THE ABSORPTION OF ULTRA-VIOLET LIGHT BY SOLUTIONS OF PLANT PIGMENTS

by

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STATEMENT OF PROBLEM

The purpose of this research was to determine the absorption of ultra-violet light by solutions of various plant pigments, and to determine the absorption bands within the visible spectrum.

DISCUSSION OF THE LITERATURE OF PLANT PIGMENTS

The general groups of plant pigments are: the chlorophylls or greens; the carotinoids and anthoxanthins which cause the yellows, oranges, and brownish reds; the anthocyanins which cause red, blue, and violet colors; phycoerythrin which causes red in sea weeds; and phycophaein which is in brown algae. The last two groups were not considered in this work.

Some plant pigments, as the chlorophylls and carotinoids, occur in the chloroplasts, and others, as anthocyanins, are in solution in the cell sap. Chlorophyll is

the substance which makes photosynthesis possible. So these chloroplasts have the ability to change the radiant energy of the sun into the potential energy of the carbohydrates. Some investigators think the pigment is dissolved in an oily substance which is held in the meshes of the plastids;others believe it is in the form of a precipitate. It is soluble in alcohol, and alcohol solutions are generally used for spectroscopic studies. Willstatter (6) has made the most detailed study of chlorophyll, and he states that the absorption spectra of alcohol solutions depend upon the concentrations of the solutions, and the material from which they were prepared. If the leaves have been placed in boiling water before being placed in alcohol the solution has different properties than one prepared without the hot water treatment.

According to Haas and Hill (2), Tsweet (1910) observed that the absorption bands of the spectrum of the living leaf was displaced toward the red end of the spectrum as compared with the bands in the spectrum of extracted chlorophyll.

Closely associated with the chlorophylls are the carotinoids. They are insoluble in the cell sap, and occur in separate chromoplasts, or with the chlorophyll in the chloroplasts. There are four carotinoids. The most important are carotin and xanthophyll which are yellow. Lycopin

or lycopersicin is a third carotinoid found in tomatoes. It may be yellow or red depending on the temperature. For example, tomatoes ripened at a very high temperature would be yellow instead of the normal red. The fourth carotinoid is fucoxanthin. It is brownish red and is found in seaweeds. According to some authors the carotinoids have their origin in a particular kind of elongated mitochondria; others regard them as decomposition products of chlorophyll. They may occur in crystalline or amorphous form. It is very difficult to obtain appreciable quantities in the crystalline form, so the pigments are usually studied in solution. Carotin in 95 per cent alcohol gives yellow to golden solutions. Palmer (4) found two and in some cases three absorption bands in the green and blue parts of the spectrum. He found that the width and intensity of the absorption bands varied with the concentration of the solution, and with the thickness of the layer. He observed that the solar F-line was practically in the center of one band, and he used this to distinguish the carotin spectrum from the other carotinoids.

Anthoxanthins include a number of yellow pigments which are found in the vegetative organs, and in the petals of plants. They are closely related to the anthocyanins which cause blue and red colors. Some of these pigments are of

considerable value as dye stuffs. They are widely distributed among the higher plants. They are most abundant in plants which grow in conditions of insolation, unless there is a thick cuticle or hairs for protection against light rays of shorter wave length. There is sometimes an interchange between the anthoxanthins and anthocyanins. When the plant is young, it may contain red anthocyanin which changes to anthoxanthin in the mature plant. At leaf fall the anthocyanin may reappear. They are soluble in acids and alkalies, giving yellow to red solutions.

Anthocyanins occur in the cell sap. They are often present in quantities sufficient to entirely mask the green color of the chlorophyll which may also be present. If they are in a neutral solution, they have a violet tint. If the solution is acid, they have a red tint, and if in an alkaline solution a blue color results. Thus the pigment seems to be an indicator within the plant itself, showing whether the cell sap is neutral, acid, or alkaline. The presence of anthocyanin is affected by light of high intensity. For example, apples and other fruits, and the vegetative organs of certain plants will not assume a red color if kept in the dark. On the other hand light does not seem to be of much importance in the formation of anthocyanin in the roots of

beets. The spectrum is complementary to that of chlorophyll, the main absorption bands being in the yellow and yellow green, with minor ones in the blue end of the spectrum. Thus anthocyanin absorbs those rays which are not absorbed by chlorophyll, and which are the least harmful to the chlorophyll. However, it does not absorb the rays in the green region which are most harmful to chlorophyll. So it would seem that the red color is not a protective screen as some people have thought.

The appearance of anthocyanin is closely related to the sugar content of the tissues in which it occurs. The red dye will appear in certain aquatic plants if they are placed in a weak sugar solution and exposed to strong sunlight at ordinary temperatures. This red color does not appear if the plants are grown in water or in diffuse daylight.

According to Haas and Hill (2), Overton experimented with the effect of temperature in addition to light and sugar. At low temperatures the red pigment formation was promoted, which accounts for the red color prevalent in Alpine plants, and may account for autumnal coloring. Red coloring matter often appears in stems or leaves which have been injured. The injury interrupts the transfer of carbohydrates from the leaf so sugar accumulates above the cut.

According to Haas and Hill (2), Pick thought the dye was a filter to separate from the light falling on the leaf certain rays which would hinder the translocation of the starch. Keeble found that in leaves which had the dye on one side but not on the other, the difference in temperature was about 2°C., so he concluded that it was of value as a protection against the heating effect of strong sunlight. Stahl thought it absorbed heat and so increased transpiration. Ewart thought it was to protect the chlorophyll against the action of too strong sunlight. He also thinks that in the beet root it has no special function, and may be a waste product of metabolism.

Anthocyanin may be combined with anthoxanthin or with some of the yellow plastid pigments. The resultant color may be brown, crimson, scarlet, or orange-red. Purple anthocyanin and chlorophyll in plastids may produce dark brown or black. These two pigments are complementary and one absorbs what the other does not.

GENERAL METHOD OF PROCEDURE

Briefly, the general method of procedure was to tear fresh flower petals into small pieces and then extract the pigment with a suitable solvent. The resulting solution was filtered and placed in a quartz cell. Spectrographs were then taken, using a carbon arc as the source of light. First the bare arc was photographed, then the cell containing the solution was placed in front of the collimator. Exposures were made varying the time so as to observe (1) the absorption bands which would appear on short exposure, and (2) the wave lengths which were not transmitted regardless of the length of exposure.

APPARATUS

The spectrograph used was a Bausch and Lomb quartz spectrograph designed for spectra between 7000 A^{o} and 2100 A^{o}. See figure 1 for a view of the spectrograph with the arc and cell in position.

Fig. 1

Any spectrograph is composed of the following essential parts: (1) the slit, (2) the collimator lens system, (3) the dispersing system, and (4) the recording system.

The slit was operated by a micrometer screw which read to 0.01 mm. For nearly all of this work it was opened to 0.03 mm. Just in front of the slit there was a slide with a long, horizontal V-shaped opening. This was adjusted so that the vertical length of the slit was 6 mm. This allowed six exposures with the wave length scale adjacent to each, on a plate. The slit was protected by a thin quartz cover glass, and in front of this was the shutter.

In the lens system the light passed through the slit, was diffracted, and passed down the collimator tube through a quartz lens which bent the beams into parallel rays. This lens was mounted on a casting which was attached to the base and permanently fixed in its proper location. Its focal length for 5893 A^{o} was 610 mm., and its aperture was 50 mm.

The dispersion prism was mounted on an adjustable table supported by a separate casting. There were adjustments to bring it into proper rotation to the axis. There was also an adjustment to put it in the position of minimum deviation.

The dispersing prism was of the Cornu type, made of two 30° x 60° x 90° prisms, one of right quartz and one of left quartz, cemented together in optical contact. The camera lens had the same focal length and aperture as the collimator lens.

The plate holder support could be moved up or down by means of a screw. A scale divided into millimeters was provided so exposures could be evenly spaced on the plate. The plate holder was made of aluminum. It had a metal shield and springs on the back cover forced the plate against curved metal seats so that it brought the entire spectrum into focus at once.

A wave length scale was provided which had its own light for illumination when the scale was brought into position for exposure by means of a lever on the outside of the case. The scale was graduated from 7000 A° to 2100 A°. Between 7000 A° and 4000 A° a line could be estimated to 10 A°. From 4000 A° to 2100 A° the line could be estimated to 1 A°.

The entire optical system was completely enclosed in a metal case which was light tight. Most of the parts were supported from the heavy base which was provided with leveling screws. The interior was divided so far as possible

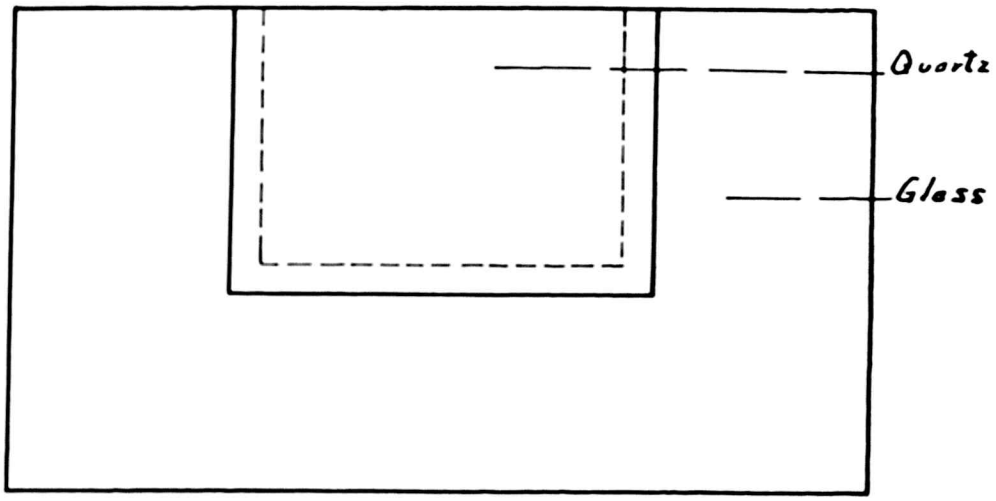
into three compartments, thus reducing the possibility of reflected light.

Panchromatic plates 4 x 10 inches were used. They were developed for 2.5 minutes in a developer made as follows:

16 ounces water
0.6 gram elon
5 grams hydroquinone
15 grams sodium sulphite
15 grams sodium carbonate
1 gram potassium bromide

They were then washed in clear water and placed in a fixing bath for 15 minutes. The fixing bath was made by dissolving 0.5 of a pound of Hypo in 32 ounces of water. They were then placed in running water for 15 minutes, dried at room temperature, and stored in a cool, dry place. The prints were made on sensitized paper using the same developing and fixing baths.

The quartz cell was made by cutting a rectangular piece out of the side of a piece of plate glass. Then quartz plates were attached on each side of this opening. with waterglass. The sketch shows the cell drawn full size. It held a layer of solution 9 mm. thick.



Another cell, 27 mm. thick, was made of hard wood with quartz sides.

The source of light was a carbon arc. Two carbon electrodes were connected across a 220 volt circuit, with a suitable resistance in series. It was arranged so that the positive terminal was in line with the collimator slit since it gave the brightest light. The negative terminal was in a vertical position and was raised just enough that it did not interfere with the light leaving the positive terminal in the direction of the slit. The spectrum thus obtained ranged from 7000 A^{o} to 2200 A^{o} and contained a great many lines rather uniformly distributed. These

characteristics made it a suitable source of light for this experiment.

PREPARATION OF SOLUTIONS OF PLANT PIGMENTS

The choice of solvents was one of the first questions. It had to be something which would not absorb the ultraviolet light itself. Figure 2 shows the spectra of the solvents used.

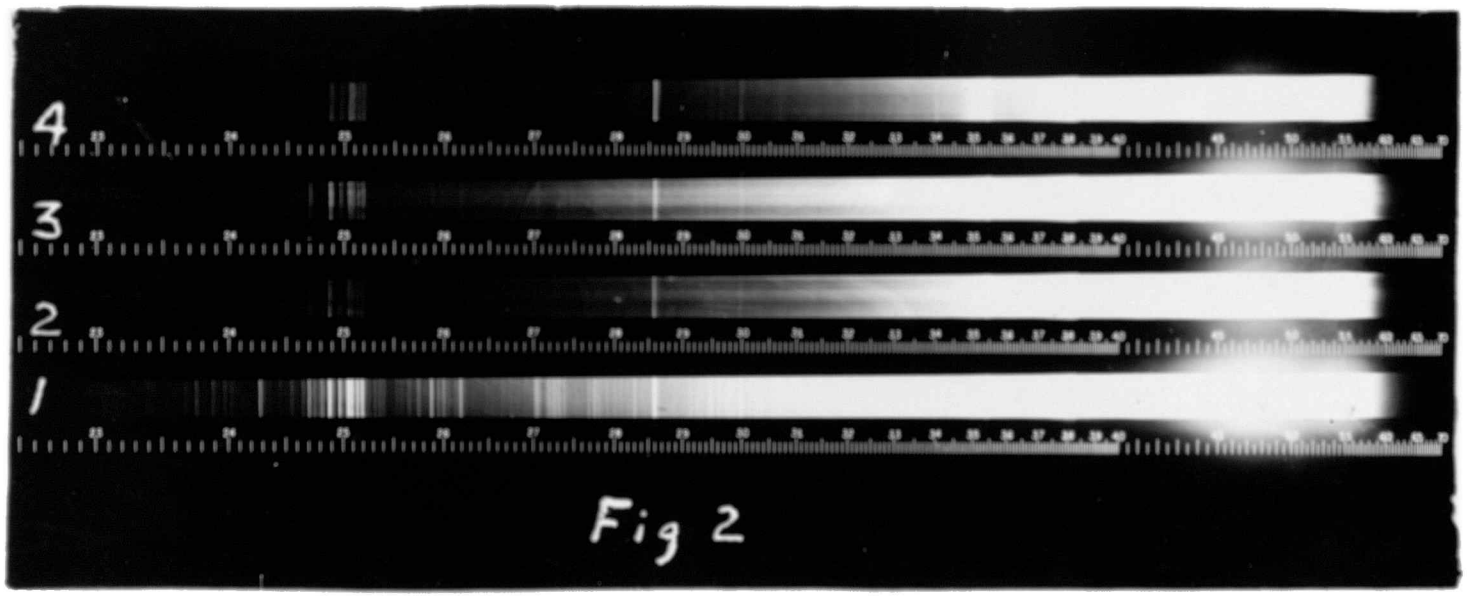


Fig 2

No. 1 is the bare arc. No.2 is the arc with the quartz cell interposed. No.3 is the arc with the cell filled with alcohol interposed. No.4 is the arc with the cell filled with distilled water interposed. This figure shows that alcohol and water do not have absorption bands in the ultra-violet and so were suitable solvents so far as their spectra were concerned. Alcohol was the most satisfactory. Hot water was found to be better in a few cases. Ether also extracted some pigments very well but it was difficult to obtain sufficient solution for experimental purposes if this solvent was used. This was due to the fact that the solution was very concentrated, and could not be diluted by the addition of more of the solvent.

The pigments were obtained entirely from the petals and leaves of the plants. The material was torn into small pieces and sufficient solvent added to cover them. These were then stirred and crushed with a glass rod until the color was removed. The solution was then filtered into a beaker and was ready for use.

At first the solutions were made up and stored for later used. However, some of them showed distinctly that light faded them. In other cases the odor indicated that decomposition had taken place. Storing in the dark was

also tried but under these conditions color changes occurred. On that account it was decided to use only fresh solutions.

Since it takes a large quantity of petals and complicated chemical processes to obtain the pure pigment, and consequently a standard solution, the concentration of the solutions used were determined largely by their intensity of color.

TABLES

The following pages contain tabulations of the data obtained from the spectrographs of the various solutions.

Table I.-- Data from spectra of red flowers

Name of flower	Pigment	Solvent	Time of exposure Sec.	Absorption bands: Partial	Absorption bands: Complete	Limit of transmission	Plate
Dark Red Snapdragon	Anthocyanin	Alcohol	2	---	---	5900 A°	3
	Anthocyanin	Alcohol	32	4600-5100 A°	5100-5700 A°	4600	
Carnation	Anthocyanin	Alcohol	2	---	---	5900 A°	4
	Anthocyanin	Alcohol	32	4250-4900 A°	4900-5650 A°	4250	
Tulip	Anthocyanin	Alcohol	2	4450-4550 A° 4750-5500	4550-4750 A°	4450 A°	
	Anthocyanin	Alcohol	32	4100-4300 4450-4600	4300-4450 4600-4750	4100	
Coxcomb	Anthocyanin	Water	10	4300-5400 A° 5100-5300	--- ---	4300 A°	
	Anthocyanin	Water	40	4200-4500	---	4200	
Geranium	Anthocyanin	Alcohol	2	4000-4900 A° 3950-4000	4900-5400 A°	4000 A°	
	Anthocyanin	Alcohol	16	4900-5400	---	3950	
Dark Red Hollyhock	Anthocyanin	Alcohol	10	3850-4050 A° 5100-5350	---	3800 A°	
	Anthocyanin	Alcohol	40	3850-4000	---	3750	
Cyclamen	Anthocyanin	Alcohol	2	3860-5700 A°	---	3810 A°	1
	Anthocyanin	Alcohol	32	3860-3950	3560-3690 A°	3550	
Woolflower	Anthocyanin	Water	20	3850-4000 A°	5100-5500 A°	3760 A°	
	Anthocyanin	Water	40	3850-4000	5100-5500	3760	
Poppy	Anthocyanin	Alcohol	10	3850-4000 A°	3560-3700 A°	3550 A°	
	Anthocyanin	Alcohol	20	3850-4000	3560-3700	3550	
Japonica	Anthocyanin	Alcohol	2	3850-5000 A°	5000-5500 A° 3560-3630	3550 A°	2
	Anthocyanin	Alcohol	32	3560-3600	---	3540	

9 mm. cell.

Table II.-- Data from spectra of yellow flowers

Name of flower	Pigment	Solvent	Time of exposure Sec.	Absorption bands: Partial	Absorption bands: Complete	Limit of transmission	Plate
	Carotinoid	Alcohol	10	---	---	4950 A°	
Zinnia	Carotinoid	Alcohol	20	---	---	4950	
	Carotinoid	Alcohol	2	---	---	4950 A°	5
Jonquil	Carotinoid	Alcohol	32	---	---	4900	
	Carotinoid	Alcohol	2	---	---	5000 A°	6
Calendula	Carotinoid	Alcohol	32	---	---	4900	
	Carotinoid	Alcohol	2	---	---	4800 A°	
Iris	Carotinoid	Alcohol	32	---	---	4800	
	Carotinoid	Alcohol	2	---	---	4750 A°	7
Rose	Carotinoid	Alcohol	32	4450-4550 A°	4550-4700 A°	4450	
	Carotinoid	Alcohol	5	4200-5300 A°	---	4200 A°	
Marigold	Carotinoid	Alcohol	20	4200-4500	---	4200	
	Carotinoid	Alcohol	2	4100-4800 A°	---	4100 A°	
Tulip	Carotinoid	Alcohol	32	----	---	4100	
	Carotinoid	Alcohol	2	4100-4800 A°	---	4100 A°	8
Dandelion				3970-4300	4300-4450 A°		
	Carotinoid	Alcohol	32	4500-4600	4600-4750	3970	
	Carotinoid	Alcohol	2	3860-4300 A°	3560-3700 A°	3550 A°	
Snapdragon	Carotinoid	Alcohol	15	3860-4200	3560-3650	3550	

9 mm. cell.

Table III.-- Data from spectra of blue and purple flowers

Name of flower	Pigment	Solvent	Time of exposure	Absorption bands: Partial	Absorption bands: Complete	Limit of transmission	Plate
			Sec.				
	Anthocyanin	Alcohol	2	4100-5000 A°	---	4100 A°	12
Lilac	Anthocyanin	Alcohol	32	4050-4100	---	4050	
Morning Glory	Anthocyanin	Alcohol	10	5100-5400 A°	---	4050 A°	
	Anthocyanin	Alcohol	20	5200-5300	---	3930	
	Anthocyanin	Alcohol	2	4000-5200 A°	5200-6000 A°	4000 A°	9
Stock	Anthocyanin	Alcohol	32	5350-5600	---	3940	
	Anthocyanin	Alcohol	2	4900-5850 A°	----	3940 A°	10
Iris	Anthocyanin	Alcohol	32	---	---	3850	
	Anthocyanin	Alcohol	10	3900-4000 A°	3860-3900 A°	3850 A°	
Petunia	Anthocyanin	Alcohol	20	3900-3950	3860-3900	3830	
				5700-5800 A°			
	Anthocyanin	Alcohol	2	3850-4000	---	3810 A°	11
Cineraria	Anthocyanin	Alcohol	32	3850-3900	---	3770	

9 mm. cell.

Table IV.-- Comparison of spectra of petals and solutions

Name of flower	Pigment	Solvent	Time of exposure	Absorption bands: Partial	Absorption bands: Complete	Limit of Transmission	Plate
Red Rose sol	Anthocyanin	Alcohol	32 sec.	---	---	4100 A°	13
				3850-4100 A°	4700-5600 A°		
Red Rose petal	Anthocyanin	---	2 min.	4200-4700	6600-7000	3800 A°	13
Red Geranium sol	Anthocyanin	Alcohol	16 sec.	5600-6600 A° 5000-5400	---	4000 A°	
Red Geranium petal	Anthocyanin	---	2 min.	6000-6800 A°	6800-7000 A°	6000 A°	
Yellow Iris sol	Carotinoid	Alcohol	32 sec.	---	---	4050 A°	13
Yellow Iris petal	Carotinoid	---	2 min.	4200-4900 A°	3850-4100 A° 6600-7000	3800 A°	13
Yellow Jonquil sol	Carotinoid	Alcohol	32 sec.	---	---	4900 A°	
Yellow Jonquil petal	Carotinoid	---	2 min.	5050-6700 A°	6700-7000 A°	5050 A°	
Blue Iris sol	Anthocyanin	Alcohol	32 sec.	---	---	4050 A°	14
Blue Iris petal	Anthocyanin	---	2 min.	4050-5000 A° 5850-6700	5000-5850 A° 6700-7000	4100 A°	14
Blue Petunia sol	Anthocyanin	Alcohol	20 sec.	3900-3950 A°	3860-3900 A°	3830 A°	
Blue Petunia petal	Anthocyanin	---	2 min.	4100-4700	4700-7000	4100 A°	
Green Geranium sol	Chlorophyll	Alcohol	32 sec.	3470-3700 A° 3950-4450 4550-4750 6750-6850	---	3470 A°	14
Green Geranium leaf	Chlorophyll	---	2 min.	5150-6500 A°	6500-7000 A°	5150 A°	14
Green Spirea sol	Chlorophyll	Alcohol	32 sec.	---	6600-6800 A°	4750 A°	
Green Spirea leaf	Chlorophyll	---	2 min.	5200-5650 A°	5650-7000	5200 A°	

9 mm. cell.

Table V.-- Effect of ultra violet light on chlorophyll

	Treatment	Time of exposure	Absorption bands: Partial	Absorption bands: Complete	Limit of transmission	Plate
Geranium	30 days	2 sec.	3850-4250 A° 4700-4850	3580-3770 A° 4250-4700 6600-6900	3570 A°	15
Experiment I	10 min. 36 ins.	32 sec.	3450-3550 A° 3560-3700 3850-4800	6700-6800 A°	3410 A°	
	Control	2 sec.	3500-3550 A° 3570-3700 3850-4250 4450-4900	3430-3500 A° 4250-4450 6600-6800	3420 A°	16
		32 sec.	3350-3500 A° 3600-3700 3850-4050 4200-4400	6650-6750 A°	3350 A°	
Experiment II	7 days	2 sec.	4900-6500 A°	6500-7000 A°	4900 A°	17
	10 min. 27 ins.	32 sec.	3550-3720 A° 3850-4400	6700-6750 A°	3480 A°	
	Control	2 sec.	3370-3550 A° 3560-3700 3850-4400 4600-4750	---	3370 A°	18
		32 sec.	3300-3550 A° 3560-3700 3850-4500	---	3300 A°	

Table V. Continued

Experiment III	16 days	2 sec.	4500-4750 A°	6600-6900 A°	4500 A°	19
	10 min. 27 ins.	32 sec.	4400-4750 A°	6750-6850 A°	4400 A°	
	Control	2 sec.	3560-3800 A° 4450-4550	3850-4450 A° 4550-4750 6600-6900	3550 A°	20
		32 sec.	3500-3550 A° 3700-3800 3850-4400	3560-3700 A° 6750-6800	3500 A°	

9 mm. cell.

Table VI.-- Effect of thickness and concentration

	Treatment	Thickness of cell	Time of exposure	Absorption bands: Partial	Absorption bands: Complete	Limit of transmission	Plate
Spirea Alcohol Solution	Grown outdoors	27 mm.	2 sec.	5000-6000 A°	6000-7000 A°	5000 A°	21
			32 sec.	6000-6400 A°	6400-6900 A°	4950 A°	
		9 mm.	2 sec.	---	6600-6900 A°	4850 A°	22
			32 sec.	---	6600-6800 A°	4750 A°	
Geranium Alcohol Solution	Grown indoors but in bright sun.	27 mm.	2 sec.	4450-4800 A°	6600-6900 A°	4450 A°	23
			32 sec.	3850-4150 A° 4200-4400	6700-6800 A°	3800 A°	
		9 mm.	2 sec.	4400-4800 A°	3850-4150 A° 4200-4400 6650-6800	3820 A°	24
			32 sec.	3850-4400 A° 4600-4700	3560-3770 A° 6750-6800	3550 A°	
	Window open part of the time.	27 mm. Conc.=1/3 of that used above	2 sec.	4550-4800 A°	3850-4150 A° 4200-4450 6600-6800	3820 A°	25
			32 sec.	3850-4400 A° 4550-4750	6750-6800 A°	3750 A°	
Geranium Leaf	Same as those used for above solutions	---	1 min.	5100-6500 A°	6500-7000 A°	5100 A°	26
			4 min.	5100-6500 A°	6500-7000 A°	5100 A°	

DISCUSSION OF DATA

The data for flowers which contained red pigments are shown in Table I and in Plates I to IV.

The dark red Snapdragon solution on a 2-second exposure absorbed all wave lengths shorter than 5900 A^{o}. Only the orange and red were transmitted. On a 32-second exposure there was partial transmission of the blues and greens, but the yellow greens and yellows from 5100 A^{o} to 5700 A^{o} were still completely absorbed. The Carnation showed almost the same absorption for a 2-second exposure as the Snapdragon and for a 32-second exposure the blues and blue greens were partially transmitted, and the greens and yellows completely absorbed. The red Tulip showed complete absorption in the blue from 4550 A^{o} to 4750 A^{o} with partial absorption on each side of this band. All wave lengths shorter than 4450 were absorbed. When the exposure was increased to 32 seconds the same band showed up in the blue and another from 4300 A^{o} to 4450 A^{o} . The limit of transmission was extended to 4100 A^{o}.

The Coxcomb did not show any complete absorption bands but showed partial absorption of the blues, greens, and yellow greens. On longer exposure a band in the blue green from 4500 A^{o} to 5100 A^{o} was partially transmitted. The

limit of transmission at about 4200 A° varied slightly. The red Geranium showed a complete absorption band in the green from 4900 A° to 5400 A° on a 2-second exposure. The blues and violets below this band were partially absorbed. On longer exposure this complete absorption band became a partial absorption band and the blues and violets were transmitted. There was partial transmission of violet from 3950 A° to 4000 A° . The dark red Hollyhock had no complete absorption bands. But it showed partial absorption of a narrow band in the violet and another in the yellow greens. The limit of transmission was 3800 A°.

The Cyclamen had no complete absorption bands for a 2-second exposure but partially absorbed all the violets, blues, greens, and yellows from 3860 A° to 5700 A° . There was complete transmission of a narrow band of violet from 3810 A° to 3860 A°. On longer exposure a few lines from 3550 A° to 3560 A° were transmitted but the violets were completely absorbed from 3560 A° to 3690 A° and again from 3860 A° to 3950 A° . The Woolflower showed the same characteristics for long and short exposure; complete absorption of the greens and partial absorption of a band in the violets. The limit of transmission is very near that of the longest ultra-violet wave lengths. The Poppy showed

the same partial absorption in the violet that the Woolflower showed, and a complete absorption in the ultra-violet from 3560 A^{o} to 3700 A^{o} . A very narrow band of ultra-violet around 3555 A^{o} was transmitted. The Japonica completely absorbed the greens and yellow greens on short exposure and also absorbed a band in the ultra-violet from 3560 A^{o} to 3630 A^{o} . On long exposure this last band became a partial absorption band. A narrow band of ultra-violet around 3550 was transmitted here. The two last solutions were the only red ones that transmitted any ultra-violet.

The limit of transmission in the red solutions varies widely from 5900 A^{o} to 3540 A^{o} . Of the ten flowers examined only two transmitted any ultra-violet. There were no absorption bands which showed up continuously. Many of the solutions showed a band from 3850 A^{o} to 4000 A^{o} ; another from 4200 A^{o} to 4500 A^{o} showed in several cases. A few cases showed a band centering around 5100 A^{o}.

The yellow solutions containing carotinoids showed more similarity. (See Table II and Plates V to VIII.) The Zinnia, Jonquil, Calendula, and Iris showed no bands. The limit of transmission was near 4900 A^{o} with complete transmission up to 7000 A^{o} . The Rose showed no bands on

short exposure but on long exposure the limit of transmission was 4450 A°. A few lines came through here, then there was absorption either partial or complete for most of the blues. The Marigold and Tulip showed partial absorption of the blues and blue greens, but no complete absorption. The limit of transmission is about 4100 A°. The Dandelion completely absorbed a band in the blue-violet and another in the blue-green. The rest of the violet and blue was partially absorbed. The yellow Snapdragon is the only one in this group that transmitted ultra-violet light. It transmitted a narrow band at 3550 A°, then completely absorbed to 3650 A°, transmitted to 3860 A° and partially transmitted to 4200 A°. Absorption bands in the yellow solutions were between 3860 A° and 4500 A° or the violets and blues, with the yellows, oranges and reds transmitted.

The blue and purple solutions are given in Table III and Plates IX to XII. The Stock and Petunia are the only ones that show complete absorption bands. On short exposure the Stock absorbed all the greens, yellows, and oranges, but on long exposure all the colors were transmitted except a partial band in the yellow. The Petunia showed a band in the violet from 3860 A° to 3900 A° with partial absorption from there to 4000 A°. All of these

solutions showed partial absorption bands, one centering around 3900 and another around 5200. The limit of transmission does not vary so widely, being from 4100 A^{o} to 3770 A^{o}. But no ultra-violet is transmitted. The bands have more definite limits in this group than in any other even though there is only partial absorption.

In all the groups, as would be expected, a band which shows complete absorption on short exposure, shifts to partial absorption on long exposure. Sometimes the limits changed, and sometimes were almost identical.

The effect of acids and alkalies on the solutions was tried. HCl added to a red solution made it brighter red, and if added to a red solution which had faded it brought back the red color. $NaCO_3$ turned the red solution a yellow green with considerable sediment in it. HCl turned a blue solution red and $NaCO_3$ turned it yellow green. It has been known for some time that anthocyanin was red or blue depending upon whether it was in an acid or alkaline cell sap. Wheldale (5). HCl turned a yellow solution a clear green and $NaCO_3$ left it yellow but a milky yellow instead of clear.

The absorption bands were changed by these additions, so it seems that addition of certain materials to the soil

in which the plant is grown might result in decided color changes. A few examples of this are known. Hydrangeas can be changed to red or to blue by the addition of certain salts to the soil. Several chemicals are known which will cause a deeper green in the leaves of the plant.

The spectra through the petal and the corresponding solutions were compared, but there did not seem to be any general similarity between them. Two samples each of red, yellow, blue, and green were examined. (See Table IV and Plates XIII and XIV.)

Plate XIII shows (4 and 5) the spectra through a red Rose solution and the red Rose petal. There is a complete transmission through the solution from 4100 A^{o} to 7000 A^{o} but the petal completely absorbs the blues, greens, and yellows and only partially transmits the violets. No ultraviolet is transmitted in either case. The yellow Iris solution and petal are shown on Plate XIII (2 and 3). The solution transmits to 4050 A^{o} but the petal partially absorbs wave lengths shorter than 4900 A^{o} . It transmits some to 3800 A^{o}.

The green Geranium solution and leaf are shown in Plate XIV (4 and 5). The solution transmitted up to 3470 A^{o}.. with four partial absorption bands, one in the longer ultraviolet, one in the violet, one in the blue, and one in the

red. This band in the red was absorbed in nearly all chlorophyll solutions examined. The leaf partially transmitted the wave lengths from 5200 A^{o} to 5650 A^{o} or the greens and yellows.

The blue Iris solution and petal are shown in Plate XIV (2 and 3). The solution had no bands and transmitted to 4050 A^{o} . The petal transmitted to about the same limit but showed two complete absorption bands, one in the green and yellow, and one in the red. Two partial absorption bands, one in the violet and blue, and one in the red and red orange. There were no lines completely transmitted. Probably the thickness of the petal is a large factor in causing differences in the spectra of the solution and petal. In some cases on long exposure lines of shorter wave length were transmitted through the petal than were transmitted through the solution. But there were always absorption bands in the petal spectrum that did not show in that of the solution. These varied greatly in location, hence they did not seem to be due to any material common to all petals. It cannot be attributed to the color because the solution and the petal are of the same color.

The effect of ultra-violet light on green leaves was studied briefly. (See Table V and Plates XV to XX.) A

geranium plant was treated 10 minutes a day for 30 days at a distance of 36 inches from the mercury arc lamp. The rest of the time it was kept with an untreated control plant in front of a window exposed to the sunlight. The concentration of the solution used here and in all other chlorophyll solutions was regulated by using equal leaf area each time with a fixed amount of alcohol. There was not a decided difference in the spectra taken through the solutions made from the two plants, but the lines came through a little more distinctly in the untreated solution. There was transmission up to about 3400 A^{o} with complete absorption bands showing from 3400 A^{o} to 3700 A^{o} in the ultra-violet, one in the blue-violet, and the one in the red centering around 6800 which always showed up. The four partial bands which were noted above showed in these solutions.

The treatment was continued for seven days with the distance reduced to 27 inches. At this stage some of the leaves were turning a darker, shiny green, and in spots looked burned. The two spectra were decidedly different this time. The control transmitted many lines that the treated plant did not. The control is shown in Plate XVIII and is very similar to the control plant in the first experiment shown in Plate XVI. The treated plant shown in Plate XVII

transmitted up to 3480 A° with complete absorption of the usual red band and partial absorption from 3550 A° to 3720 A° in the ultra-violet and partial absorption in the violets, and blues from 3850 A° to 4400 A°. From 3900 A° to 5000 A° there was much more absorption than with the untreated plant.

Two new plants were then brought from the greenhouse and placed in the same window. This was later in the spring and the sun was much brighter and the windows were open the greater part of the day so the plants were not shielded from the direct rays of the sun. The test plant was treated 10 minutes a day for 16 days at 27 inches. Again the control transmitted a considerable number of lines that the other solution absorbed. It is shown in Plate XX. It was interesting to note that the control plant was now absorbing more lines than before. The spectrum was very similar to that of the treated plant in the first test. (See Plate XV.) From this it appears that treatment with the ultra-violet must change the chlorophyll pigment in such a way that it is able to absorb energy which otherwise it could not; and that exposure to strong sunlight produces the same change. The treated plant transmitted only to 4400 A° with partial absorption from there to 4750 A° for a 32-second exposure.

There was complete transmission from there to 7000 A° except for the usual narrow band in the red which was completely absorbed.

At another time solutions were made from Spirea leaves grown in the open and Geranium leaves grown indoors but exposed a part of each day to the direct sunlight. (See Table VI and Plates XXII and XXIV.) The Spirea solution absorbed many lines that were transmitted by the Geranium solution and the limit of transmission was at 4750 A° for 32-second exposure, while the Geranium transmitted to 3550 A°. This seems to indicate that plants grown in strong sunlight will absorb lines which those grown in less intense light will transmit. Both solutions showed the red absorption band and the Geranium solution also showed a band in the ultra-violet from 3560 A° to 3770 A°.

The effect of the thickness of the layer of solution and the concentration of the solution were studied briefly. Spirea and Geranium solutions were used in cells 9 mm. and 27 mm. in thickness. (See Table VI and Plates XXI to XXVI.) The thick cell absorbed lines which were transmitted by the thin cell with both solutions. In the 27 mm. cell the absorption band in the red was much wider and with short exposures the wave lengths longer than 6000 A° were all

absorbed. The lines from 4750 A^{o} to 5000 A^{o} were transmitted with the 9 mm. cell but absorbed by the 27 mm. cell. Then some of the Geranium solution was diluted to one-third concentration and placed in the 27 mm. cell. This was equivalent to the concentrated solution in the 9 mm. cell. The two spectra were very much alike. (See Plates XXIV and XXV.) The limit of absorption changed from 3550 A^{o} for the 9 mm. concentrated solution to 3750 A^{o} for the 27 mm. diluted solution. The bands were almost identical except for partial transmission of the ultra-violet from 3560 A^{o} to 3770 A^{o} for the 9 mm. layer which was completely absorbed by the 27 mm. diluted layer.

The spectrum was then photographed through the leaf. The leaf was considerably thicker than the petals and very little light was transmitted. There was partial transmission from 5200 A^{o} to 6100 A^{o} or the greens, yellows, and oranges. (See Plate XXVI.)

CONCLUSIONS

Nearly all of the ultra-violet is absorbed by the flower petals and by the solutions of the pigments, even when the concentration is quite dilute. The concentration of the solution affects the resulting spectra. The thickness of the layer of solution also affected the resulting spectra for the two thicknesses tested. (9 mm. and 27 mm.)

There is a difference in the spectra through the solution and through the petal. In some ways the spectra through the petal might be considered the more important since the materials are in their normal state there. But on the other hand the absorption is affected in that case by many other materials than the pigment. There were other materials than pigments in the solutions also.

The time of exposure must be worked out very carefully as the amount of absorption varies greatly with the time.

The solutions should be used as soon as prepared as decomposition takes place rapidly in some cases.

Treatment with ultra-violet light or exposure to sunlight changes the chlorophyll pigment so that it absorbs more energy than it did before treatment.

Treatment with acids and alkalies will change the color of the solution and the resulting spectra. Hence

color and absorption both depend upon the acidity or alkalinity of the material in the petal.

SUGGESTIONS FOR FURTHER WORK

This study has brought up several questions which might be developed. Since the ultra-violet light treatment changed the chlorophyll spectra, it might also have an effect on the other pigments. It would be interesting to grow some plants and flowers which received no light except the ultra-violet and compare them with plants grown in the sunlight.

If pure pigments could be obtained in sufficient quantities for experimental work, it would be much more satisfactory, since materials other than the pigments are dissolved in the solvents by ordinary extraction. Willstatter (6), Palmer (4), and Wheldale (5) have all shown that it is a complicated process to obtain the pure pigments and large quantities of raw materials are required. If pure pigments were used the concentration could be determined quantitatively, instead of qualitatively by the intensity of the color.

Since the addition of acids and alkalies change the color of the solution feeding the plant with various compounds might result in color changes and consequently different absorption of energy.

The pigments from the vegetative parts of plants such as fruit, berries, tubers, and roots might be used instead of the petal pigments. They are supposedly just the same pigments but it would be interesting to know whether they have the same spectra.

The thickness of the layer of solution could be varied through wide limits. Possibly with very thin layers such as one mm. bands might show in the ultra-violet instead of almost complete absorption.

ACKNOWLEDGMENTS

I wish to express my appreciation to Prof. J. O. Hamilton, Head of the Department of Physics, for his direction of this study, and for his help and suggestions in organizing the material.

I also want to thank Dr. E. C. Miller of the Department of Botany and Plant Pathology for his suggestions, and for his careful reading of the completed manuscript.

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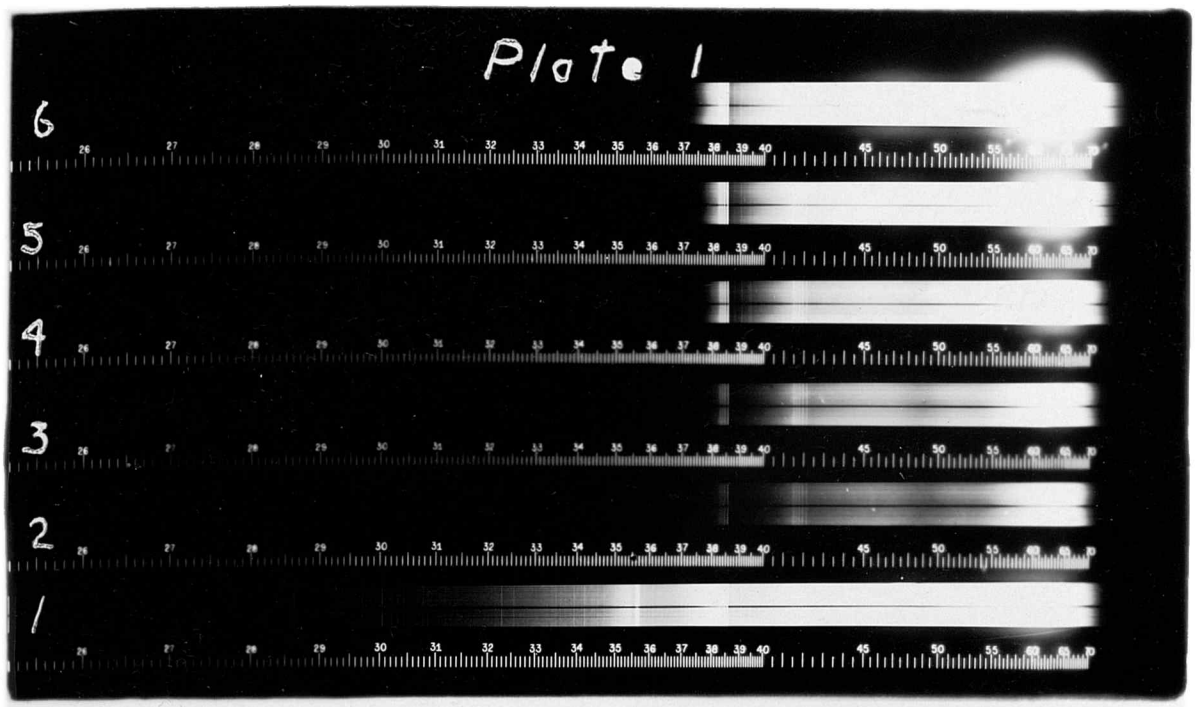
(Trans. from German to English by Schertz, F. M. and Merz, A. R.)

Plate 1.

	Sec.
1. Bare arc	2
2. Arc through Cyclamen solution (alcohol)	2
3. Arc through Cyclamen solution (alcohol)	4
4. Arc through Cyclamen solution (alcohol)	8
5. Arc through Cyclamen solution (alcohol)	16
6. Arc through Cyclamen solution (alcohol)	32

Plate 2.

	Sec.
1. Bare arc	2
2. Arc through Japonica solution (alcohol)	2
3. Arc through Japonica solution (alcohol)	4
4. Arc through Japonica solution (alcohol)	8
5. Arc through Japonica solution (alcohol)	16
6. Arc through Japonica solution (alcohol)	32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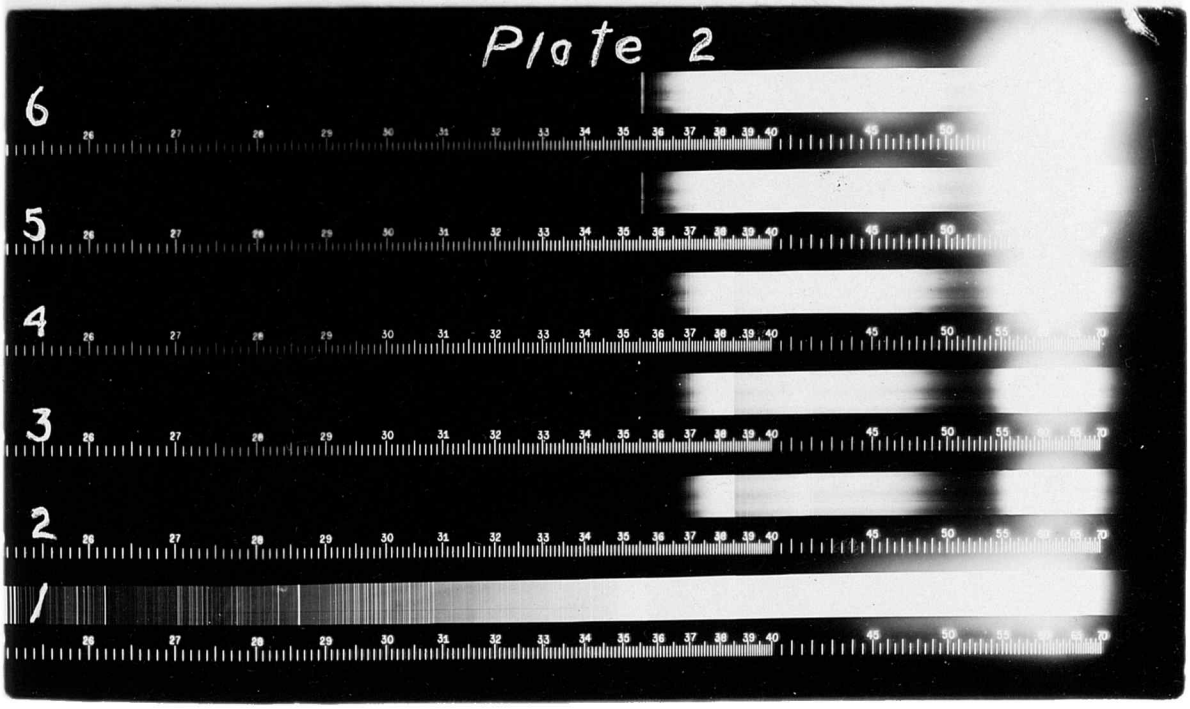
Plate 2
6
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2
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Plate 3.

	Sec.
1. Bare arc	2
2. Arc through Snapdragon solution (alcohol)	2
3. Arc through Snapdragon solution (alcohol)	4
4. Arc through Snapdragon solution (alcohol)	8
5. Arc through Snapdragon solution (alcohol)	16
6. Arc through Snapdragon solution (alcohol)	32

Plate 4.

	Sec.
1. Bare arc	2
2. Arc through Carnation solution (alcohol)	2
3. Arc through Carnation solution (alcohol)	4
4. Arc through Carnation solution (alcohol)	8
5. Arc through Carnation solution (alcohol)	16
6. Arc through Carnation solution (alcohol)	32

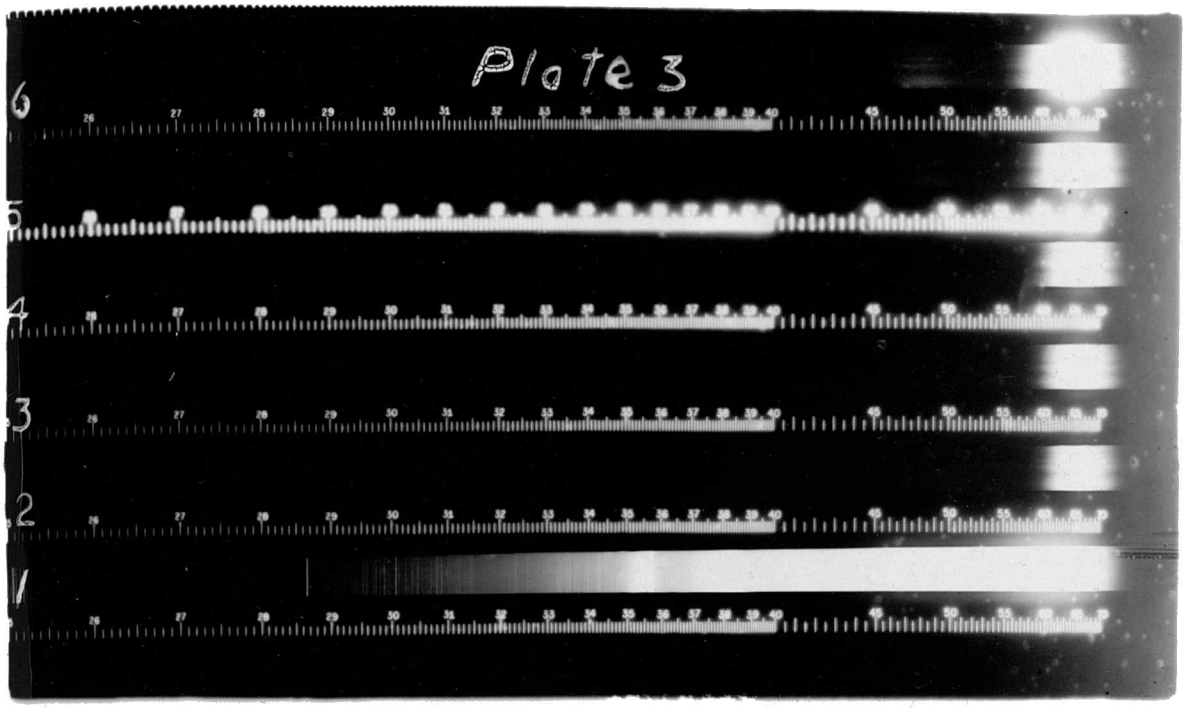
Plate 3
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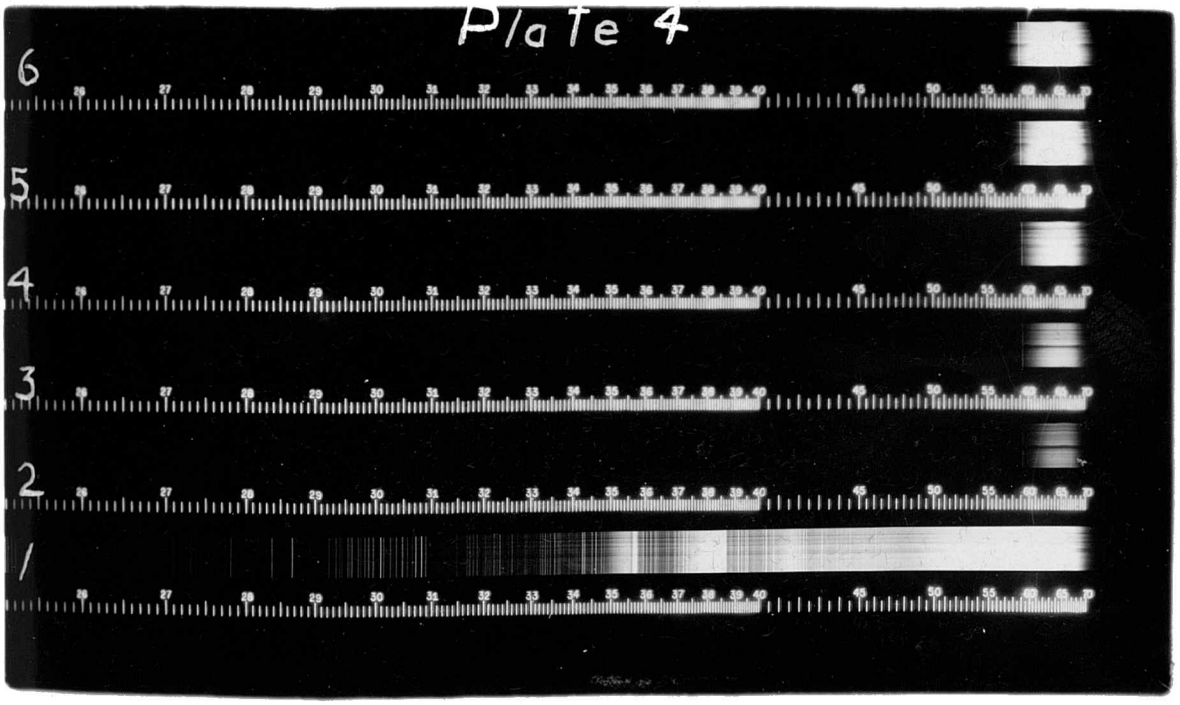
Plate 4
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2
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Plate 5.

	Sec.
1. Bare arc	2
2. Arc through Jonquil solution (alcohol)	2
3. Arc through Jonquil solution (alcohol)	4
4. Arc through Jonquil solution (alcohol)	8
5. Arc through Jonquil solution (alcohol)	16
6. Arc through Jonquil solution (alcohol)	32

Plate 6.

	Sec.
1. Bare arc	2
2. Arc through Calendula solution (alcohol)	2
3. Arc through Calendula solution (alcohol)	4
4. Arc through Calendula solution (alcohol)	8
5. Arc through Calendula solution (alcohol)	16
6. Arc through Calendula solution (alcohol)	32

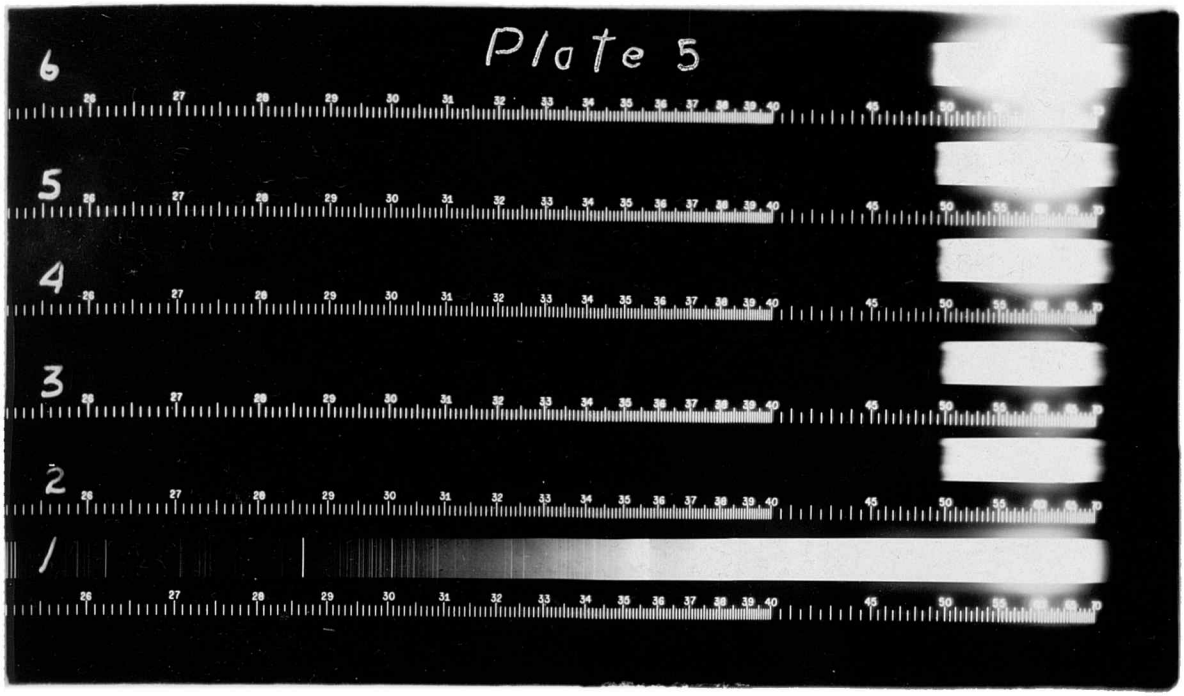
Plate 5
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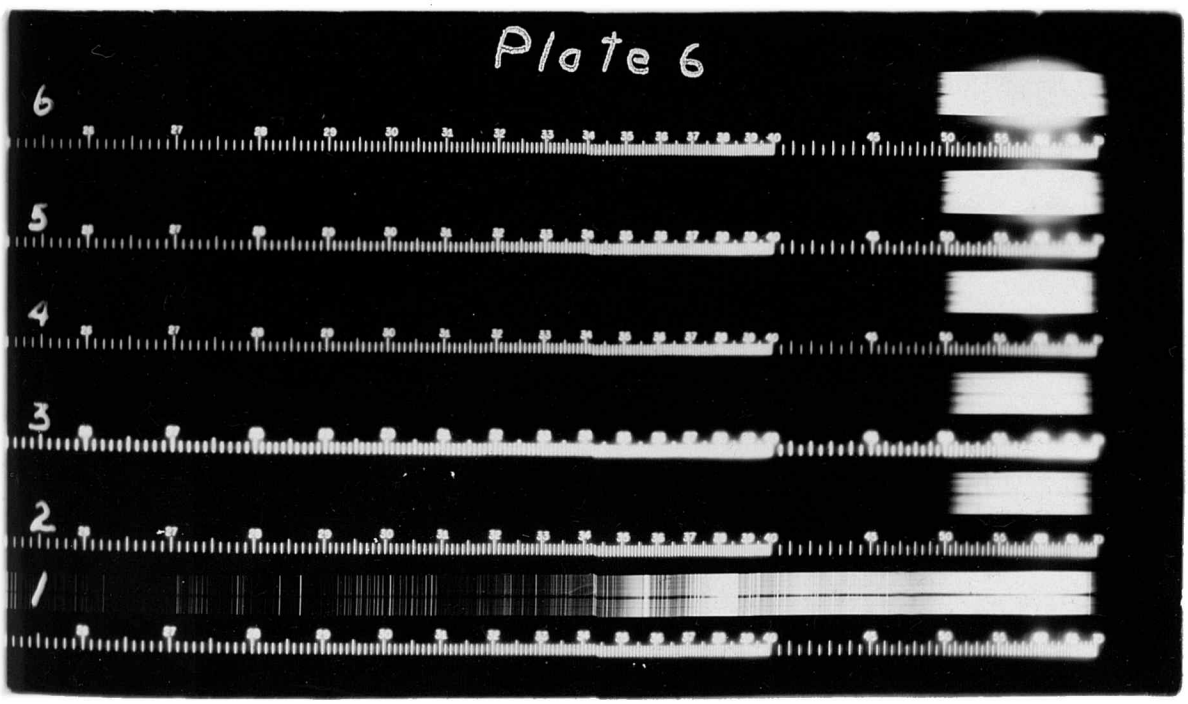
Plate 6
6
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Plate 7.

	Sec.
1. Bare arc	2
2. Arc through Rose solution (alcohol)	2
3. Arc through Rose solution (alcohol)	4
4. Arc through Rose solution (alcohol)	8
5. Arc through Rose solution (alcohol)	16
6. Arc through Rose solution (alcohol)	32

Plate 8.

	Sec.
1. Bare arc	2
2. Arc through Dandelion solution (alcohol)	2
3. Arc through Dandelion solution (alcohol)	4
4. Arc through Dandelion solution (alcohol)	8
5. Arc through Dandelion solution (alcohol)	16
6. Arc through Dandelion solution (alcohol)	32

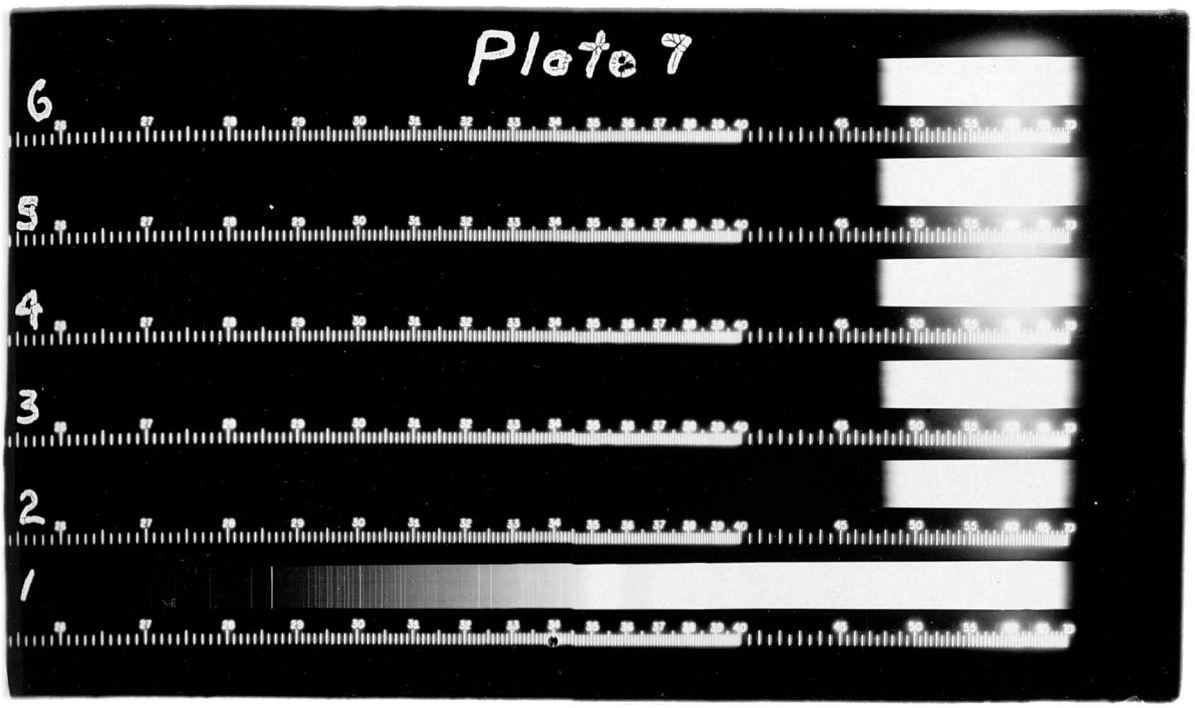
Plate 7
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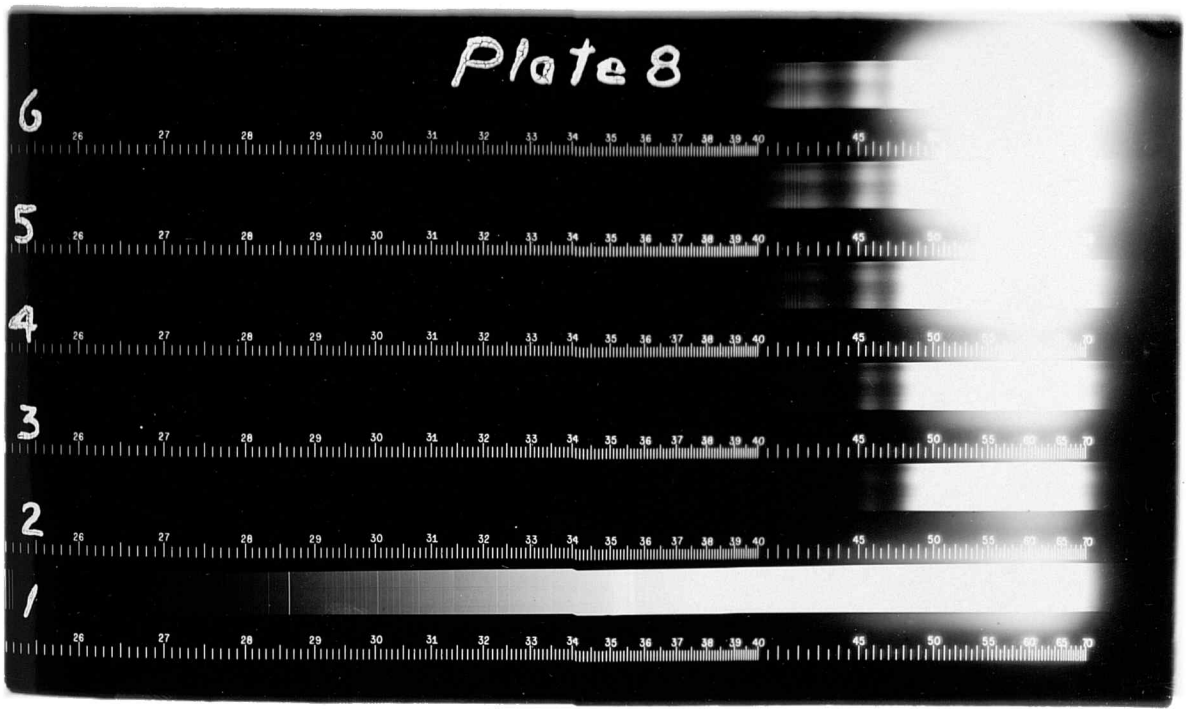
Plate 8
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Plate 9.

	Sec.
1. Bare arc	2
2. Arc through Stock solution (alcohol)	2
3. Arc through Stock solution (alcohol)	4
4. Arc through Stock solution (alcohol)	8
5. Arc through Stock solution (alcohol)	16
6. Arc through Stock solution (alcohol)	32

Plate 10.

	Sec.
1. Bare arc	2
2. Arc through Iris solution (alcohol)	2
3. Arc through Iris solution (alcohol)	4
4. Arc through Iris solution (alcohol)	8
5. Arc through Iris solution (alcohol)	16
6. Arc through Iris solution (alcohol)	32

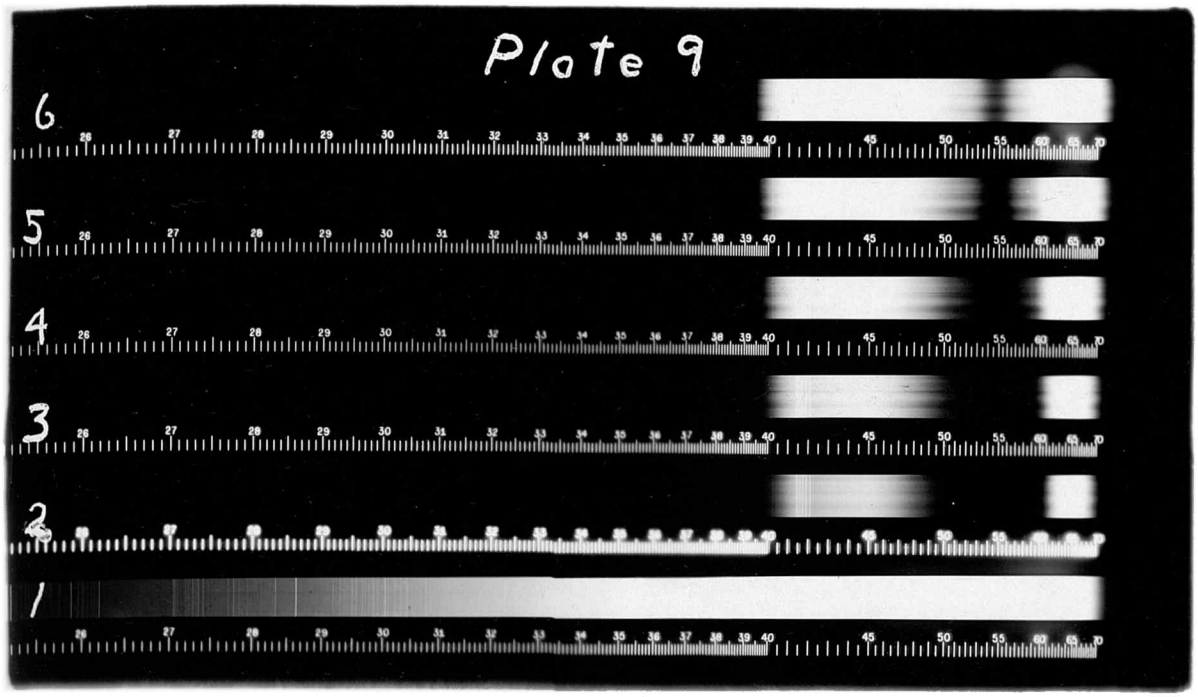
Plate 9
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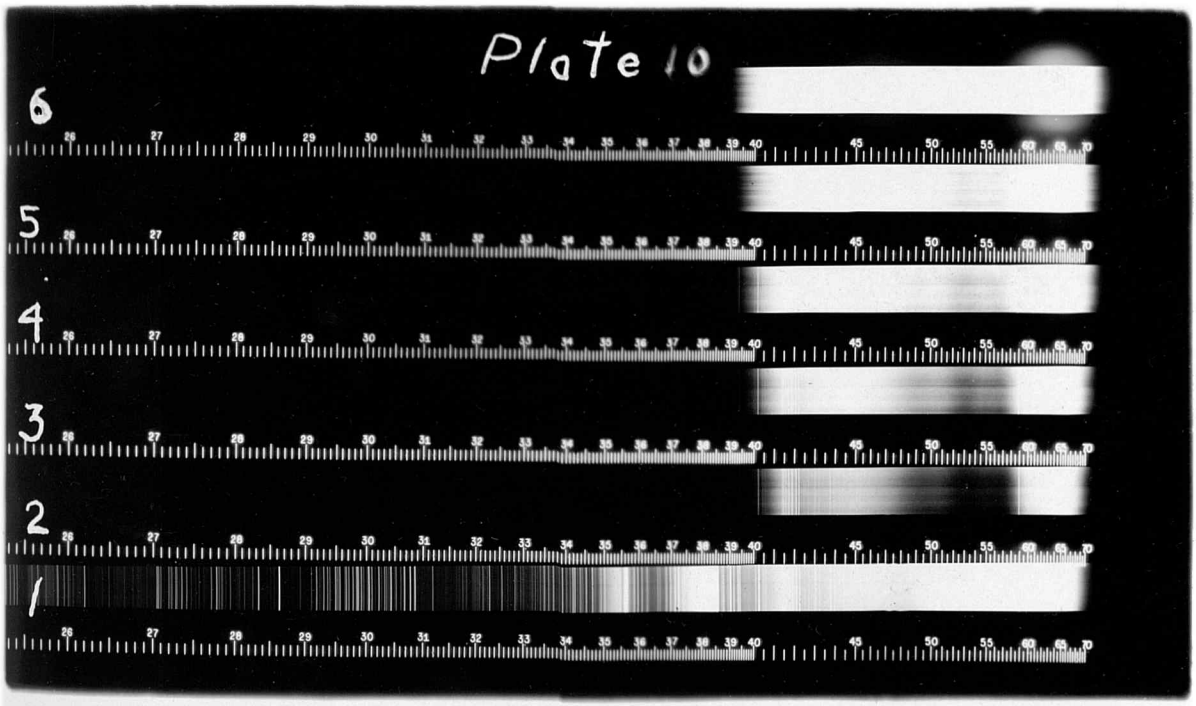
Plate 10
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Plate 11.

	Sec.
1. Bare arc	2
2. Arc through Cineraria solution (alcohol)	2
3. Arc through Cineraria solution (alcohol)	4
4. Arc through Cineraria solution (alcohol)	8
5. Arc through Cineraria solution (alcohol)	16
6. Arc through Cineraria solution (alcohol)	32

Plate 12.

	Sec.
1. Bare arc	2
2. Arc through Lilac solution (alcohol)	2
3. Arc through Lilac solution (alcohol)	4
4. Arc through Lilac solution (alcohol)	8
5. Arc through Lilac solution (alcohol)	16
6. Arc through Lilac solution (alcohol)	32

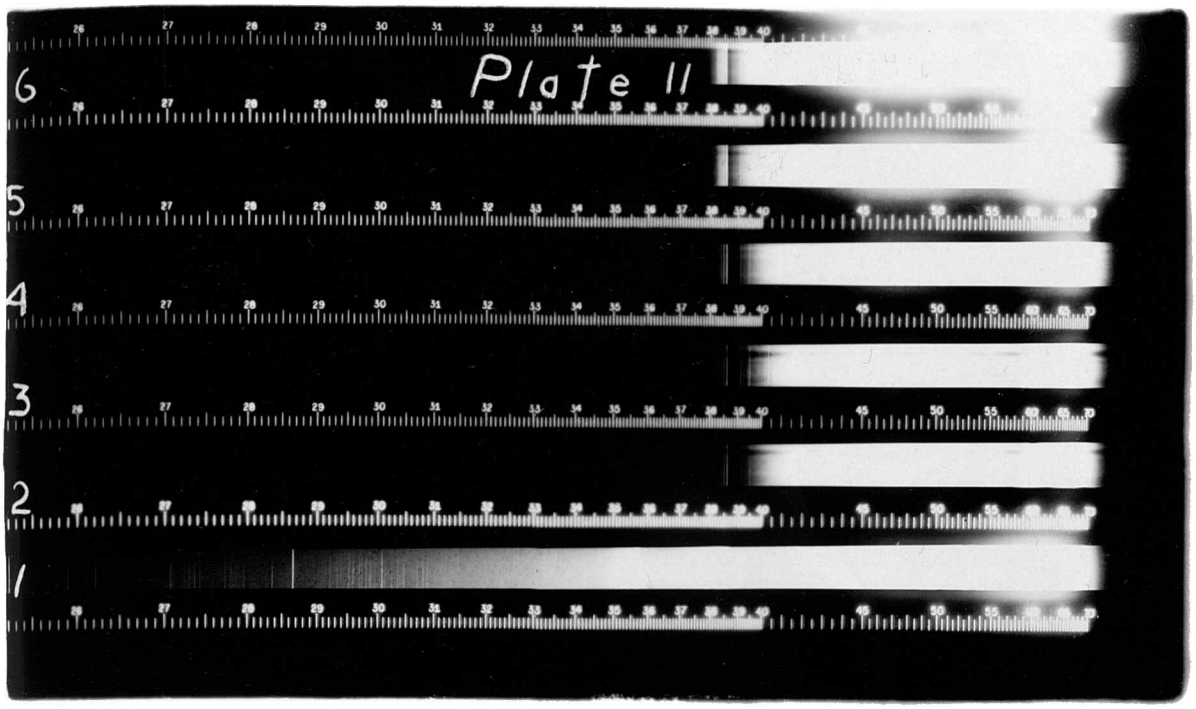
Plate 11
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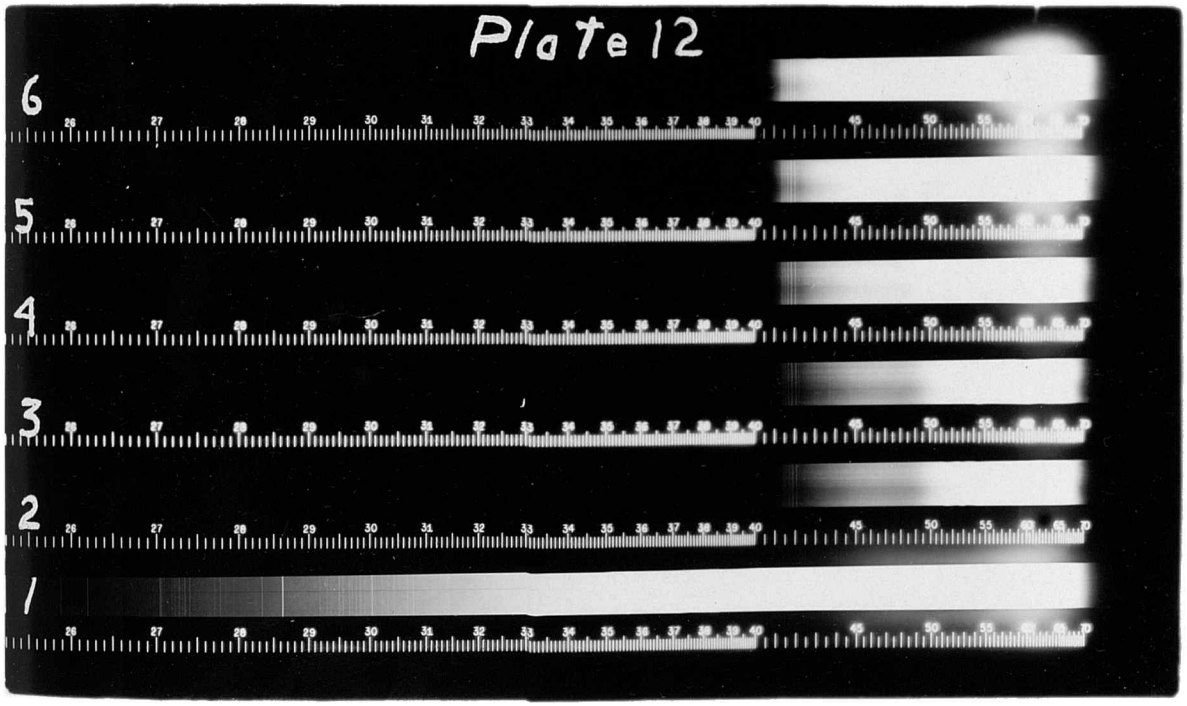
Plate 12
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Plate 13.

1. Bare arc	2 sec.
2. Arc through Yellow Iris solution (alcohol)	32 sec.
3. Arc through Yellow Iris petal	2 min.
4. Arc through Red Rose solution (alcohol)	32 sec.
5. Arc through Red Rose petal	2 min.

Plate 14.

1. Bare arc	2 sec.
2. Arc through Blue Iris solution (alcohol)	32 sec.
3. Arc through Blue Iris petal	2 min.
4. Arc through Green Geranium solution (alcohol)	32 sec.
5. Arc through Green Geranium leaf	2 min.

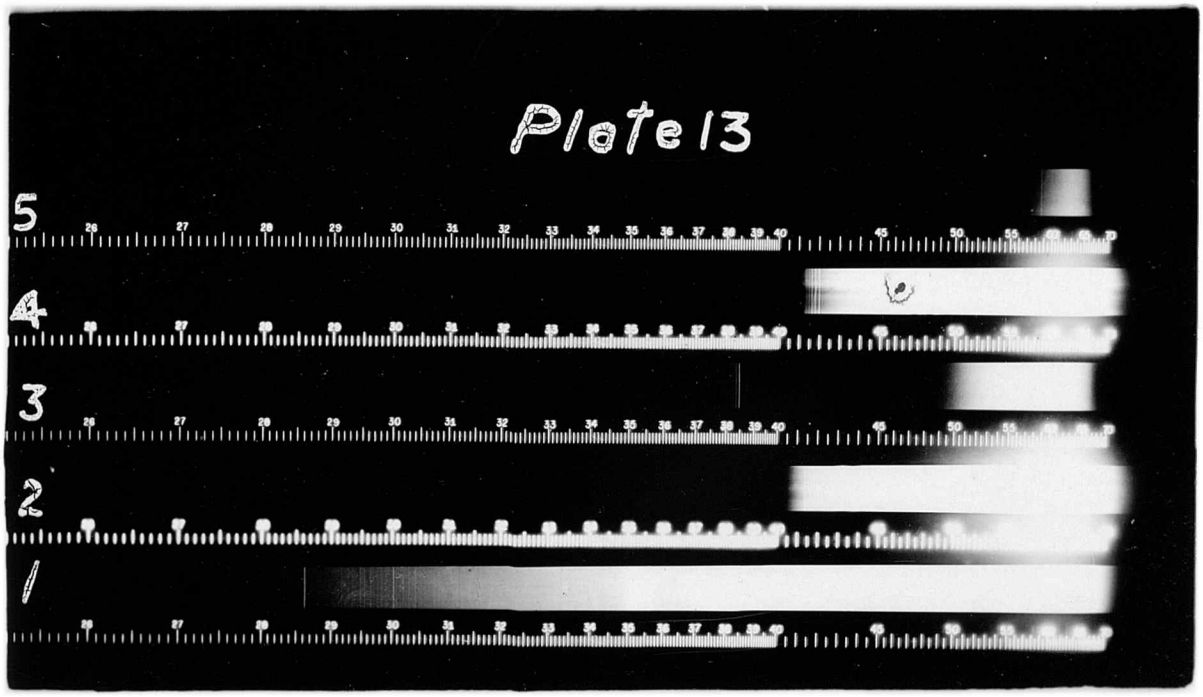
Plate 13
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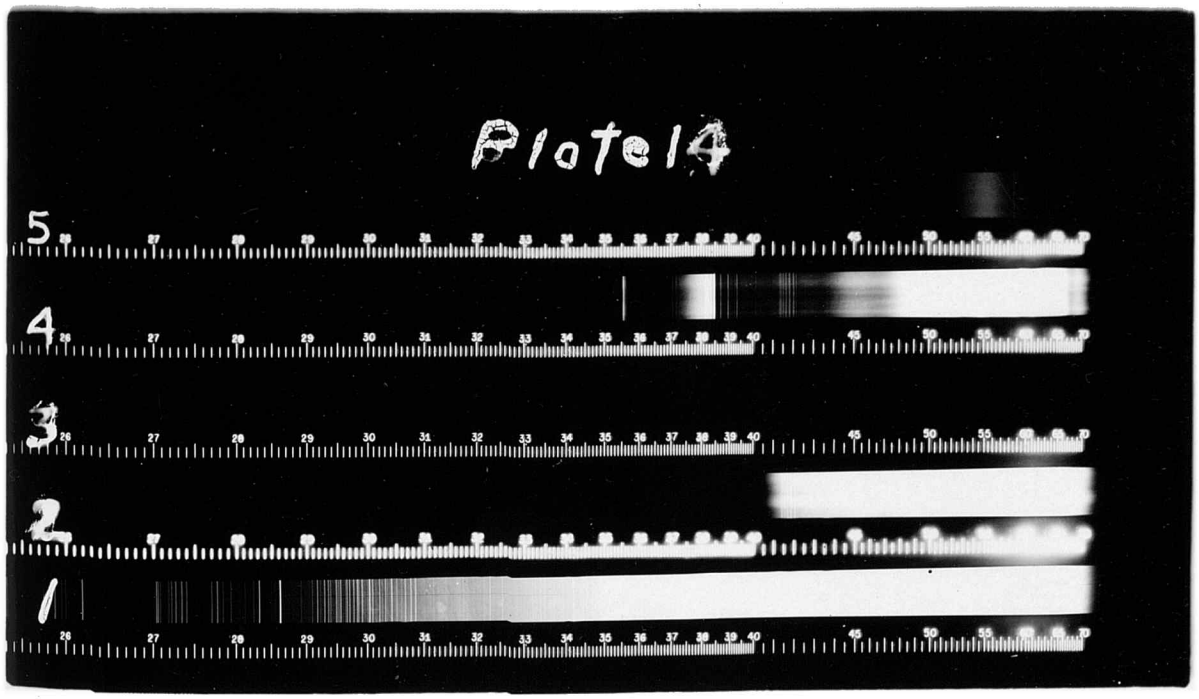
Plate 14
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Plate 15.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Above plant was treated with ultra-violet light for 10 min., 30 days, at 36 inches.

Plate 16.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Control for plant in Plate 15.

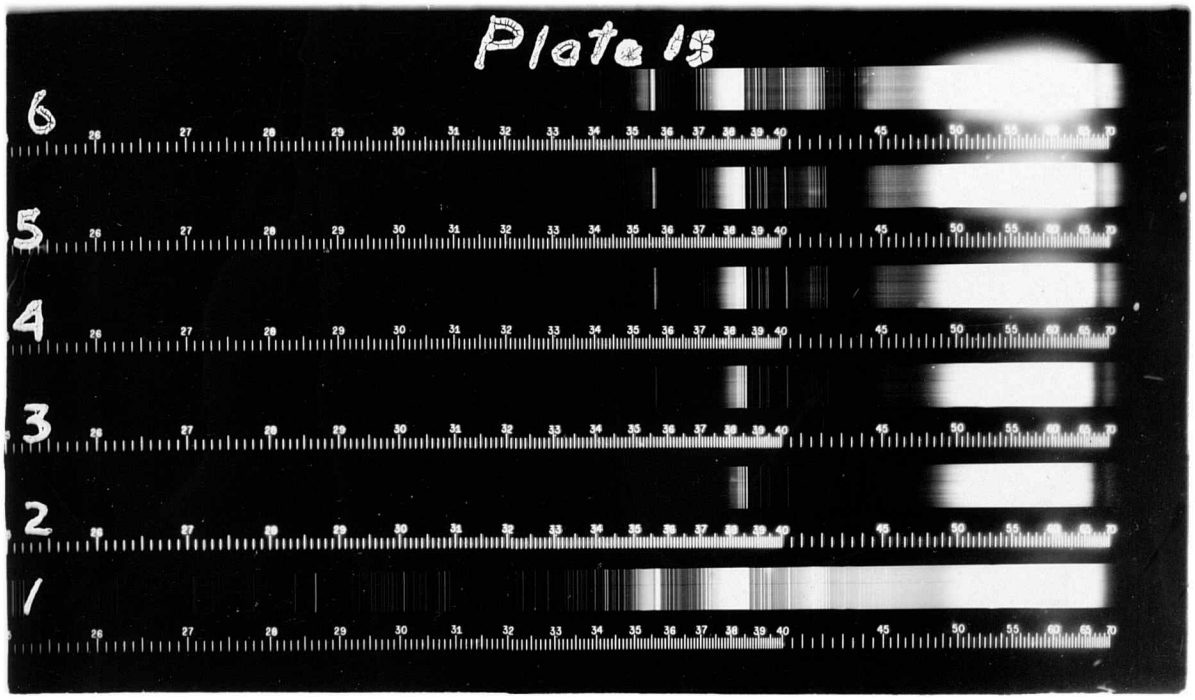
Plate 15
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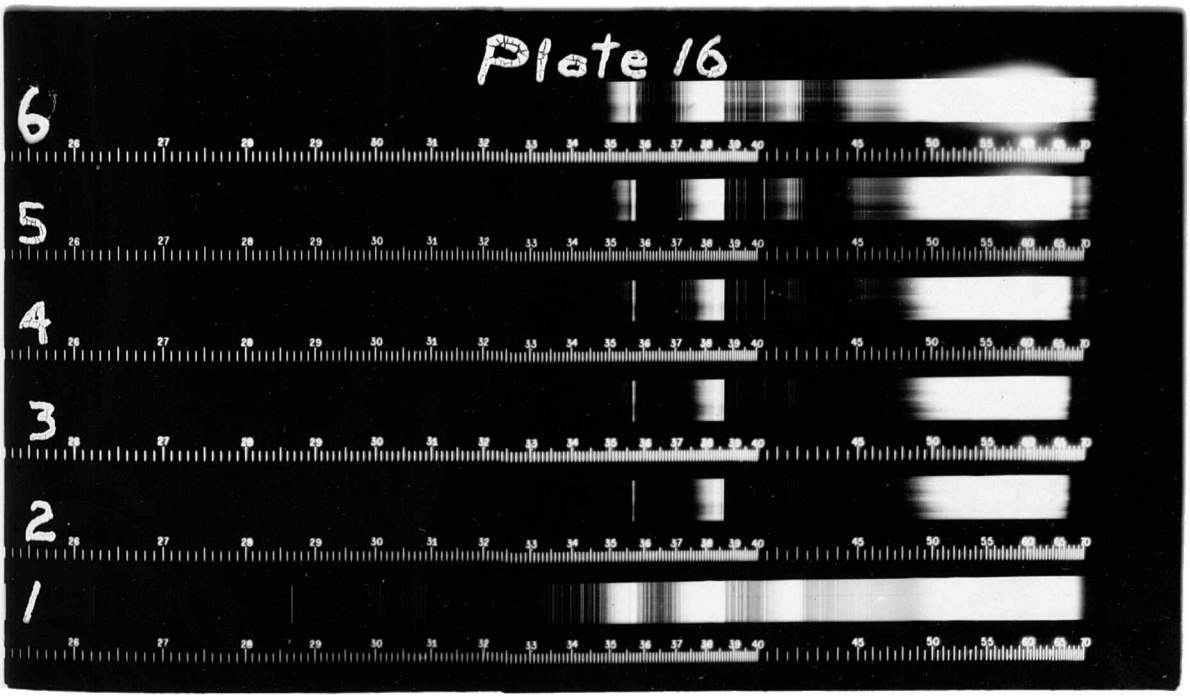
Plate 16
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Plate 17.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Above plant is plant from Plate 15 with continued ultra-violet treatment for 10 min., for 7 days at 27 inches.

Plate 18.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Control for plant in Plate 17.

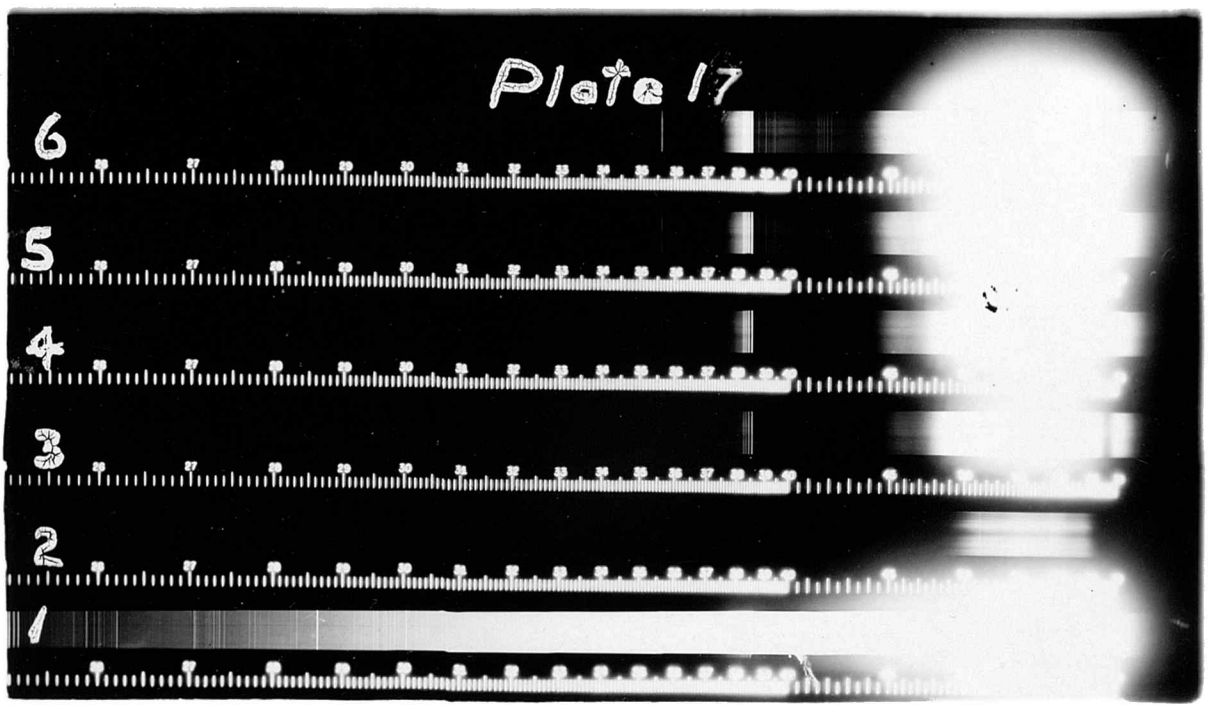
Plate 17
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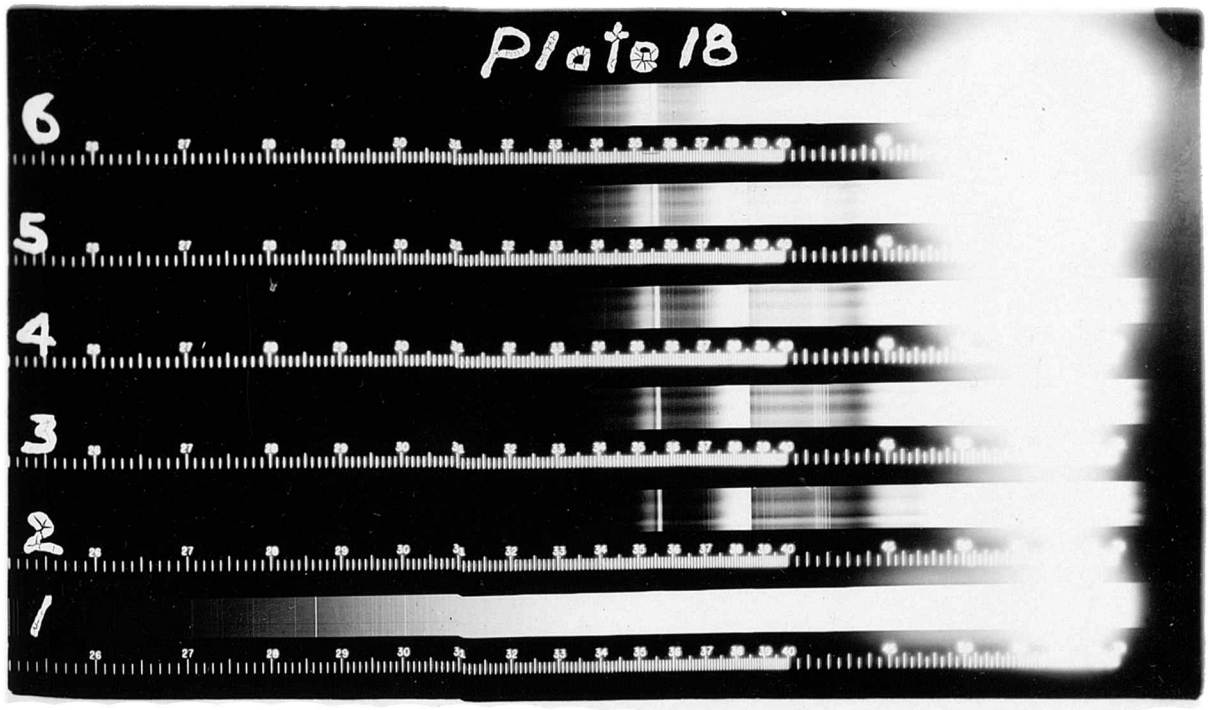
Plate 18
6
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Plate 19.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Above plant treated with ultra-violet light for 10 min., for 16 days at 27 inches.

Plate 20.

	Sec.
1. Bare arc	2
2. Arc through Chlorophyll solution (alcohol)	2
3. Arc through Chlorophyll solution (alcohol)	4
4. Arc through Chlorophyll solution (alcohol)	8
5. Arc through Chlorophyll solution (alcohol)	16
6. Arc through Chlorophyll solution (alcohol)	32

Control plant for Plate 19.

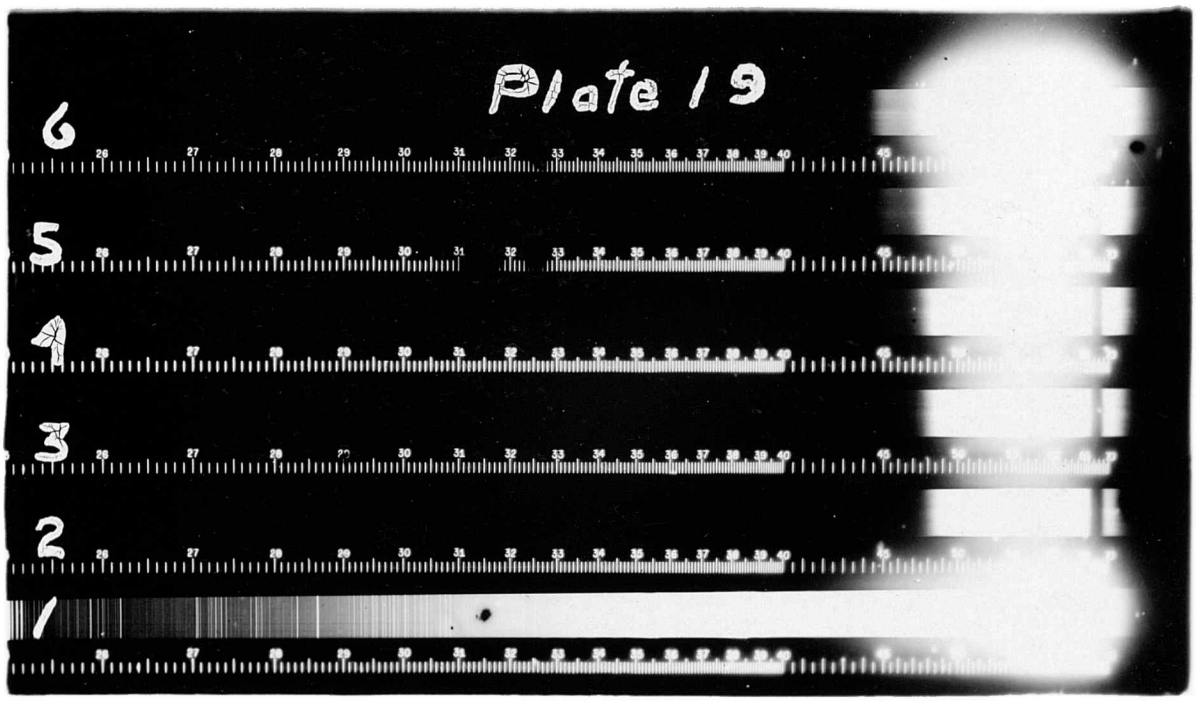
Plate 19
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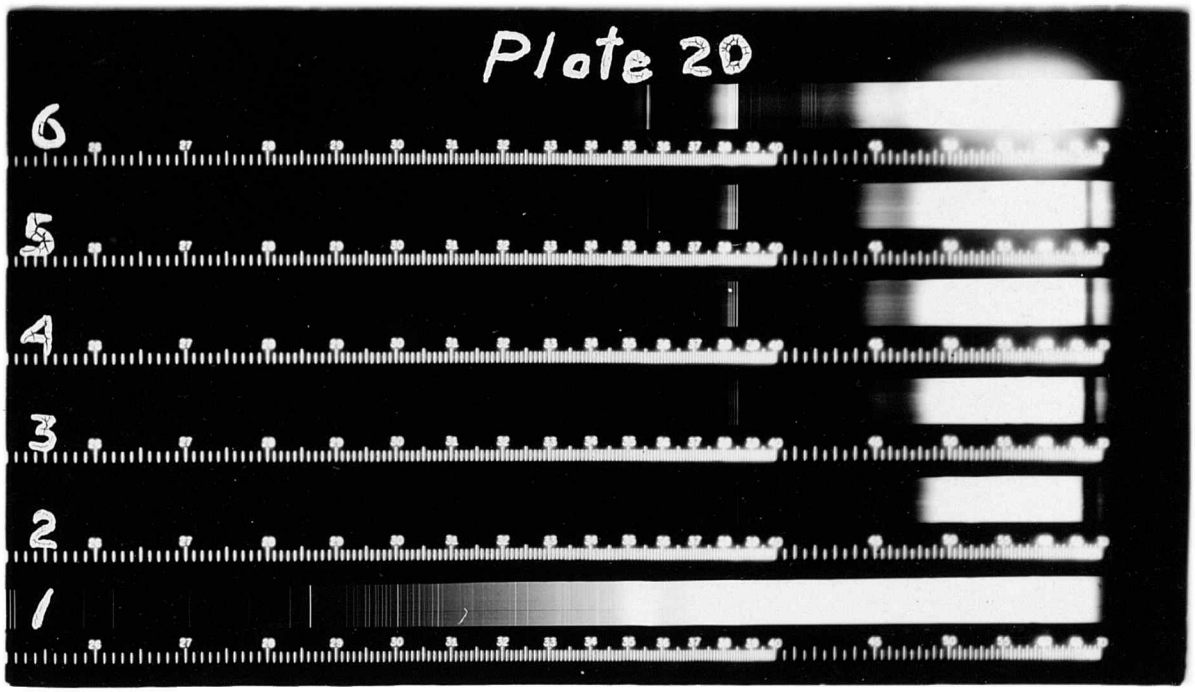
Plate 20
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Plate 21.

	Sec.
1. Bare arc	2
2. Arc through Spirea Chlorophyll solution (alcohol)	2
3. Arc through Spirea Chlorophyll solution (alcohol)	4
4. Arc through Spirea Chlorophyll solution (alcohol)	8
5. Arc through Spirea Chlorophyll solution (alcohol)	16
6. Arc through Spirea Chlorophyll solution (alcohol)	32

27 mm. cell.

Plate 22.

	Sec.
1. Bare arc	2
2. Arc through Spirea Chlorophyll solution (alcohol)	2
3. Arc through Spirea Chlorophyll solution (alcohol)	4
4. Arc through Spirea Chlorophyll solution (alcohol)	8
5. Arc through Spirea Chlorophyll solution (alcohol)	16
6. Arc through Spirea Chlorophyll solution (alcohol)	32

9 mm. cell.

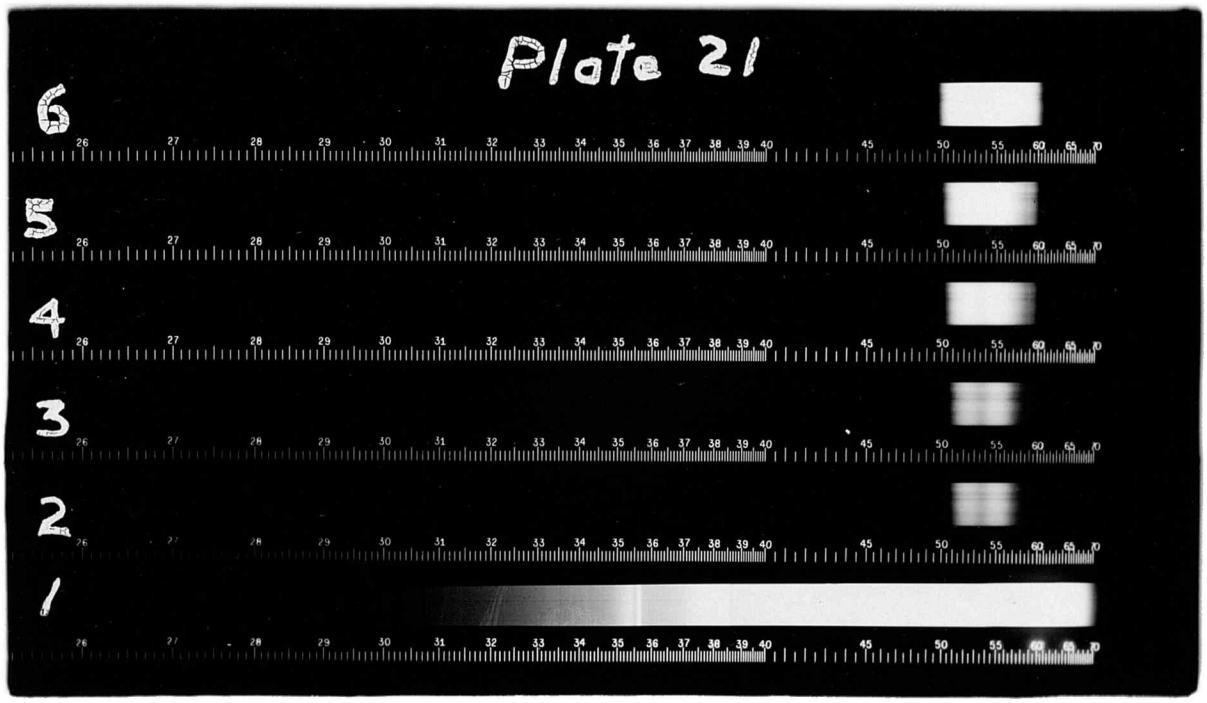
Plate 21
6
5
4
3
2
1

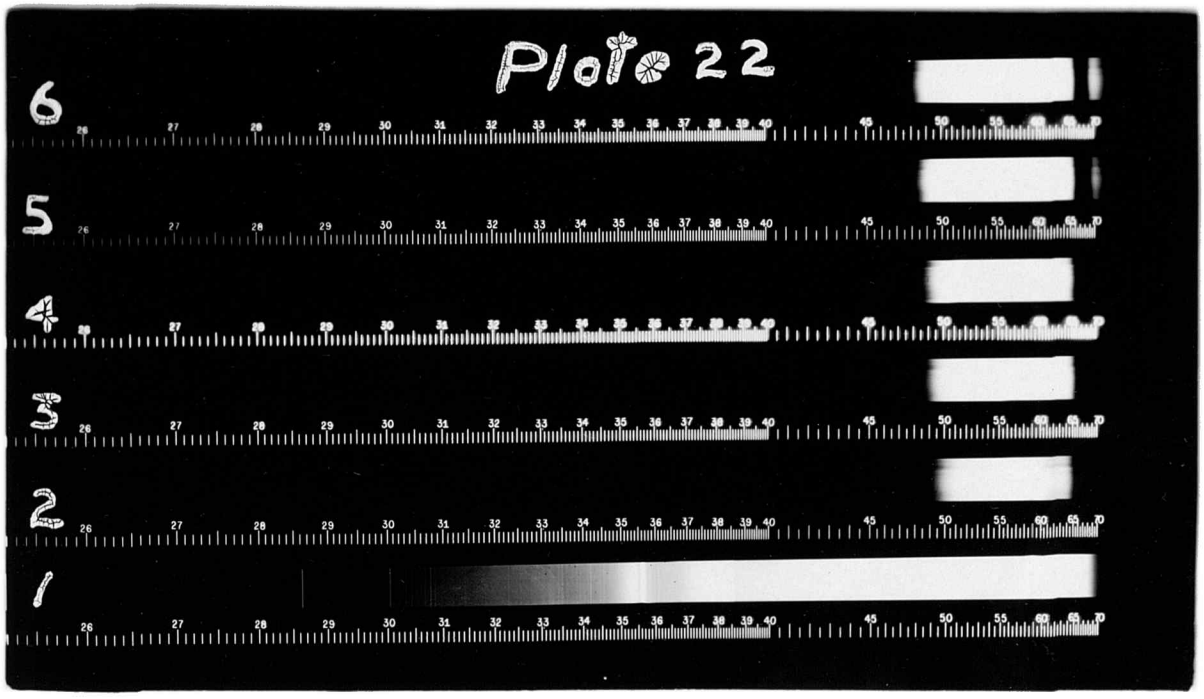
Plate 22
6
5
4
3
2
1

Plate 23.

	Sec.
1. Bare arc	2
2. Arc through Geranium Chlorophyll solution (alcohol)	2
3. Arc through Geranium Chlorophyll solution (alcohol)	4
4. Arc through Geranium Chlorophyll solution (alcohol)	8
5. Arc through Geranium Chlorophyll solution (alcohol)	16
6. Arc through Geranium Chlorophyll solution (alcohol)	32

27 mm. cell.

Plate 24.

	Sec.
1. Bare arc	2
2. Arc through Geranium Chlorophyll solution (alcohol)	2
3. Arc through Geranium Chlorophyll solution (alcohol)	4
4. Arc through Geranium Chlorophyll solution (alcohol)	8
5. Arc through Geranium Chlorophyll solution (alcohol)	16
6. Arc through Geranium Chlorophyll solution (alcohol)	32

9 mm. cell.

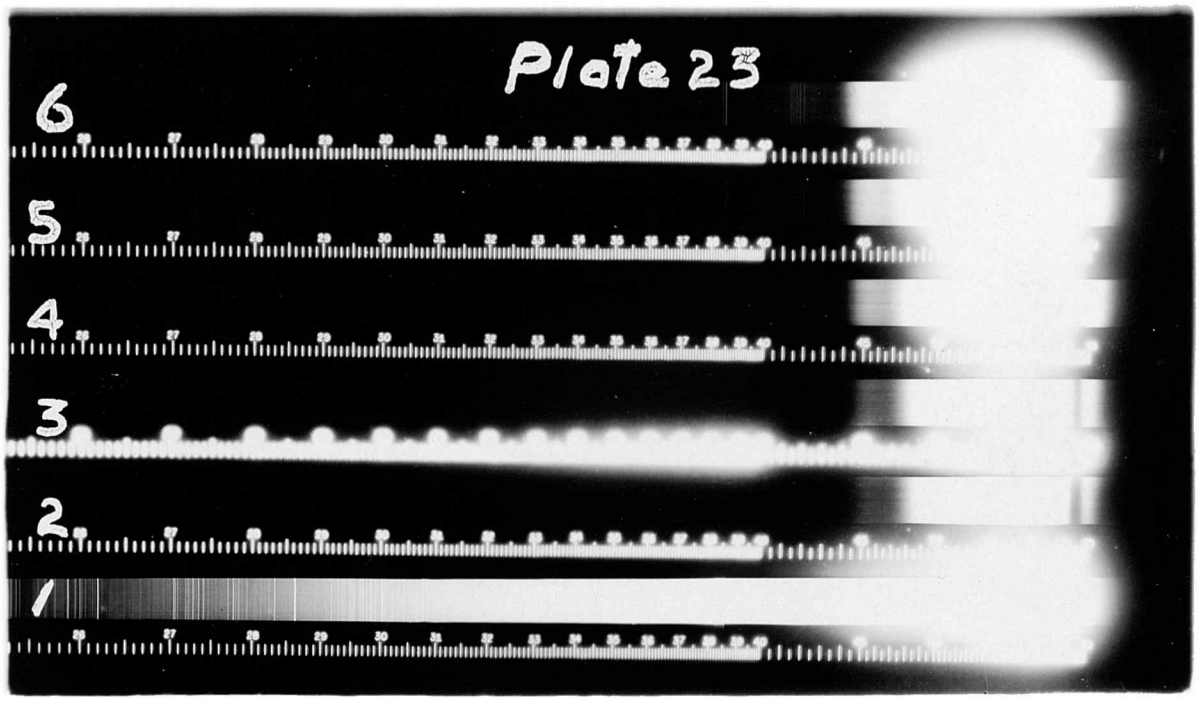
Plate 23
6
5
4
3
2
1

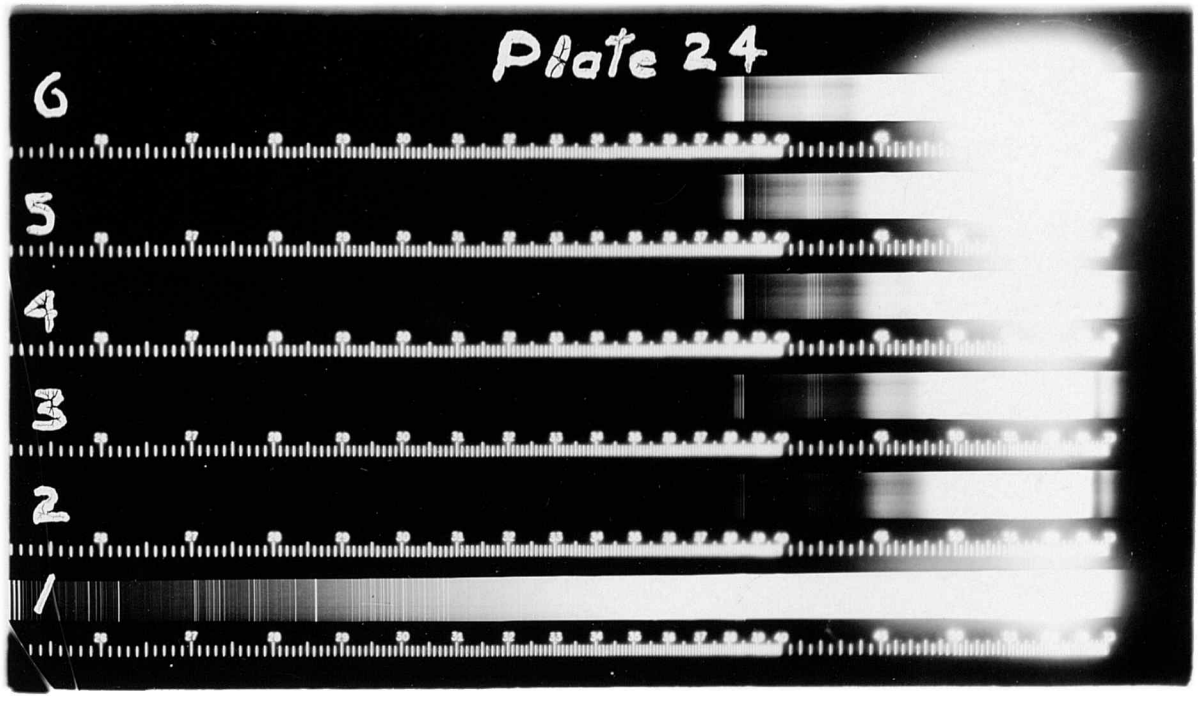
Plate 24
6
5
4
3
2
1

Plate 25.

1. Bare arc 2 sec.
2. Arc through Geranium Chlorophyll solution (alcohol) 2 sec.
3. Arc through Geranium Chlorophyll solution(alcohol) 4 sec.
4. Arc through Geranium Chlorophyll solution(alcohol) 8 sec.
5. Arc through Geranium Chlorophyll solution(alcohol) 16 sec.
6. Arc through Geranium Chlorophyll solution(alcohol) 32 sec.

27 mm. cell.

Solution from Plates 23-24 diluted to 1/3 concentration.

Plate 26.

1. Bare arc 2 sec.
2. Arc through Geranium leaf 1 min.
3. Arc through Geranium leaf 2 min.
4. Arc through Geranium leaf 3 min.

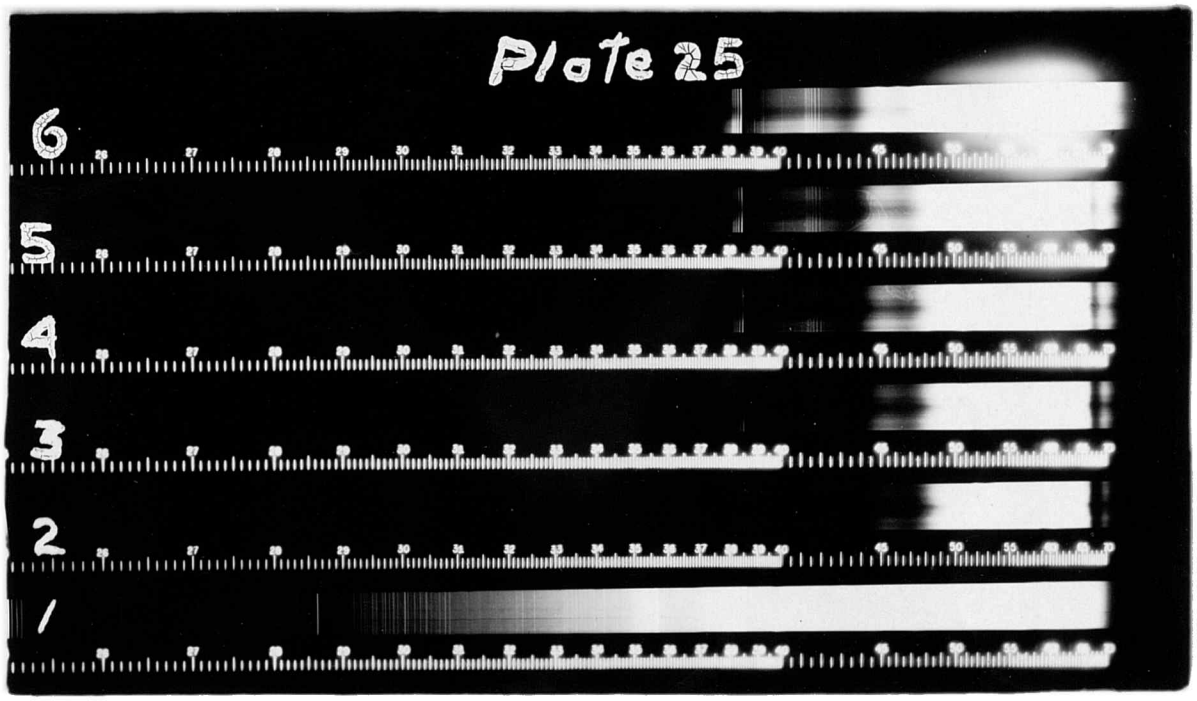
Plate 25
6
5
4
3
2
1

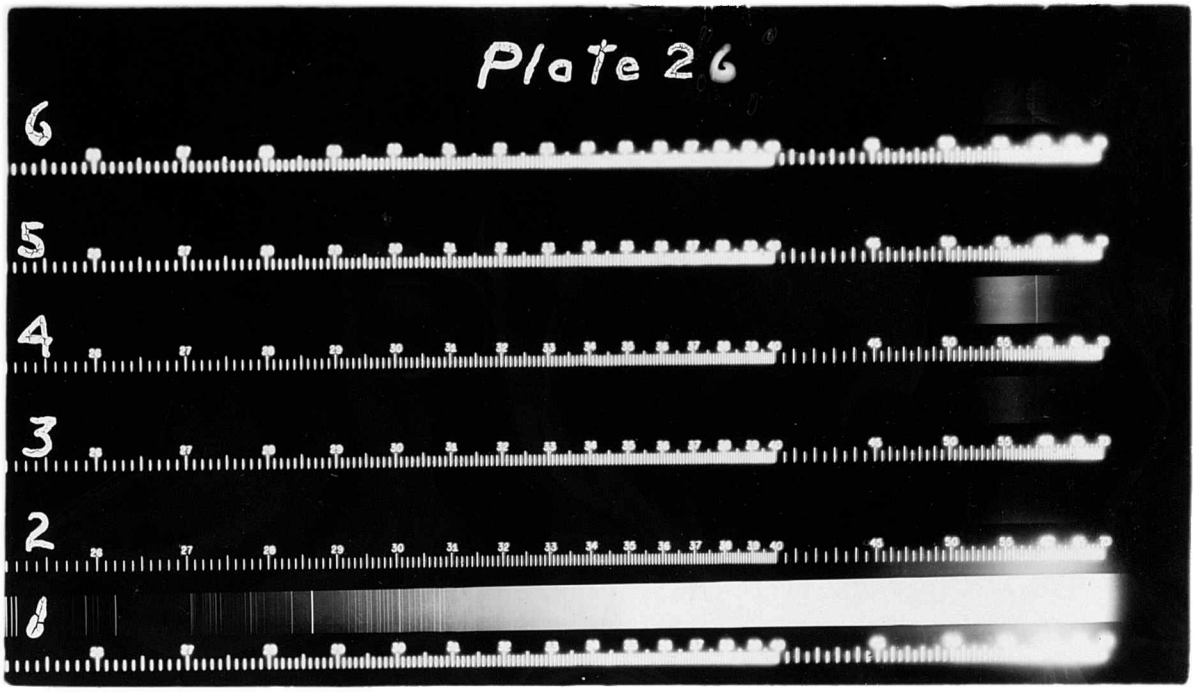
Plate 26
6
5
4
3
2
1