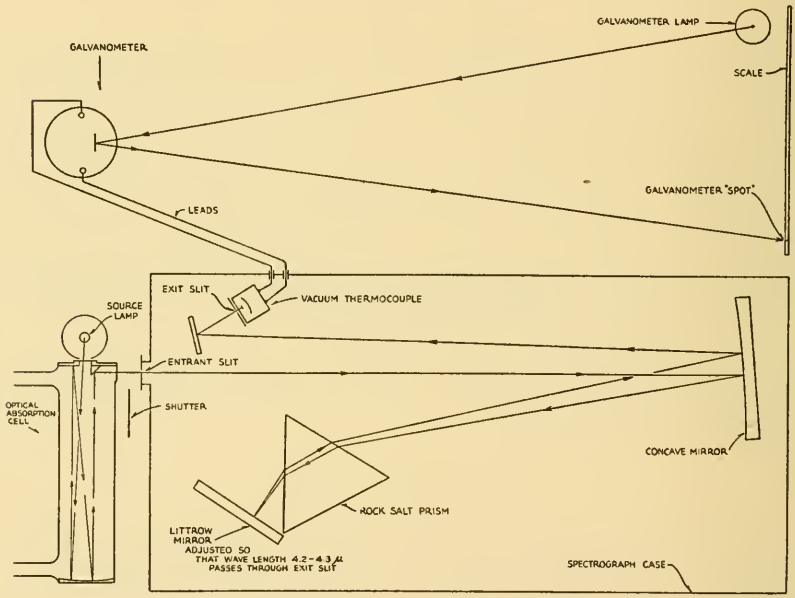


FIG. 1.—Optical system employed in spectrographic method for determination of carbon dioxide.

Control of relative humidity.—The relative humidity of the circulating air was controlled by saturating it with water vapor at one temperature and then warming it to another. These temperatures could be adjusted to give any desired relative humidity and air temperature. It should be mentioned, however, that no means of actually measuring the relative humidity of the air was provided, so that there may exist some uncertainty as to whether the calculated humidity was actually obtained. Experiments designed to study the influence of humidity on respiration indicated that, under the conditions prevailing, this factor was of minor significance.

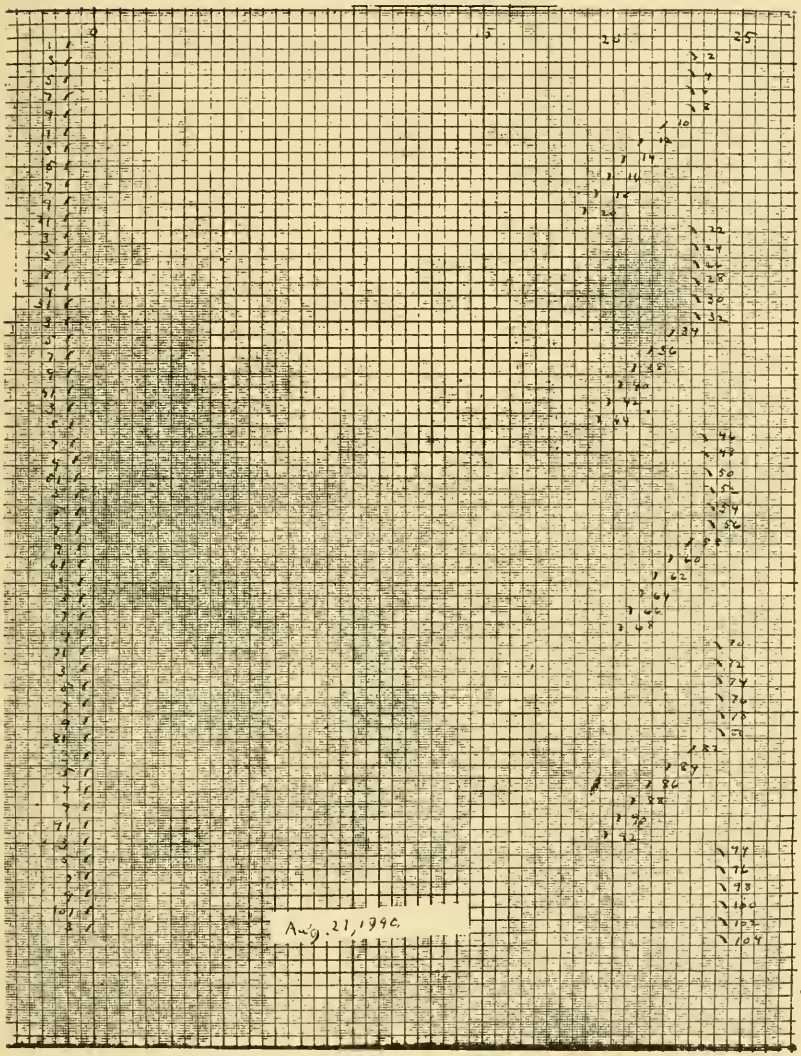


FIG. 2.—Photographic record of galvanometer deflections. Increasing carbon dioxide concentrations are represented by smaller deflections. The record shows four successive 30-minute measurements of respiration separated by "zero" periods of carbon dioxide-free air. Curves plotted from this record are shown in figure 3.

Illumination and measurement of light intensity.—For the experiments on the influence of light, visible radiation having roughly the spectral distribution of sunlight was employed (see fig. 4). This was obtained by passing the radiation from a tungsten-filament projection lamp through a filter composed of Noviol shade O, medium shade heat-resisting, and pyrex glasses, plus 15 mm. of distilled water. A semicylindrical reflecting surface, made of strips of silvered glass mirrors, was placed behind the plant chamber to better equalize the illumination of all surfaces of the leaves. The intensity of the light incident on the leaves was measured by a photocell which had been recently calibrated by the manufacturer.

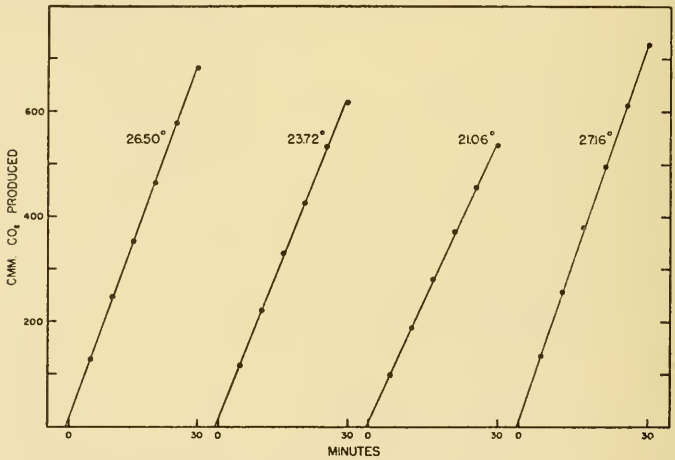


FIG. 3.—Carbon dioxide production by a single set of etiolated barley seedlings at different temperatures. Plotted from the record shown in figure 2.

Culture of plants.—A pure line of barley (variety Hannchen, C.I. No. 531), generously supplied by Dr. Merritt N. Pope, of the United States Department of Agriculture, has been used exclusively. After removal of the glumes, the seeds were soaked in tap water in darkness for a few hours, after which they were repeatedly rinsed[1] with sterile water and planted individually upon slants of one percent agar in tap water. The plant holders and method of support in the respiration chamber are illustrated in figure 5.

The seeds were germinated at 26.5° C. in a light-tight thermostatted germinator in which the humidity of the air was maintained at satura-

[1] The incidence of bacterial and fungal infections was quite low after this treatment. The application of microbicidal dusts such as ethyl mercuric phosphate (New Improved Semesan, Jr.) appeared to affect the subsequent respiration of the seedlings.

tion. After 3 or 4 days the seedlings were examined briefly in light of very low intensity (a masked flashlight was used) in order to select a uniform set and to discard any contaminated specimens. At this time the plant holders were inserted in the stopper (S in fig. 5), so that all subsequent manipulations could be performed in total darkness, and were returned to the germinator for at least 40 hours before the respiration measurements were begun. No indication that the preliminary illumination had an appreciable influence on the respiration 2 days later was obtained in special experiments undertaken to test this point. On the fifth or sixth day, when the shoots

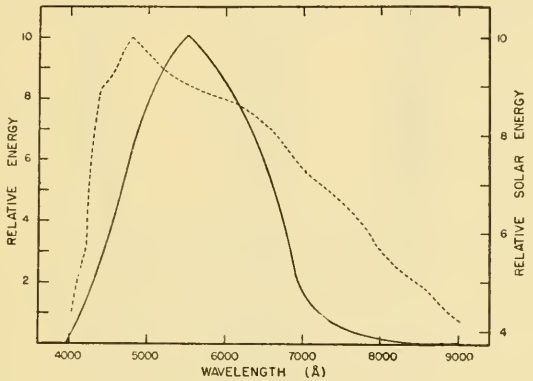


FIG. 4.—Spectral distribution of radiation. ——— artificial source (ordinates at left); - - - - - sun (smoothed curve for average day at Washington, D. C., and air mass = 1).

were 12 to 16 cm. long, the plants were introduced into the double-walled respiration chamber, which accommodated 19 seedlings.

Measurement of respiration.—The carbon dioxide content of the circumambient atmosphere was measured at 5-minute intervals over a 30- to 90-minute period. Preceding and following this the apparatus "zero" was determined for CO_2-free air, usually during half-hour periods. This establishes a base datum which permits correction for any constant drift in the filament emission which may be caused by change in the battery current. During the intervals between the respiration measurements the plants were exposed to CO_2-free air.

From the series of measurements for each respiration period, the rate of carbon dioxide production was calculated by the method of least squares. Unless otherwise stated all the rates have been corrected to a uniform temperature of 26.5° C.

CONSTANCY OF RESPIRATION RATE IN DARK

In order to assess the significance of changes in respiration rates accompanying altered conditions, it was essential first to establish the degree of constancy of the rate under fixed conditions.

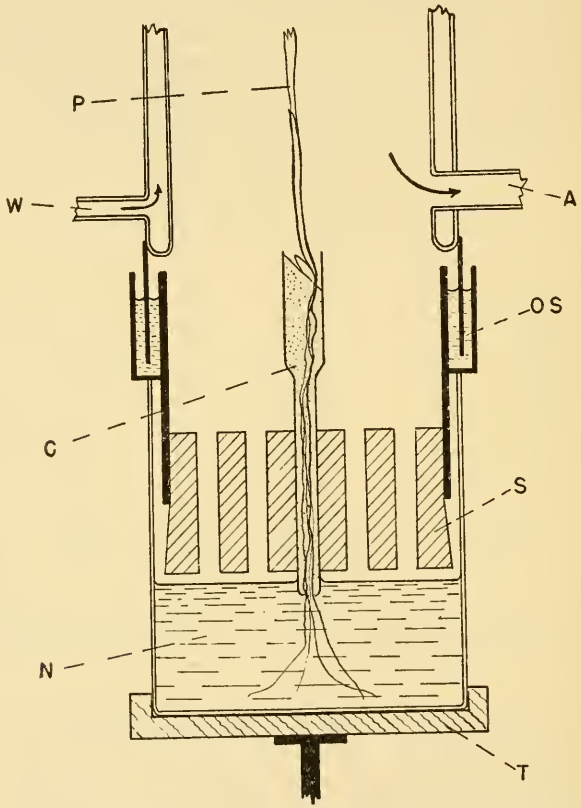


FIG. 5.—Diagrammatic view of respiration chamber in longitudinal section. S, perforated rubber stopper; C, glass tube containing agar substrate; P, plant; N, nutrient solution; T, turntable; OS, oil seal; A, outlet for circulating air which enters at top of chamber; W, inlet for water circulating between double walls of chamber ($\frac{1}{2}$ natural size).

The constancy of the dark respiration rate for an individual set of plants was determined by measuring the respiration in successive 30- or 60-minute periods, separated by half-hour periods during which the plants were maintained in CO_2-free air. Table 1 gives the results of six experiments, each with a different lot of seedlings.

The differences in absolute rates among the various experiments are due chiefly to differences in size of the plants of the individual sets.

TEMPERATURE COEFFICIENT OF RESPIRATION

Inasmuch as small variations in the temperature of the air were unavoidable, especially when the plants were exposed to light of high intensity, the temperature coefficient of respiration in darkness was determined so that correction could be made. The photographic record obtained in such an experiment is reproduced in figure 2. Figure 3 presents the data, after conversion to volumes of carbon dioxide.

TABLE 1.—*Carbon dioxide production by etiolated barley seedlings in darkness*

Experiment	Period	Respiration rate (cmm. CO_2/min./plant)	Mean rate for each experiment	Maximum deviation from mean, percent
8/12/40	1	1.396		
	2	1.395	1.385	1.6
	3	1.363		
8/14/40	1	1.434		
	2	1.498	1.479	3.0
	3	1.505		
8/19/40	1	1.314		
	2	1.284	1.295	1.5
	3	1.286		
9/30/40	1	1.401		
	2	1.458	1.429	2.0
	3	1.429		
10/30/41	1	1.506		
	2	1.434		
	3	1.492	1.469	2.4
	4	1.444		
11/4/41	1	1.110		
	2	1.099		
	3	1.098	1.104	0.5
	4	1.109		
				Average = 1.8

Over the temperature interval covered in this experiment, the rate of respiration varies linearly with the temperature. The temperature coefficient (Q_{10}) is equal to 1.6.

It is recognized that temperature coefficients for biological processes may be affected by a number of factors, including radiation (see Bělehrádek, 1935). However, in view of the relatively small variations of temperature (of the order of a few tenths of a degree) actually encountered in the experiments herein reported, it is considered justifiable to disregard this possibility for the present.

INFLUENCE OF CARBON DIOXIDE ON RESPIRATION

In the majority of experiments in which the respiration was measured for periods as long as an hour, it was observed that the rate of carbon dioxide evolution did not remain constant during this time but usually decreased somewhat. Table 2 presents representative data

TABLE 2.—*Variation of carbon dioxide production with time*

Experiment	Portion of run	Respiration rate (cmm. CO_2/min./plant)	Rates relative to first 30-min. interval of run
1	1st 30 min.	1.403	100
	2d "	1.400	99.8
2	1st "	1.419	100
	2d "	1.387	97.7
3	1st "	1.389	100
	2d "	1.362	98.0
4	1st "	1.419	100
	2d "	1.453	102.4
5	1st "	1.495	100
	2d "	1.494	99.9
6	1st "	1.551	100
	2d "	1.503	96.9
7	1st "	1.344	100
	2d "	1.288	95.8
8	1st "	1.302	100
	2d "	1.283	98.5
9	1st "	1.292	100
	2d "	1.259	97.4
10	1st "	1.389	100
	2d "	1.413	101.7
11	1st "	1.466	100
	2d "	1.432	97.7
12	1st "	1.465	100
	2d "	1.372	93.6

to exemplify the general finding. In each case the rates for the first and last 30-minute portions of an hour's run have been calculated separately.

For the 12 runs the average rate during the second 30-minute interval is 98.3 percent of that during the first 30-minute period. This difference is very nearly the same as that found, on the average, between successive runs, but it is significant that the difference is predominantly a *decrease* in rate with time.

Such a result is what would be expected if the rate of carbon dioxide evolution is the resultant of the rate of production of this gas by the respiring tissue and of the rate of its diffusion into the atmosphere. The method of measuring the carbon dioxide evolution in a closed system has the disadvantage that the external concentration of the

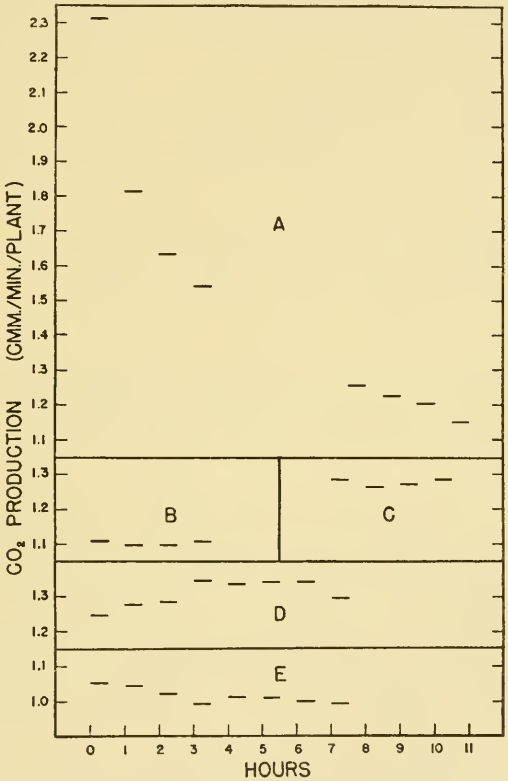


FIG. 6.—Carbon dioxide production by 5-day-old etiolated barley seedlings in darkness following exposure to various concentrations of carbon dioxide. A, in 5 percent CO_2 for 17½ hours; B, in normal air for 17½ hours; C, in normal air for 5 days; D, in CO_2-free air for 18 hours; E, in CO_2-free air for 18 hours.

gas is continuously augmented. In the present experiments the concentration increased from zero to 0.03-0.04 percent (approximately equal to the concentration in normal air) during a 30-minute run and to twice this value in 60 minutes.

The situation appears to be somewhat more complex than this, however, inasmuch as evidence has been obtained that the rate of carbon dioxide excretion at any given moment may be influenced not only by the CO_2 concentration in the environment at that particular

time, but also by the conditions to which the plant has been subjected previously. It was found that seedlings exposed to a high concentration of carbon dioxide exhibited subsequently a protracted falling rate of carbon dioxide excretion. The result of an experiment which illustrates this effect is shown in figure 6 A. Although the absolute rates of carbon dioxide excretion by different lots of plants are not strictly comparable (see table 1), it will be noted that conditioning the seedlings in air containing 5 percent CO_2 has resulted in an initial rate much greater than the normal range of variation. Similar, although less pronounced, results have been found also after conditioning in air of much lower carbon dioxide content (as low as 0.35 percent). In our earlier experiments, performed before this factor was appreciated, relatively large numbers of seedlings were grown in the closed germinator; it is calculated that, under the conditions employed, the carbon dioxide concentration could have risen to values of the order of 0.5 percent, which is sufficiently high to cause a marked after-effect.

Figure 6 C portrays the respiration of seedlings which had been grown in a box continuously aerated with normal air, and figure 6 B that of seedlings grown for 4 days in a closed box and then aerated for 17.5 hours.

Figure 6 A illustrates also the relatively long period of time required for the attainment of a constant rate of respiration following exposure to high concentrations of carbon dioxide.

These findings are very similar to those reported by Spoehr and McGee (1924) and by Willaman and Beaumont (1928). Although the mechanism of this carbon dioxide effect is not established, a number of possibilities suggest themselves. Unilluminated leaves of many species, including barley, are known to absorb carbon dioxide reversibly, by physicochemical processes unrelated to the vital activities (see Smith, 1940). The absorption is believed to be due to the water, dissolved buffer substances, and insoluble alkaline earth carbonates in the leaf which act as a sort of reservoir for carbon dioxide. In living leaves, Smith found absorption of the carbon dioxide and its evolution in vacuo to be rather rapid, only a few minutes being required for the attainment of equilibrium. Although this appears to be in sharp contrast with the results described above, it should be borne in mind that different conditions obtained, in that the evolution of carbon dioxide in our experiments occurred at atmospheric pressure. A more delayed attainment of equilibrium was observed by Hamon (1936) in the case of potato tubers, and by Gerhardt and Ezell (1934) with fruits of pear and apple.

In addition to its possible participation in such a physicochemical process, it is well established that carbon dioxide influences the respiratory process itself (see, e.g., Kidd, 1916; Thornton, 1933 *a, b,* 1935, 1937; Hamon, 1936). It is doubtful, however, that an effect of this kind plays a role in our results. On the other hand, it is conceivable that carbon dioxide might influence the gaseous exchange indirectly as, for example, by affecting the behavior of the stomata or the cellular permeability.

If there exists in the barley seedlings a CO_2 reservoir, it might be anticipated that maintenance of the plants in the absence of carbon dioxide would deplete the reservoir to such an extent that there would occur a deficit to be satisfied when CO_2 was again made available. Experiments in which plants were conditioned in CO_2-free air for several hours following a 4-day sojourn in the non-aerated growth chamber did not yield consistent results. Slowly increasing rates of carbon dioxide evolution were indeed found in a considerable percentage of such experiments, whereas in others the respiration appeared to be very constant. In a few cases, furthermore, decreasing rates were observed. Examples are illustrated in figures 6 D and 6 E. It should be noted that the various lots of plants were doubtless subjected to different conditions with respect to the CO_2 concentration during the first 3 or 4 days, depending upon the number and size of the plants present in the germinator and the number of times it was opened during this period. In view of the protracted influence shown to be exerted by a high concentration of carbon dioxide during the early part of the culture period, it is conceivable that a carry-over of the early treatment might manifest itself in a falling rate, even after several hours in CO_2-free air. On the other hand, if the early exposure to CO_2 had been somewhat less, the period in CO_2-free air might be just sufficient to deplete the reservoir to the extent that a relatively constant rate of CO_2 excretion could ensue.

The 30- or 60-minute intervals of exposure to CO_2-free air, which were customarily alternated with the respiration runs, appear to have been too short to have produced measurable effects on the subsequent respiration. This conclusion is supported by a number of experiments in which these intervals were extended, shortened, or replaced by periods of exposure to normal air.

In any event, while a clear understanding of the situation must await further experimentation, it is obvious that, in experiments in which plant respiration is measured by carbon dioxide excretion, the previous history of the plants with respect to this gas cannot safely be ignored.

INFLUENCE OF LIGHT ON RESPIRATION

In the experiments on the effect of illumination on the respiration of etiolated barley seedlings, the general procedure has been to measure the rate of carbon dioxide production first in darkness for a number of hourly or half-hourly periods, in order to ascertain whether the rate remains constant under these conditions. The plants

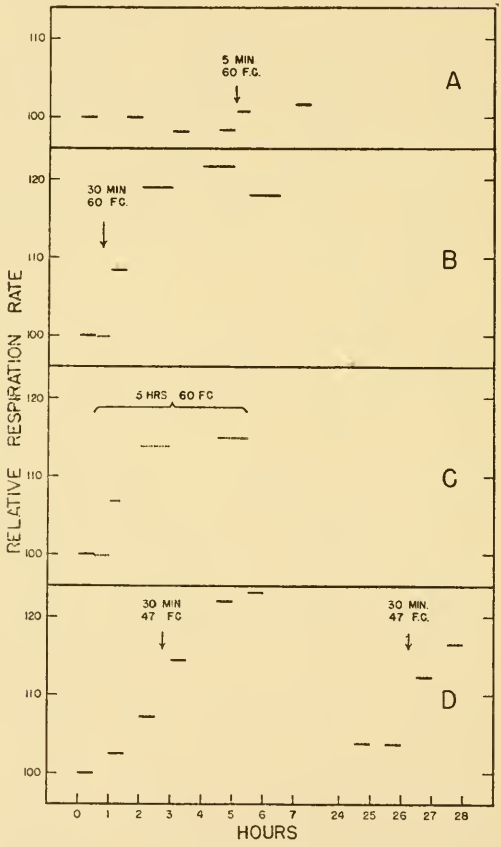


FIG. 7.—Influence of duration of illumination on carbon dioxide production by etiolated barley seedlings. ——— measured in dark; ..... measured in light.

were then illuminated, either with or without simultaneous measurements of the respiration, and following this the plants were again darkened and further measurements made. In some instances additional light exposures were given later.

A considerable amount of the accumulated data has been discarded because in the earlier experiments the marked influence of the pretreatment of the plants was not fully appreciated, and the initial dark

runs were not continued sufficiently to establish the absence of possible small drifts in respiratory rate. However, a few such experiments, of which the results are entirely in accord with those of unequivocal experiments, have been retained. For this reason, while the general trend of the results to be presented is unquestionable, the quantitative data are not regarded as having a high degree of precision.

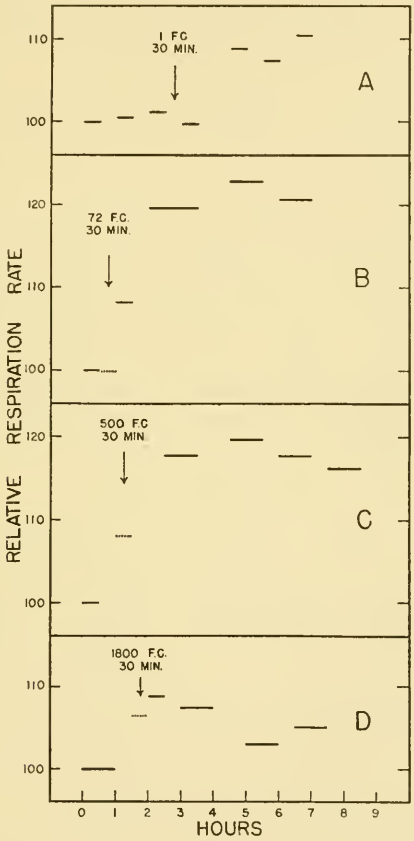


FIG. 8.—Influence of intensity of illumination on carbon dioxide production by etiolated barley seedlings. ——— measured in dark; ..... measured in light.

The illumination of etiolated barley seedlings with white light results in an increased rate of carbon dioxide production. We have performed 30 experiments, all of which have confirmed this finding. Some information concerning the effect of light intensity and exposure time on the magnitude and time course of the respiration response has been obtained. Representative results are illustrated in figures 7 and 8.

On exposure to light of fairly low intensity (180 foot-candles or less) no significant alteration of the rate of carbon dioxide excretion was usually apparent during the first 30-minute period. Following this, the plants, whether continued in light or darkened, exhibited a rate of respiration that increased to a maximum only after some hours and then gradually decreased (figs. 7 B and 8 B). The precise course of the declining rate has not been worked out; however, by 24 hours after a half-hour period of illumination the dark respiration was usually substantially the same as that found before exposure. At this time a second exposure to light again resulted in an increased rate of carbon dioxide production similar to that previously observed (fig. 7 D).

The magnitude of the respiratory stimulation is dependent upon the intensity and duration of the irradiation.

At an intensity of 60 foot-candles, exposures of 5 or 10 minutes elicited only insignificant effects (fig. 7 A), whereas illumination for 20 minutes or longer resulted in marked increases of the rate of carbon dioxide evolution (figs. 7 B and 7 C). On prolonged exposure the course of events is complicated by the formation of chlorophyll and the concomitant onset of photosynthesis; several hours of illumination, at the intensities employed, are required, however, for the latter process to attain a measurable value.

With an exposure time of 30 minutes, the magnitude of the increase in respiration rate appears to be relatively independent of the intensity, in the range of 50 to 500 foot-candles, and is of the order of 20 percent (figs. 7 B, 8 B, and 8 C). Smaller stimulations appear to be evoked by either higher or lower intensities (figs. 8 A, 8 D). Neither the upper nor the lower limit of effective intensity has been determined. There is some indication also that the higher intensities occasion a more prompt manifestation of the respiratory increase than do the lower intensities; thus in figures 8 C and 8 D, the stimulation is apparent during the illumination period itself.

There is considerable evidence that the respiration of a great variety of plant tissues is influenced by radiation, although very little is known of the mechanism of these effects (for literature see Weintraub, 1944). A number of characteristics of the effect here described indicate that the increased rate of carbon dioxide excretion is the result of a complex of responses and not merely of a simple photochemical reaction; these are the initial latent period, the prolonged character of the response, the peculiar relationship of the stimulation to the duration and intensity of the inciting radiation, and the lack of obedience of the reciprocity law. In certain respects these are very suggestive of

the characteristics of the so-called photodynamic processes (see Blum, 1941).

Among the possible indirect mechanisms which might be involved is the stomatal behavior. If the rate of gaseous exchange were limited by the size of the stomatal apertures and if the illumination resulted in an opening of the stomata, the increased respiration could conceivably be explained on this basis. However, we have been unable to demonstrate, by direct microscopic observation, such an effect of the light.

SUMMARY

The illumination of etiolated barley seedlings with white light occasions an increase in the subsequent rate of carbon dioxide evolution, whether measured in light or in darkness. As a result of the irradiation, the respiratory rate rises relatively slowly to a maximum and remains substantially at the new level for several hours. The magnitude of the stimulation and its time course appear to depend upon the duration and intensity of the exposure.

In the absence of light, the rate of carbon dioxide excretion is influenced also by alterations of the carbon dioxide content of the atmosphere in which the seedlings have been confined.

LITERATURE CITED

Bělehrádek, J.
1935. Temperature and living matter. (Protoplasma Monograph No. 8.) Berlin.

Blum, H. F.
1941. Photodynamic action and diseases caused by light. Reinhold Publishing Corp., New York.

Gerhardt, F., and Ezell, B. D.
1934. Retention of carbon dioxide gas in the intercellular atmosphere of pears and apples. Science, vol. 80, pp. 253-254.

Hamon, F.
1936. Influence de l'acide carbonique sur la respiration des tissus végétaux et de la levure. Ann. Physiol. Physicochim. Biol., vol. 12, pp. 940-982.

Kidd, F.
1916. The controlling influence of carbon dioxide. III. The retarding effect of carbon dioxide on respiration. Proc. Roy. Soc. London, ser. B., vol. 89, pp. 136-156.

McAlister, E. D.
1936. A spectrographic method of measuring carbon dioxide concentration. Phys. Rev., vol. 49, p. 704.
1937a. Spectrographic method for determining the carbon dioxide exchange between an organism and its surroundings. Plant Physiol., vol. 12, pp. 213-215.
1937b. Time course of photosynthesis for a higher plant. Smithsonian Misc. Coll., vol. 95, No. 24.

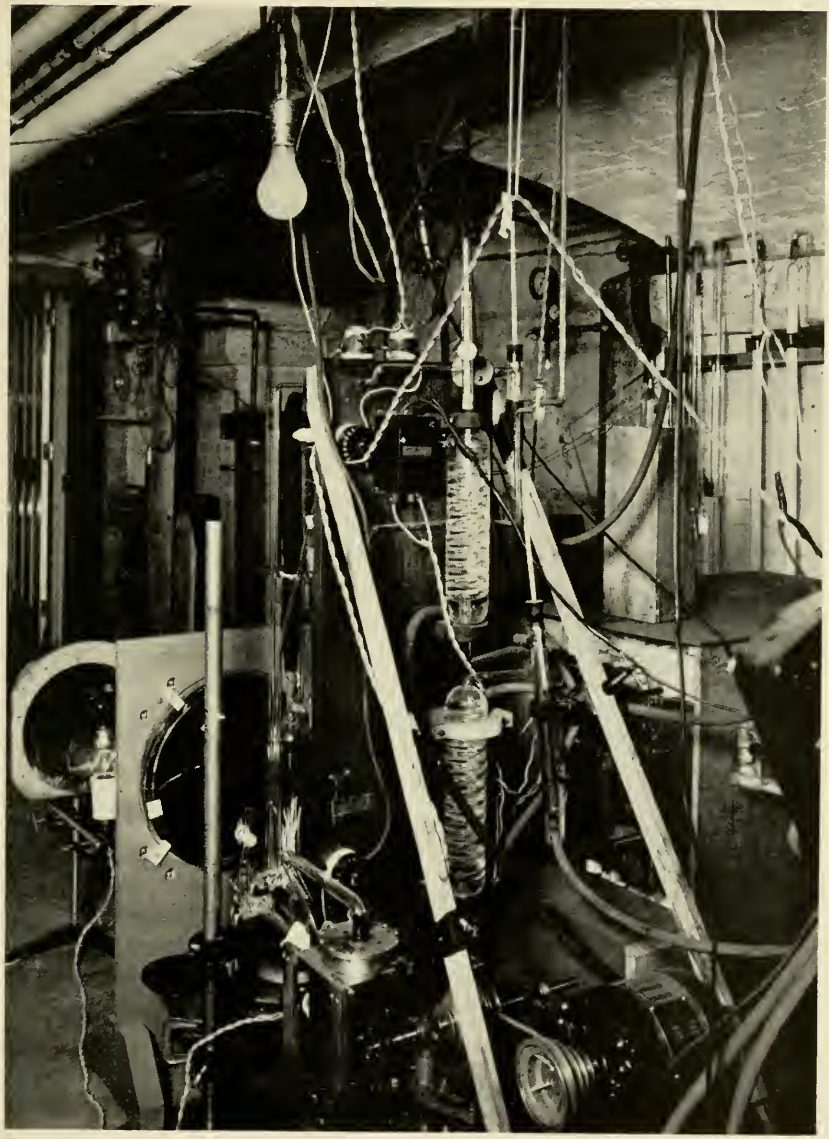
Smith, J. H. C.
1940. The absorption of carbon dioxide by unilluminated leaves. Plant Physiol., vol. 15, pp. 183-224.

Spoehr, H. A., and McGee, J. M.
1924. The effect of fluctuations in the CO_2 content of the atmosphere on the rate of respiration of leaves. Amer. Journ. Bot., vol. 11, pp. 493-502.

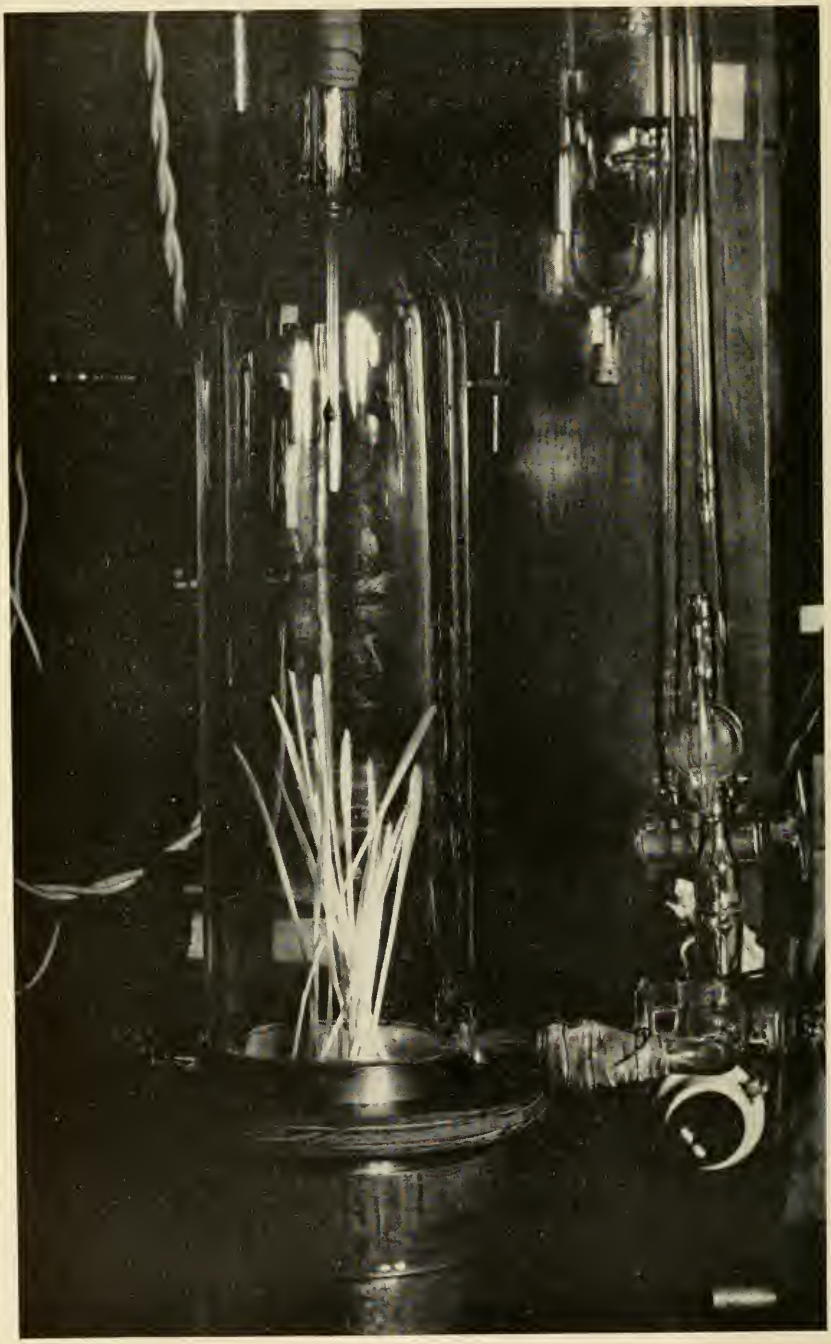
Thornton, N. C.
1933a. Carbon dioxide storage. III. The influence of carbon dioxide on the oxygen uptake by fruits and vegetables. Contr. Boyce Thompson Inst., vol. 5, pp. 371-402.
1933b. Carbon dioxide storage. V. Breaking the dormancy of potato tubers. Contr. Boyce Thompson Inst., vol. 5, pp. 471-481.
1935. Carbon dioxide storage. VIII. Chemical changes in potato tubers resulting from exposure to carbon dioxide. Contr. Boyce Thompson Inst., vol. 7, pp. 113-118.
1937. Carbon dioxide storage. X. The effect of carbon dioxide on the ascorbic acid content, respiration, and pH of asparagus tissue. Contr. Boyce Thompson Inst., vol. 9, pp. 137-148.

Weintraub, R. L.
1944. Radiation and plant respiration. Bot. Rev. (in press).

Willaman, J. J., and Beaumont, J. H.
1928. The effect of accumulated carbon dioxide on plant respiration. Plant Physiol., vol. 3, pp. 45-61.



VIEW OF APPARATUS USED FOR MEASUREMENT OF RESPIRATION



VIEW OF RESPIRATION CHAMBER WITH SEEDLINGS IN PLACE