

Investigation on the Influence of Ultra-violet Rays on the Physiological Activities of Azotobacter.

III. Influence of Ultra-violet and Monochromatic Rays on the Pigment Production.

By

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This investigation deals with the influence of ultra-violet and monochromatic rays on the pigment production of *Azotobacter chroococcum* by using the natural light, Hanovia mercury lamp and vitalite lamp as the source of rays.

The previous investigations dealing with the pigment production of *Azotobacter* reported that such factors as oxygen supply^{1,2)} source of nitrogen³⁻⁶⁾ and carbon^{1-3,5)}, calcium carbonate^{1,7,8)}, temperature¹⁾, and reaction^{6,8,9)} are responsible for the pigment production. JONES²⁾, WINOGRADSKY¹⁰⁾ and others have reported that the pigment production is caused by the lack of energy so that the carbon supply has a great influence on its production. Also the alteration of reaction by an addition of calcium carbonate is considered to accelerate the pigment production. In regard to the chemical constituent of the pigment, RIPPPEL and LUDWIG⁷⁾ claimed that it is melanin made up of tyrosin, which seems to be most widely accepted.

POTTHOFF¹¹⁾ exposed microorganisms to the ultra-violet rays and noted that they lose their power to produce pigment but they recover the power on repeated exposure, and it is anticipated that *Azotobacter* may behave similarly although it remains to be experimented.

KATSER¹²⁾ investigated *Azotobacter agilis* as to the influence of light rays and found that among the visible rays, yellow and green color stimulated their growth and nitrogen fixing capacity while violet color has the opposite action.

Experimental.

The experimental procedure was the same as used in the preceding experiments¹³⁾. The culture of *Azotobacter chroococcum* either on ASHBY's agar slant or solution, was exposed to the rays from Hanovia mercury lamp, and the results were examined microscopically and macroscopically. The solution culture was grown in Erlenmeyer hard glass flask, quartz and hard glass test-tubes.

I. *Influence of Ultra-violet Rays on the Growth and Pigment Production of Azotobacter chroococcum.*

The growth was determined by the direct counting under microscope with an aid of MEISSNER'S solution, as described previously, and the pigment production as well as the growth on the agar medium was observed macroscopically.

The changes in number of bacteria exposed to the ultra-violet rays for different intervals, in Erlenmeyer flask are shown in Table I.

Table I.
Change in Bacterial Number.

Tests No.	Time in hours.	Length of exposure.					
		Control.	1 Min.	5 Min.	10 Min.	30 Min.	1 Hour.
I	Initial.	107	107	107	107	107	107
	24	1,043	1,128	1,410	1,043	761	1,043
	48	4,512	5,865	3,948	3,102	2,961	2,566
	72	10,886	9,475	11,590	8,629	7,755	7,219
	96	14,185	15,736	14,101	13,621	12,436	11,140
	120	17,794	18,217	16,920	16,723	16,554	14,382
II	Initial.	185	185	185	185	185	185
	24	1,994	2,022	1,805	1,580	1,168	1,184
	48	7,953	6,373	7,681	6,783	5,076	4,287
	72	11,675	12,098	13,621	9,842	9,408	10,434
	96	16,469	17,090	16,808	16,385	15,792	15,567
	120	18,951	19,881	20,220	19,938	16,469	14,636

Notes: The data show the number of organisms in 1cc. expressed by thousand.

Table II.
Experimental Results in Test-tubes of Hard Glass.

Test No.	Hours of incubation.	Length of exposure.											
		Growth.						Pigment production.					
		Con-trol.	1 Min.	5 Min.	10 Min.	30 Min.	1 Hour.	Con-trol.	1 Min.	5 Min.	10 Min.	30 Min.	1 Hour.
I	24	+	+	+	+	+	—	—	—	—	—	—	—
	48	++	++	++	++	+	—	—	—	—	—	—	—
	72	##	##	##	##	++	—	—	±	±	±	±	—
	96	##	##	##	##	++	+	+	+	++	++	++	—
	120	##	##	##	##	++	+	++	++	##	##	##	—
	168	##	##	##	##	++	+	##	##	##	##	##	—
II	24	+	+	+	+	+	—	—	—	—	—	—	—
	48	++	++	++	++	+	—	—	—	—	—	—	—
	72	##	##	##	##	+	±	±	±	±	+	±	—
	96	##	##	##	##	++	+	+	++	++	##	++	—
	120	##	##	##	##	++	+	++	##	##	##	##	—
	168	##	##	##	##	++	+	++	##	##	##	##	—

Notes: Number of + indicates the rate of growth and pigment production; — none; ± scant.

As Table I indicates, one minute exposure seems to be the optimum which was the same in the previous experiment, and the number decreased on longer exposure.

The results obtained on the solid medium in the test-tubes of hard glass, are shown in Table II and Plate VI, Fig. 1.

As shown above, no appreciable difference was found up to ten minutes exposure and more pigment was produced where the better growth took place as a whole but no regularity was observed. It seems that ten minutes exposure is the optimum for pigment production which is much longer than that for the growth.

The similar experiment as the preceding test was carried out in the quartz test-tubes and the results are given in Table III.

Table III.
Experimental Results in Quartz Test-tubes.

Test No.	Length of exposure.	Hours of incubation.											
		Growth.						Pigment production.					
		24	48	72	96	120	168	24	48	72	96	120	168
I	Control.	+	++	+++	+++	+++	+++	-	-	-	+	++	++
	1 Min.	+	++	++	++	++	++	-	-	-	-	+	++
	5 Min.	-	-	-	-	+	+	-	-	-	-	-	-
II	Control.	+	++	+++	+++	+++	+++	-	-	+	+	++	++
	1 Min.	+	++	+++	+++	+++	+++	-	-	+	+	++	++
	5 Min.	-	-	-	+	+	+	-	-	-	-	-	-

Notes: The rate of growth and pigment production is proportional to the number of +.

As the data in Table III indicate, the influence of ultra-violet rays is very markedly manifested, and one minute exposure deteriorates both the growth and pigment production.

II. Influence of Ultra-violet and Monochromatic Rays on the Growth and Pigment Production of *Azotobacter chroococcum*.

Azotobacter chroococcum was exposed to the natural light through different colored glass filters by using quartz, 'Acme' ultravit glass, hard glass test-tubes, Erlenmeyer flasks and Petri dishes.

A. Experiment under the Natural Rays:

The experiments were carried out in our green-house and also on the south side of our laboratory. The preparation was placed in a box of 40 cm. wide and 70 cm. high, which is covered with different colored glass.

The results are given in Plate VI, Fig. 2 and Table IV.

Table IV.
Experimental Results in Green-house.

Method of exposure.	Hours of incubation.	aH.	Growth.									Pigment production.								
			Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.	Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.
Quartz.	24	3.0	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	
	48	4.5	++	-	++	++	++	+	++	++	++	-	-	-	-	-	-	-	-	
	72	2.5	##	-	##	++	##	++	##	##	##	-	-	-	-	-	-	-	-	
	96	2.5	##	-	##	++	##	++	##	##	##	-	-	±	-	-	-	-	-	
	120	3.5	##	-	##	++	##	++	##	##	##	-	-	+	-	±	±	-	±	
	144	4.5	##	-	##	++	##	++	##	##	##	±	-	+	-	+	+	+	+	
	168	2.0	##	-	##	++	##	++	##	##	##	+	-	+	-	+	+	+	+	
'Acme' ultravit glass.	24	3.0	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	
	48	4.5	++	-	++	++	++	+	++	++	++	-	-	-	-	-	-	-	-	
	72	2.5	##	-	##	++	##	+	##	++	##	-	-	-	-	-	-	-	-	
	96	2.5	##	-	##	++	##	+	##	##	##	+	-	-	-	-	-	-	+	
	120	3.5	##	-	##	++	##	+	##	##	##	++	-	±	-	±	±	±	++	
	144	4.5	##	-	##	++	##	+	##	##	##	##	-	+	-	+	+	+	##	
	168	2.0	##	-	##	++	##	+	##	##	##	##	-	+	-	+	+	+	##	
Petri dish.	24	3.0	+	-	++	+	++	+	++	++	++	-	-	-	-	-	-	-	-	
	48	4.5	++	-	++	++	++	++	++	++	++	-	-	-	-	-	-	-	±	
	72	2.5	##	-	##	++	##	++	##	##	##	-	-	±	-	-	-	-	+	
	96	2.5	##	-	##	++	##	++	##	##	##	+	-	+	-	±	±	±	++	
	120	3.5	##	-	##	++	##	++	##	##	##	++	-	++	-	+	+	+	##	
	144	4.5	##	-	##	++	##	++	##	##	##	++	-	++	-	++	+	+	##	
	168	2.0	##	-	##	++	##	++	##	##	##	++	-	++	-	++	+	+	##	
Hard glass.	24	3.0	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	
	48	4.5	++	-	++	++	++	++	++	++	++	-	-	-	-	-	-	-	-	
	72	2.5	##	-	++	++	++	++	##	##	##	-	-	-	-	-	-	-	-	
	96	2.5	##	-	##	++	++	++	##	##	##	±	-	±	-	±	±	±	±	
	120	3.5	##	-	##	++	##	++	##	##	##	+	-	+	±	±	+	±	+	
	144	4.5	##	-	##	++	##	++	##	##	##	##	-	++	+	++	+	+	##	
	168	2.0	##	-	##	++	##	++	##	##	##	##	-	++	+	++	+	+	##	

Notes: Number of + indicates the rate of growth and pigment production; - none; ± scant.

The results shown in Plate VI, Fig. 2 and Table IV were obtained in our green-house, which indicate that those exposed to ultra-violet, black and red rays

as well as the control kept in the incubator grew vigorously and produced good pigment while no pigment and growth was produced in which was kept in the green-house, and very scant growth and slight pigment were produced under orange color. As Table IV indicates, little difference was noted among the tests carried out under different conditions.

Since the foregoing tests were carried out in the green-house where the temperature sometime rised above 50°C. and it went down as low as 19°C., in other cases, the following tests were carried out-of-doors on south side of the laboratory where the average temperature was about 28°C.

The results obtained as to the change of bacterial number is given in Table V and the relation between the growth and pigment production is noted in Table VI respectively.

Table V.
Change in the Number of Cells.

Test No.	Hours of incubation.	aH.	Color of glass.								Ultra-violet.
			Con-trol.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	
I	Initial.	—	133	133	133	133	133	133	133	133	133
	24	1.0	1,895	1,895	3,181	2,121	3,181	1,918	2,707	2,279	2,391
	48	1.5	4,174	4,264	6,249	3,835	5,076	3,091	3,813	4,670	6,791
	72	1.5	4,670	4,715	6,994	3,793	6,046	4,535	4,715	4,941	6,881
	96	1.5	6,069	6,069	8,573	5,640	7,806	5,347	6,588	6,046	7,039
	120	1.5	7,874	6,588	10,468	6,994	8,957	6,249	6,381	6,136	9,408
II	Initial.	—	81	81	81	81	81	81	81	81	81
	24	1.5	1,534	1,647	2,391	1,692	2,030	1,421	1,579	1,827	1,850
	48	1.5	2,910	3,023	4,444	2,437	3,226	2,391	2,775	3,001	3,339
	72	1.0	3,339	3,407	4,670	3,452	3,610	2,775	3,362	3,181	3,519
	96	1.5	4,264	3,610	5,076	3,655	4,377	3,249	3,790	3,790	4,444
	120	2.0	5,189	5,189	7,919	5,347	6,971	5,640	6,069	4,941	6,136

Notes: Data in the table indicate the number of organisms in 1 cc. by thousand.

(See Table VI on next page.)

As the above results indicate, the good growth and abundant number of bacteria were obtained under ultra-violet, black and red rays, which were better than the control. On the other hand, in the open room and under white light as well as orange and violet, the growth was worse. Again the cultures on the solid medium under different conditions showed the similar results as in the solution culture. It is worthy to note that the growth in the control which was kept in the incubator was worse than that under ultra-violet, black and red rays, out-of-doors, which may be possibly due to the fluctuation of temperature in case of the out-of-doors or in other words the fluctuation of temperature caused the stimulation.

Table VI. (Continued.)

Method of exposure.	Test No.	Hours of incubation.	aH.	Growth.									Pigment production.								
				Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.	Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.
Hard glass.	I	24	1.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		48	1.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		72	1.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		96	1.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		120	1.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		168	3.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		240	5.0	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
	II	24	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		48	1.5	##	+	##	##	##	##	##	+	+	##	##	##	##	##	##	##	##	##
		72	1.0	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		96	1.5	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		120	2.0	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		168	3.0	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
		240	4.0	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
				##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##

Notes: Number of + indicates the rate of growth and pigment production; - none; ± scant.

Further the growth in the hard-glass test-tubes, produced after 168 hours is shown in Plate VII, Fig. 1.

B. Experiment under the Vitalite Lamp:

The experiments in Part A were carried out under the natural light of which the quantity of ultra-violet rays varies by the hours of a day and also by the daily weather conditions¹⁴. In this experiment, the vitalite lamp of 300 W, 110 V was used as the source of ultra-violet rays, at the distance of 100 cm. and the exposure was made continuously through different colored glass plate of 17 cm. square.

The quantity of ultra-violet rays was determined as described previously and found aH for 24 hours was 1.08—1.11¹⁵. The culture on solid medium alone was used, and obtained the results which are given in Table VII and Plate VII, Fig. 2.

(See Table VII on next page.)

This experiment was carried out in our dark-room where the temperature variation was comparatively small and the average temperature was 28°—30°C. during the experiment. The results given in Table VII indicate that in all cases the results were similar to those obtained under the natural light although the degree of differences among different rays is not so marked in this case. The reason for this slight difference may be due to the weakness of intensity of rays in this experiment so that the initial difference which might have existed is nullified at the end. However as a whole good growth was obtained under ultra-

Table VII.
Results obtained under Vitalite Lamp.

Method of exposure.	Test No.	Hours of incubation.	Growth.										Pigment production.									
			Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.	V. G.	Control.	Room.	Black.	White.	Red.	Orange.	Green.	Violet.	Ultra-violet.	V. G.
Quartz.	I	24	+	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±
		48	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	II	24	+	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±
		48	+	+	±	+	±	±	±	±	±	±	±	±	±	±	+	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Hard glass.	I	24	+	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±
		48	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	II	24	+	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	±
		48	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Petri dish.	I	24	+	+	±	+	±	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		48	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	II	24	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		48	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		72	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		96	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		120	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		144	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		168	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±

Notes: V.G.= 'Acme' ultravit glass; the number of + indicates the rate of growth and pigment production; ± scant.

violet, black, red and green rays with the control, and the pigment production showed the same tendency. The results produced in Petri dish on the last day are shown in Fig. IV.

Summary.

The influence of ultra-violet and monochromatic rays on the pigment production of *Azotobacter chroococcum* was investigated by using the natural light, Hanovia mercury lamp and vitalite lamp as the source of rays, and obtained the following results:

(1) As in the case of growth experiment, the pigment production of *Azotobacter* was stimulated by a short exposure to the ultra-violet rays but the optimum exposure for the pigment production is longer than that for the growth. For example, in case of Hanovia lamp; 1 minute for the growth and 10 minutes for the pigment production.

(2) Among the monochromatic rays, black, red and green were better for the pigment production than violet and orange, which was the same in case of growth. The pigment production was very meager when the culture was left standing in a room or under white rays.

(3) As a rule, the pigment production was marked in the good growth but not always in parallel each other and sometimes it is reversed.

(4) Exposing *Azotobacter* for a long period to vitalite lamp, a marked difference was observed at the initial stage but the difference became less and less as the time passed. This seems to be due to the adaptability of the organism to the rays.

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PLATE VI.

Fig. 1. Influence of Ultra-violet Rays on Pigment Production.



Notes: (1) Control; (2) 1 Min.; (3) 5 Min.; (4) 10 Min.;
(5) 30 Min.; (6) 1 Hour.

Fig. 2. Influence of Monochromatic Rays on Pigment Production.



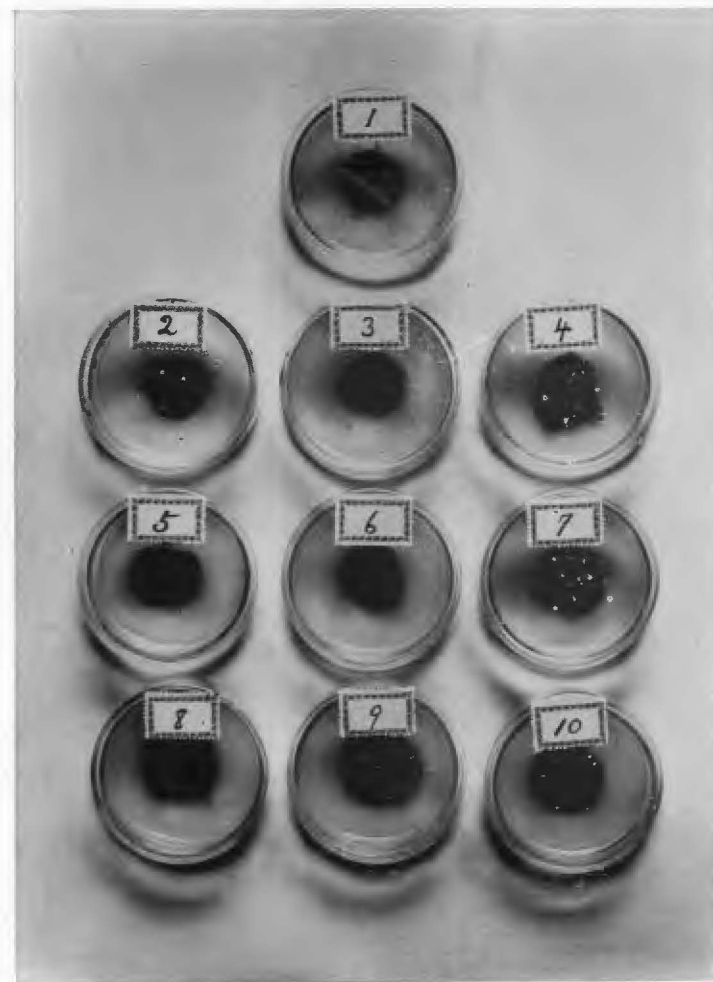
Notes: (1) Control; (2) Room; (3) Black; (4) White; (5) Red; (6) Orange;
(7) Green; (8) Violet; (9) Ultra-violet. (1 min. exposure.)

Fig. 1. Influence of Monochromatic Rays on
Pigment Production.



Notes: (1) Control; (2) Room; (3) Black; (4) White; (5) Red;
(6) Orange; (7) Green; (8) Violet; (9) Ultra-violet. (1 min.
exposure.)

Fig. 2. Influence of Continuous Exposure on
Pigment Production.



Notes: (1) Control; (2) Room; (3) Black; (4) White;
(5) Red; (6) Orange; (7) Green; (8) Violet;
(9) Ultra-violet. (1 min. exposure.) (10) V.G. =
'Acme' ultravio. glass.