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(WITH THREE PLATES)

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THE DEPENDENCE OF CARBON DIOXIDE ASSIMILATION IN A HIGHER PLANT ON WAVE LENGTH OF RADIATION

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(With Three Plates)

Growth in dry weight in autotrophic plants requires the absorption and assimilation of carbon dioxide from the air, and this is the basic physiochemical reaction of all life.

The problem of the effect of different wave lengths of light on the carbon dioxide assimilation by a plant has been the subject of investigation for many years. In most of the earlier investigations broad spectral regions were used, and not much attention was given to the distribution of energy in the various spectral regions. Thus, very little information may be obtained from these earlier investigations.

Among the first investigators to realize the importance of determining the distribution of energy in the spectral regions were Kniep and Minder (1909). They used sunlight during the middle of the day with glass filters to isolate the red and blue regions of the spectrum and a liquid filter for the green region. They came to the conclusion that red and blue light of the same intensity produced about the same rate of photosynthesis. Warburg and Negelein (1923), working with *Chlorella* suspended in solution and using a quartz mercury arc with line filters, reported that red light was the most efficient in producing photosynthesis, and blue light the least. In their experiment they assumed complete absorption of the light.

On the basis of equal energy absorption, Burns (1933) and Gabrielsen (1935), working with higher plants, both draw the conclusion that red light is the most efficient in producing photosynthesis, green next, and blue light the least. They both used rather broad spectral regions and obtained values for only a few regions of the spectrum. It is also not clear from their data that they obtained a true measure of the absorbed energy.

The purpose of the present investigation is to determine the rate of photosynthesis as a function of the wave length of light on the basis of equal values of incident radiation.

APPARATUS

The apparatus used in this experiment has been described in a paper by Hoover, Johnston, and Brackett (1933). Although the apparatus was rebuilt in order to improve the method of controlling the temperature and humidity, its general features remain the same. The plants are placed in a closed glass system in which a rapid recirculation of air is maintained. New air at a constant rate enters the system at one point and is drawn off at another for analysis.

The design of the glass which holds the nutrient solution was changed in order to permit a continuous flow of solution. The seal between the flask, holding the plant, and the growth chamber was made by the lower part of the growth chamber dipping into a cup of paraffin oil sealed to the neck of the flask. A view of the apparatus is shown in plate 1.

FILTERS

The problem of obtaining radiation of sufficient energy in narrow wave-length bands over an area large enough for even a small wheat plant is a difficult one. A good monochromator gives the best means of obtaining narrow bands, but in most cases the radiation intensity is low except for very small areas. Glass, liquid, or dyed gelatin films may be used to obtain bands of radiation of considerable intensity over a large area, but these filters transmit rather broad bands. Thus it is difficult to compare the results obtained with these filters. There are, however, certain combinations of these filters called line filters which do transmit narrow bands of radiation. The transmission of these filters is low, but when used with line sources such as the mercury arc, sufficient energy was obtained for this experiment. The following filters were used to isolate a few of the strong mercury lines.

Wave length (A)	Filter
5780	Corning monochromatic filter No. 4
5461	" " " " 5
4358	" " " " 7
4048	" " " " 8
3650	" " " " 10

The Christiansen filter described by McAlister (1935), although not an ideal filter, has certain advantages for an experiment of this nature. By merely changing the temperature of the filters it was possible to cover the entire spectral region of photosynthesis by using two filters with different proportions of carbon disulphide and benzene. In the first experiment with these filters, four filters 6 inches in diameter and 2 inches thick were used in order to illuminate the plants on four sides. The temperature of the filters was controlled by placing them in thermostated water baths. A very simple optical system was used in this case. A 1000-watt Mazda lamp was placed near the focus of a concave mirror in order to obtain a magnified image of the source at the plant. The light passed twice through the filter before reaching the plant. Figure 1 represents the energy distribution obtained with these filters under the condition of the experiment. The distribution of energy in the bands was not entirely satisfactory, owing

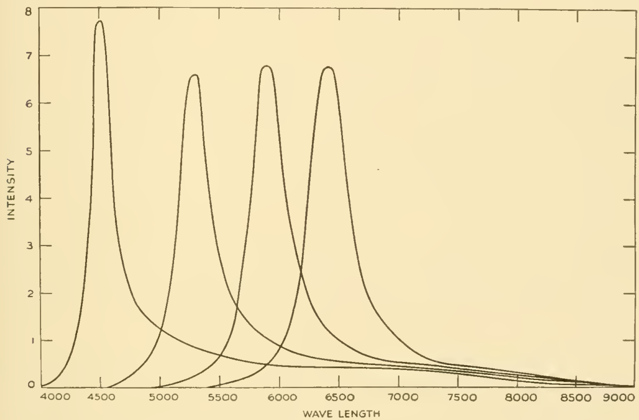

FIG. 1.—Energy transmission curves for 6-inch Christiansen filters.

to imperfections in the concave mirrors and to the fact that the filters were too thick to obtain good temperature control.

In the second experiment with the Christiansen filters sunlight was used as the source of radiation. Sunlight was obtained for the experiment by using a large coelostat located on the south side of the Smithsonian building. The moving mirror of the coelostat was 30 inches in diameter. Light reflected from this mirror was reflected by a second mirror to a third mirror located at one end of a long room. This mirror was about 60 feet from the plants. This combination of mirrors illuminated an area at the plant chamber about 15 inches in diameter. A Christiansen filter 12 x 14 inches and 1 inch thick was made in order to use most of the energy in this beam. The filter was placed 50 feet from the plant chamber. The distribution of energy in the spectral bands obtained with this filter is represented in figure 2. These bands are much narrower than those obtained with the 6-inch filters. Plate 2 shows the large coelostat and plate 3 the large filter with water bath for controlling the temperature. In order to use the entire width of the beam of light, which was five or six times as wide as the growth chamber, a system of plane mirrors was used to reflect the light to the plants. This gave a fairly uniform illumination over the entire plant.

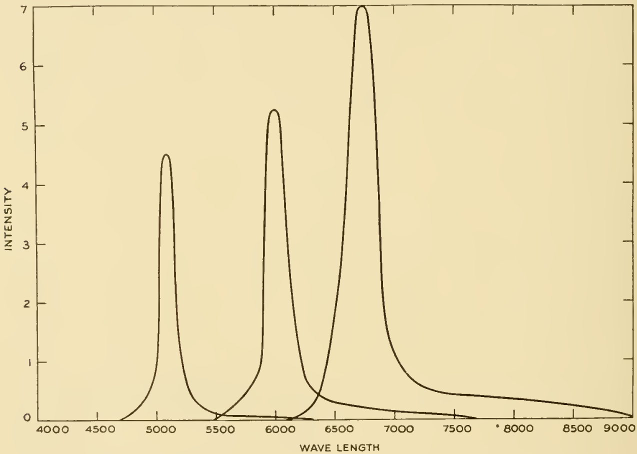

FIG. 2.—Energy transmission curves for the 12 by 14 inch Christiansen filters.

EXPERIMENTATION

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 or 3 cm, the young wheat 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 or 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 small holes in a paraffined flat cork stopper that fitted into the top of the flask of nutrient solution. The flask with the plant in place was then connected to the growth chamber in the manner explained above.

The plants were grown under controlled conditions for a few days before being used in an experiment. The temperature was about 21° C., humidity about 70 percent, carbon dioxide concentration two or three times that of normal air, the light intensity 1500 foot-candles for 18 hours a day, and the root temperature about 18° C. The plants were kept under these conditions except during the time observations were being made.

The light intensity obtained with the filters was never more than 300 foot-candles, the carbon dioxide concentration between two and three times that of normal air, and the temperature about 21° C. Under these conditions light was the limiting factor.

Using the quartz mercury arc and line filters, the ratio between the rate of photosynthesis at 5461 A and 4350 A remained about the same for the same plant on different days as well as for different plants grown under the same conditions. This furnished a means of comparing the results with various filters throughout the course of the experiment. 5461 A was used as the standard, and the ratio of the rate of photosynthesis at 5461 A and other wave lengths was determined.

In order to correct for respiration, the respiration rate was determined before and after each light observation, and appropriate corrections were applied to the photosynthetic rates observed.

RESULTS

Measurements were made at various wave lengths of the photosynthesis produced in wheat by the rays transmitted by Christiansen filters adjusted to give predominantly these several wave lengths. In each case the measurements corresponded to equivalent intensities of radiation transmitted by the filters as determined by a thermocouple placed inside the growth chamber. The direct results obtained with these filters are given in table 1 and figure 3. Curve A represents the results obtained with the large Christiansen filters and sunlight, curve B those obtained with the 6-inch Christiansen filters and Mazda lights, and the points marked X are those obtained with the line filters and a quartz mercury arc.

The curves show two maxima of photosynthesis—one in the blue about 4400 A, and the other in the red at 6500 A. The green region of the spectrum was less effective. The maxima obtained with the large filters are somewhat higher than those obtained with the small filters, owing to the fact that the wave-length bands are narrower in the first case.

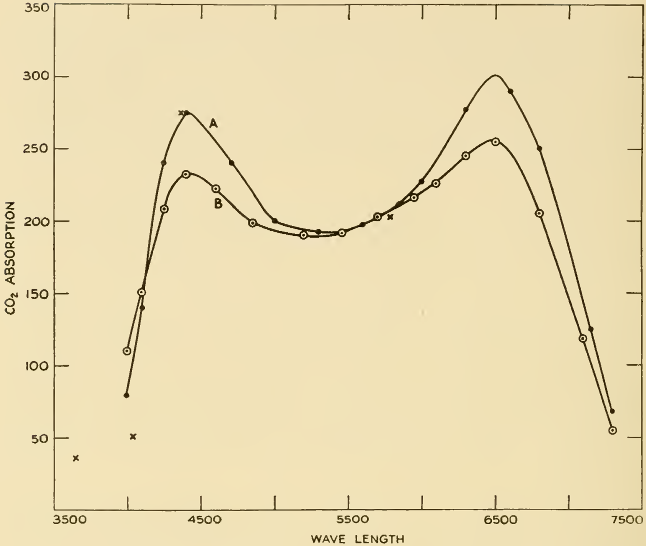

FIG. 3.—Wave-length assimilation curves. A, results obtained with large Christiansen filters and sunlight; B, results obtained with 6-inch Christiansen filters and Mazda lights. Points marked X, the results obtained with the line filters and quartz mercury arc.

TABLE 1.—*Carbon Dioxide Absorption in Different Wave-length Regions*

(Original uncorrected mean values)

λ	Large Christiansen filters	λ	Small Christiansen filters	λ	Corning line filters
4000	80	4000	110	3650	37
4100	140	4100	151	4048	52
4250	240	4250	208	4358	275
4400	275	4400	232	5461	193
4700	240	4600	222	5780	203
5000	200	4850	199		
5300	193	5200	191		
5461	193	5461	193		
5600	198	5700	204		
5850	212	5950	217		
6000	227	6100	226		
6300	277	6300	245		
6500	301	6500	255		
6600	295	6800	205		
6800	250	7100	118		
7150	125	7300	55		
7300	68				

CORRECTION OF CURVES FOR STRAY LIGHT

These curves of photosynthesis and wave length may be regarded as approximately correct, but need improvement because the Christiansen filters, besides the predominant wave lengths, transmitted others in lesser degrees.

Consider, for example, any wave length, as at the blue maximum of curve A in figure 3, connecting photosynthesis and wave length. For brevity call such a wave length W_0. On either side of this spectral point consider equal wave-length intervals of width a to be set off. Thus we reach wave lengths $[W_0-ma]$, $[W_0-(m-1)a]$, ... $[W_0-a]$, $[W_0]$ $[W_0+a]$, ... , $[W_0+(n-1)a]$, $[W_0+na]$. Consider this succession of equally spaced wave-length places to extend from the wave length $[W_0-ma]$, where the Christiansen filter ceases to transmit shorter waves appreciably, or else where photosynthesis sensibly ceases, to the wave length $[W_0+na]$ where it ceases to transmit longer waves appreciably, or else where photosynthesis sensibly ceases.

We have now to deal with two curves: 1, the approximately determined curve of photosynthesis and wave length; 2, the curve of energy transmission and wave length for that Christiansen filter which transmits predominantly the wave length W_0.

Let $[e_0]$, $[e_{-m}]$ . . . . $[e_{-1}]$, $[e_{+1}]$ . . . . $[e_{+n}]$ represent the ordinates of curve no. 1 at the wave length region W_0 and at wave lengths situated at equal intervals of width a on either side, thus covering the whole range of spectrum from the place $[W_0-ma]$ to $[W_0+na]$. Let $[C_0]$, $[C_{-m}]$ . . . . $[C_{-1}]$, $[C_{+1}$ . . . . $[C_{+n}]$ be the areas included under the curve no. 2 above described, within intervals of wave length a from the wave length $[W_0-ma]$ to the wave length $[W_0+na]$. These areas will be proportional respectively to the energy transmitted by the Christiansen filter which transmits predominantly the wave length W_0, but whose transmission extends in sensible degree all the way between wave length $[W_0-ma]$ and $[W_0+na]$. The sum total of these areas we may call q, proportional to the total amount of energy of radiation transmitted by the filter. If we divide the measured areas $[e_{-m}]$ . . . . $[e_{+n}]$ by the sum total of these areas, the new values of $[e_{-m}]$ . . . . $[e_{+n}]$ will represent the fractional part of the total energy q in each wave length interval a from the wave length $[W_0-ma]$ to $[W_0+na]$, and $\Sigma(e_{-m} \ldots\ldots\ldots e_{+n})=1$.

Inasmuch as the curve no. 1 described above represents approximately the dependence of photosynthesis on wave length, therefore the products $[e_{-m}C_{-m}]$ . . . . $[e_0C_0]$ . . . . $[e_{+n}C_{+n}]$ will each represent approximately the amount of photosynthesis contributed by an element of spectrum of width a which is transmitted by that Christiansen filter which predominantly transmits wave length W_0. The sum total of these products $\Sigma(e_{-m}C_{-n} \ldots . e_0C_0 \ldots . e_{+n}C_{+n})$ represents the total photosynthesis, originally measured as e_0. Of this sum of products only one, e_0C_0, represents the photosynthesis that is produced by radiation lying between the wave lengths $[W_0 - \frac{1}{2}a]$ and $[W_0 + \frac{1}{2}a]$. But this interval contains the fraction C_0 of the total amount of energy of radiation which was employed in the experiment at wave length W_0.

We find, then, that in the experiment, as actually performed, C_0q of energy produced

$$e_0 \times \frac{e_0C_0}{\Sigma(e_{-m}C_{-m} \ldots . e_0C_0 \ldots . e_{+n}C_{+n})}$$

of photosynthesis. Had the full unit quantity, q, of radiation of wave length W_0 been used, then the amount of photosynthesis would have been (according to the first approximation):

$$\frac{e_0}{C_0} \times \frac{e_0C_0}{\Sigma(e_{-m}C_{-m} \ldots . e_0C_0 \ldots . e_{+n}C_{+n})} .$$

Performing a similar operation at all wave lengths where photosynthetic measurements were made, we are now able to plot a new curve in place of curve no. 1, somewhat closer to the true form which the photosynthetic curve should take. This new curve may now be used as a new point of departure to obtain a still better approximation. That is to say, starting a second time with the original curve no. 1, we employ the corrected form of it, not itself, in determining new and better correction factors by which to multiply the ordinates of the original curve no. 1 to obtain from it a still better approximation to the true form desired. It has been found desirable to repeat these steps twice in case of curve A obtained with the large Christiansen filters and three times in case of curve B obtained with the small filters. After that no appreciable change in the form occurred and the process was discontinued.

A numerical example of the first corrected approximation is given in table 2, for wave length above defined as W_0. In the example W_0 is 4600 A and the wave-length intervals a are 200 A.

Using the results given in table 2,

$$\frac{e_0}{C_0} \times \frac{e_0C_0}{\Sigma(e_{-m}C_{-m} \ldots . e_0C_0 \ldots . e_{+n}C_{+n})} = \frac{257}{.700}\,\frac{257 \times .700}{249.7} = 265$$

the first approximation of the amount of photosynthesis produced at 4600 A as originally measured was 257.

In table 2, column 1 gives the wave lengths at the center of the wave-length intervals, column 2 the fractional part of the total energy transmitted by the filter in the various wave-length intervals, column 3 the measured values of photosynthesis, and column 4 the approximate amount of photosynthesis contributed by each wave-length interval. The sum total of column 4 represents the amount of photosynthesis originally measured as 257.

A similar operation was performed for each 200 A wave-length interval for the whole spectral region covered by the photosynthetic measurements. These values represent the first corrected approximation to the photosynthesis and wave length curve. Subsequent corrected approximations will be understood by what has been said in the preceding paragraphs.

In table 3, column A represents the measured values of photosynthesis determined with the large Christiansen filters, and columns A_1, A_2, and A_3 are the first, second, and third corrected approximations, respectively. Column B represents the measured values of photosynthesis obtained with the small filters, and columns B_1, B_2, B_3, and B_4 are the first, second, third, and fourth corrected approximations, respectively. Only small changes occur in the form of the curves after the first corrected approximation in case of the large filters, and after the second corrected approximation in case of the small filters.

FINAL RESULTS

The final corrected results, in each case, have been plotted in figure 4. Curve A_3, the corrected form of the curve obtained with the large Christiansen filters, is considered to be the curve nearest to the true form of the photosynthesis–wave-length curve.

The points on the original curve are based on the mean of five or more determinations of the ratio between the photosynthesis produced at 5461 A and at the wave length at the given point. Individual determinations did not vary on the average more than 4 percent from the mean ratio. Thus we may assume that the original values of the photosynthesis produced by the energy transmitted by the various filters are accurate to about $\frac{4}{\sqrt{4}}$ percent or 2 percent.

In correcting the original curve another source of error is possible. The distribution of energy transmitted by the large Christiansen filters was measured by a double monochromator on clear days, near noon. Since the distribution of energy in sunlight varies with the transparency of the air, it is not certain that the measured values

TABLE 2.—*Data Used in the Calculation of the first Corrected Approximation to the True Value of Photosynthesis at 4600 A*

$(W_{-ma}\cdots W_0\cdots$ $\cdots W_{+na})$	$(C_{-m}\cdots C_0\cdots$ $\cdots C_{+n})$	$(e_{-m}\cdots e_0\cdots$ $\cdots e_{+n})$	$(e_{-m}C_{-m}\cdots e_0C_0\cdots$ $\cdots e_{+n}C_{+n})$
4000	0	80	0
4200	.004	211	0.8
4400	.106	275	29.1
4600	.700	257	180.0
4800	.122	225	27.4
5000	.033	200	6.6
5200	.018	193	3.5
5400	.013	193	1.5
5600	.004	198	0.8
5800	0	200	0
		Sum......	249.7

TABLE 3.—*Corrected Values of Carbon Dioxide Absorption in Different Wave-length Regions*

	Large Christiansen filters				Small Christiansen filters				
λ	A	A_1	A_2	A_3*	B	B_1	B_2	B_3	B_4*
4000	80	64	51	50	110	85	65	60	55
4200	211	219	223	223	223	189	196	202	206
4400	275	289	292	292	232	267	275	280	281
4600	257	265	263	263	222	249	251	254	254
4800	225	226	226	227	203	218	218	220	221
5000	200	199	200	200	194	203	199	197	196
5200	193	189	190	190	191	199	193	191	190
5400	193	188	185	186	191	203	194	191	190
5600	198	193	190	191	198	213	203	199	198
5800	208	199	194	194	207	228	219	214	213
6000	227	224	219	218	220	247	236	228	225
6200	260	270	264	264	236	275	268	264	263
6400	295	328	329	330	253	316	321	324	325
6600	295	345	351	352	248	320	330	335	336
6800	250	301	305	307	205	275	277	279	280
7000	177	212	213	214	149	185	190	191	192
7200	105	123	124	124	86	98	100	102	103
7400	30	22	16	13	25	22	16	13	11

* Final results.

for the various filters represents the real distribution at the time of observation. This factor may account for a large part of the variations in the original observations.

In order to form an estimate of the importance of this source of error for the corrected curve, I computed two sets of energy distribution curves, one for a hazy day and the other for a clear day. Following the method given above, two forms of the corrected curve

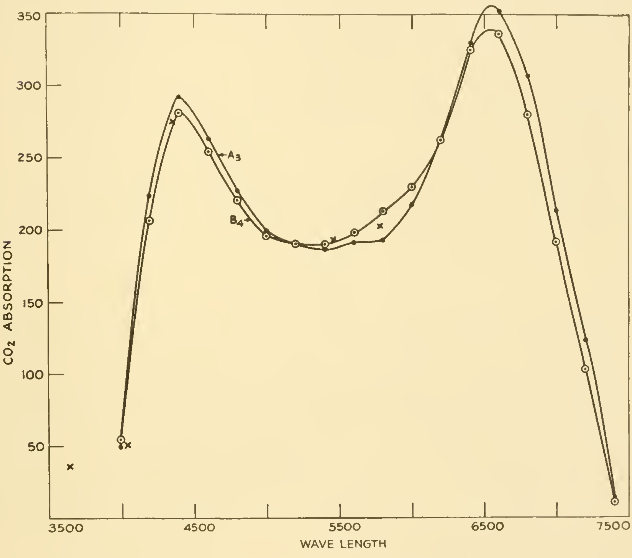

FIG. 4.—Wave-length assimilation curves. A_3, the corrected form of the curve obtained with the large Christiansen filters; B_4, the corrected form of the curve obtained with the small Christiansen filters. Points marked X, the results obtained with the line filters and quartz mercury arc.

were obtained. The maximum difference between these curves was about 3 percent. This difference represents two extreme cases; thus we may assume that the corrected curve would not be changed by more than half the error mentioned. This error added to the error of the original measurements would indicate that curve A_3 represents the true form of the photosynthesis–wave-length curve to a probable error of about 4 percent. The curve B_4, as well as the values obtained with the line filters, agree with curve A_3. The difference is 5 percent or less except in a short wave-length interval near 5800 A. Values for wave lengths longer than 6800 A are probably in error by more than 5 percent, since they are materially affected by the assumption that photosynthesis ceases at 7500 A.

Some measurements made by Dr. McAlister, in this laboratory, on the amount of light transmitted by a wheat leaf, combined with data by Shull (1929) on the reflecting power of green leaves, show that less photosynthesis may be expected in the green region, owing to the fact that the leaves transmit and reflect a larger portion of the incident radiation in this region. Although it is not possible as yet to interpret the results of my experiments in terms of equal *absorbed* energy, a correction for the reflecting power and transmission of the leaf would probably raise the photosynthetic activity of absorbed green radiation to be equal to or greater than that for the blue region. There may probably still be a maximum in the red.

The long wave-length limit of photosynthesis appears to be between 7200 A and 7500 A. Using the strong line of mercury, some slight amount of photosynthesis was still found at 3650 A in the ultraviolet.

SUMMARY

The rate of photosynthesis on the basis of equal incident energy was determined as a function of the wave length of light for a wheat plant. The entire visible spectrum is effective in producing photosynthesis. The wave-length limits, although not accurately determined, appear to be between 7200 A and 7500 A on the red end, and less than 3650 A on the blue end of the spectrum. A principal maximum occurs at 6550 A in the red, and a secondary one at 4400 A in the blue. Increased reflection and transmission of radiation in the green region by plant leaves diminish the effectiveness of incident green rays to promote photosynthesis.

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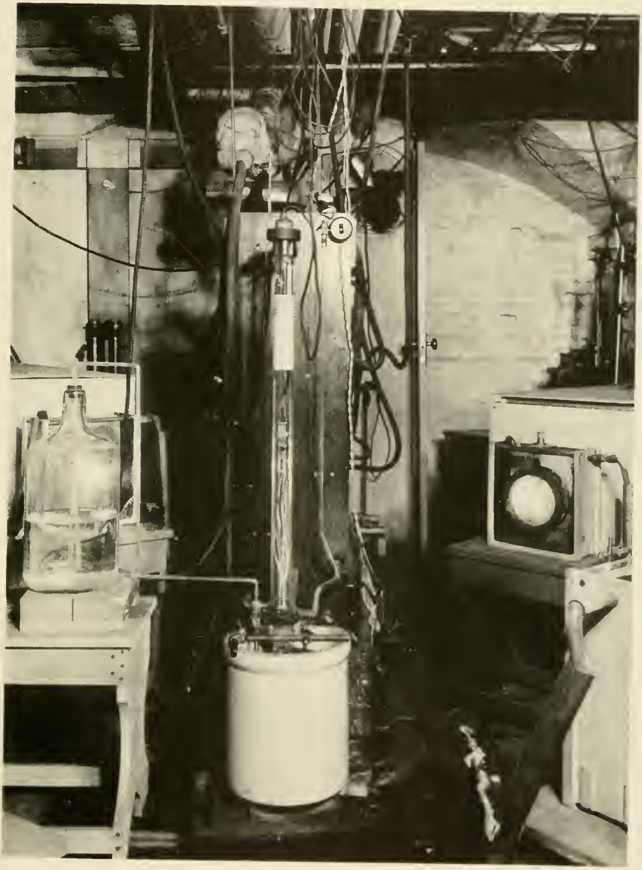
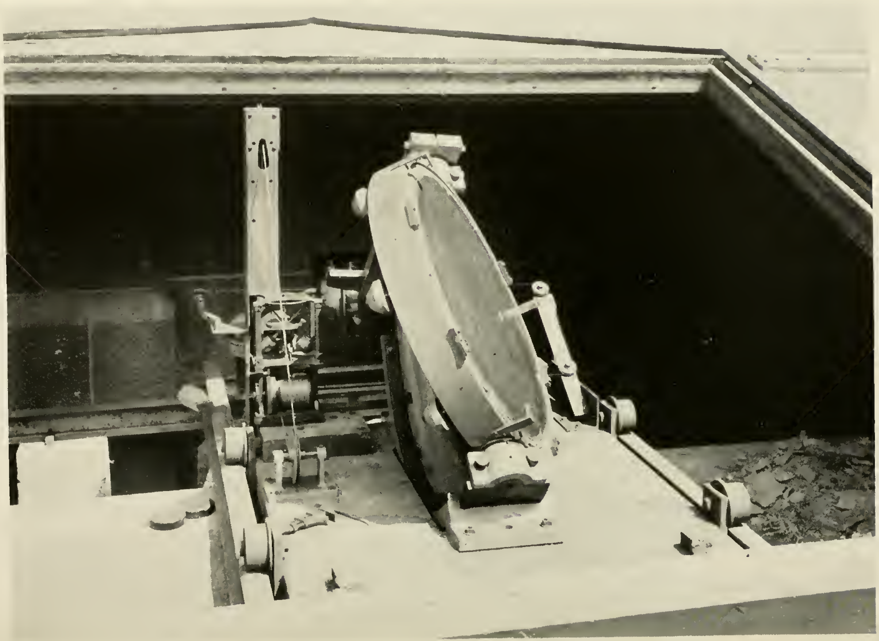


A GENERAL VIEW OF THE APPARATUS SHOWING THE GROWTH CHAMBER AND ONE OF THE 6-INCH CHRISTIANSEN FILTERS



THE 30-INCH MIRROR COELOSTAT

THE 12 BY 14 INCH CHRISTIANSEN FILTER AND TEMPERATURE CONTROL SYSTEM