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

II. On the Stimulation of Azotobacter chroococcum by Ultra-violet Rays.

By

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The preceding paper[6] dealt with the lethal action of ultra-violet rays on Azotobacter chroococcum, and in the course of investigation, it was found that a short exposure stimulated the physiological activity of Azotobacter, and in this investigation, the following points were investigated and the results are reported: 1.) Rate of stimulation by different length of exposure, determined by the number of cells and also by the change of P_H in the medium; 2.) Mechanism of stimulation, the influence of ultra-violet rays on the physical properties of medium and their subsequent influence on Azotobacter; 3.) Manner of exposure, continuous or intermittent.

In regard to the stimulating influence of ultra-violet rays, there are numerous reports in other fields. Recently Owen and Mabley[1] reported the growth and the rate of alcohol fermentation are stimulated by the action of ultra-violet rays. Nadson and Philippov[2] investigated the action of ultra-violet rays on Saccharomyces and also Mucor, and found that both of these organisms are stimulated as to their physiological activities. As to the bacteria, several investigators reported with more or less difference that bacteria are weaker than yeasts and fungi against the action of ultra-violet rays. A majority of reports concerning bacteria are in regard to the lethal action of the rays and comparatively few on the stimulating action. For this reason, this investigation was carried out on Azotobacter.

Experimental:

With an exception of the use of filter, the method employed is the same as described previously, using Hanovia mercury lamp as the source of ultra-violet rays and exposed Azotobacter chroococcum for different intervals in Ashby's

solution medium in Erlenmeyer flask of hard glass and cells were counted by the direct microscopic method stained with Meissner's solution, and P_H was determined by the quinhydrone electrode.

Results:

I. Stimulation by Ultra-violet Rays:

First the results obtained in the previous investigation were substantiated and found that the five seconds exposure for quartz tube, 30 seconds to 1 minute for Erlenmeyer flask of ordinary glass, had the stimulating effect. Then further it was investigated to find the optimum exposure by using the shorter intervals for exposure, and the change of cell number and P_H were examined and obtained the following results:

Table I.

Change in the Number of Cells.

Experiment No.	Hours.	Length of exposure.						
		Control.	5 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.
I	Initial.	221	221	221	221	221	221	221
	5	446	446	469	587	540	460	587
	24	16,056	16,244	16,526	18,685	11,549	11,549	12,207
	48	333,333	38,122	48,920	52,864	44,037	38,310	26,009
	72	40,939	46,385	65,164	79,437	64,132	48,826	49,202
	120	105,164	108,357	120,751	142,911	114,836	88,169	78,216
II	Initial.	232	232	232	232	232	232	232
	5	363	376	426	563	282	305	305
	24	17,747	17,465	20,845	19,061	10,798	9,671	7,512
	48	44,507	47,981	44,225	55,211	38,967	20,845	24,601
	72	60,235	62,347	74,460	82,348	65,916	49,953	51,268
	120	83,850	102,535	103,099	98,404	87,042	61,315	61,409
III	Initial.	272	272	272	272	272	272	272
	5	798	915	915	1,033	587	587	587
	24	8,627	8,732	12,207	14,085	9,484	10,141	9,484
	48	33,427	33,709	37,653	33,897	23,380	20,376	20,845
	72	47,981	50,704	56,244	50,798	40,563	32,301	32,301
	120	80,000	81,972	81,972	88,639	76,244	43,286	40,939

Note: Data in the table indicate the number of organisms in 1 cc. by thousands.

Table II.

Change of pH.

Experiment No.	Hours.	Length of exposure.						
		Control.	5 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.
I	Initial.	7.00	6.97	6.88	6.95	6.97	6.97	7.00
	24	6.72	6.74	6.72	6.75	6.77	6.76	6.77
	48	6.70	6.69	6.63	6.62	6.64	6.64	6.64
	72	6.68	6.58	6.59	6.56	6.58	6.59	6.60
	120	6.59	6.59	6.59	6.56	6.59	6.60	6.62
	168	6.59	6.56	6.57	6.54	6.59	6.61	6.61
II	Initial.	6.74	6.82	6.81	6.77	6.82	6.81	6.84
	24	6.61	6.62	6.60	6.58	6.65	6.61	6.67
	48	6.56	6.62	6.56	6.57	6.63	6.57	6.60
	72	6.53	6.48	6.53	6.50	6.55	6.55	6.53
	120	6.55	6.51	6.55	6.44	6.57	6.51	6.52
	168	6.53	6.51	6.58	6.48	6.59	6.55	6.52
III	Initial.	6.86	6.88	6.86	6.88	6.88	6.93	6.90
	24	6.65	6.63	6.63	6.65	6.64	6.64	6.64
	48	6.59	6.59	6.62	6.64	6.62	6.64	6.64
	72	6.58	6.59	6.55	6.55	6.57	6.63	6.61
	120	6.58	6.59	6.54	6.53	6.61	6.60	6.60
	168	6.57	6.52	6.55	6.53	6.61	6.62	6.61

The data in Table I indicate that 30 seconds and 1 minute exposure increased the number of cells and it was greatest at 1 minute exposure while 2 minutes exposure differed slightly from the control, and longer exposure than 2 minutes caused a rapid decrease in number of cells. The change of P_H was in parallel with the number of cells in general. Again the number of cells are shown graphically as follows:

(See Figure I, II and III on the page 564—565.)

The above results indicate plainly that a short exposure to the mercury lamp stimulated the organism and the optimum exposure was 30 seconds to 1 minute. Further an enquiry was made to ascertain if the stimulation is solely due to the action of ultra-violet rays or the change in temperature and some factors in the culture medium might have caused to bring about the stimulation.

Figure I.

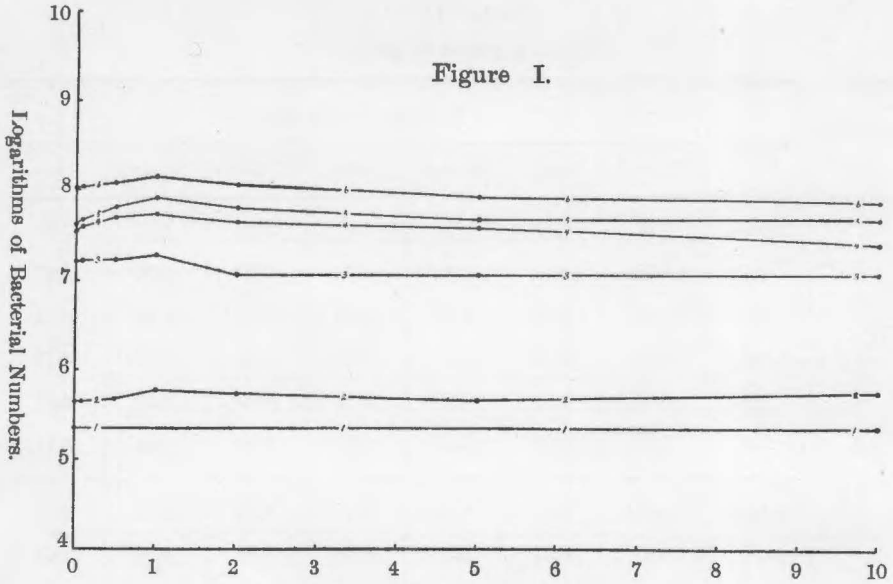
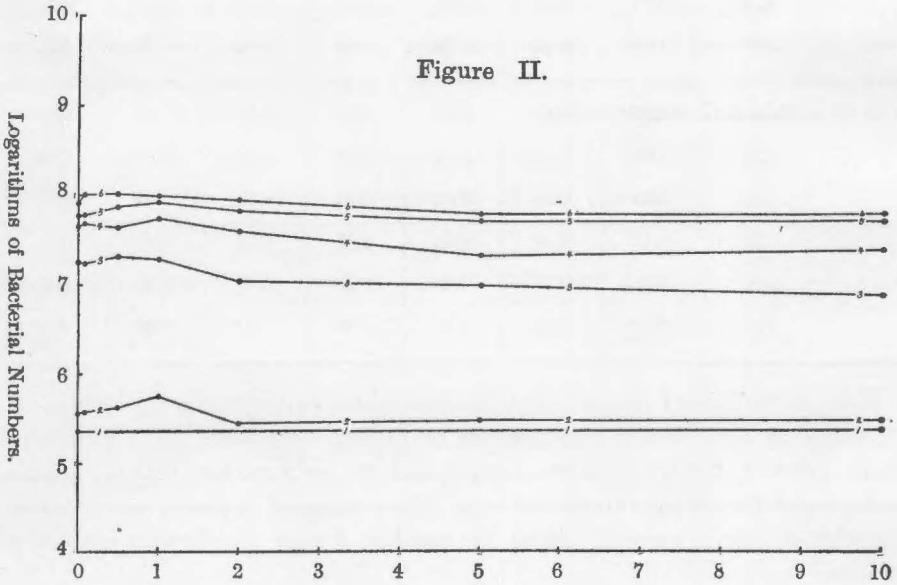


Figure II.

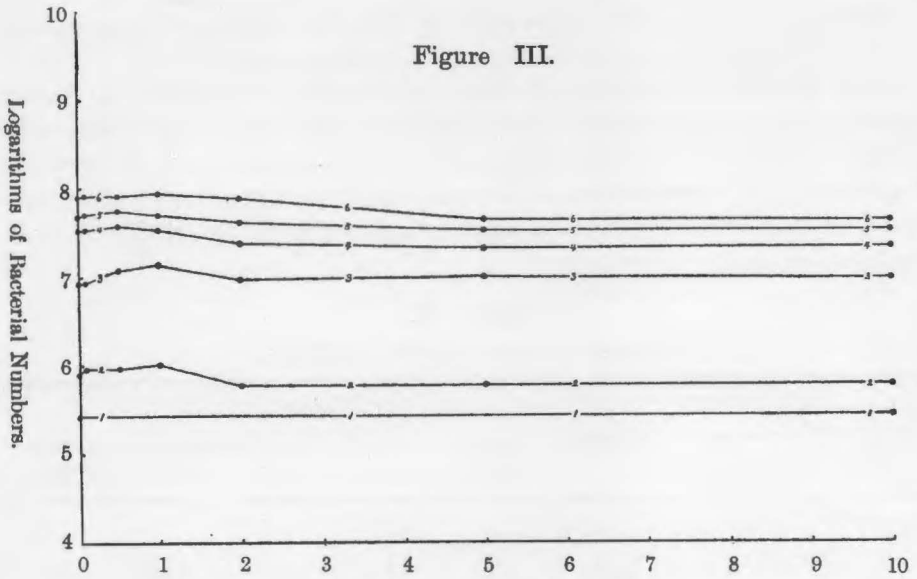


Explanations of Graph I, II and III:

Graph I, II and III represent the results of corresponding experimental number, and the numerical figure in the graphs indicate the number of hours as follows:

1—initial; 2—5; 3—24; 4—48, 5—72; 6—120 hours.

Figure III.



The experiment was carried out in the same manner as in the previous case except some flasks with the culture medium were exposure to the rays before the inoculation to test for any change may take place by the exposure, and others covered with black paper in order to test the possible influence of temperature, and obtained the following results:

Table III.

Change in the Number of Cells.

Exp't No.	Hours.	Control.	Length of exposure.					
			After inoculation.		Before inoculation.		Covered with black paper.	
			30 sec.	1 min.	30 sec.	1 min.	30 sec.	1 min.
I	Initial.	215	215	215	215	251	215	215
	24	13,521	17,277	16,995	11,455	11,996	11,737	10,798
	48	19,906	27,700	30,798	18,309	17,840	19,531	17,559
	72	49,202	65,258	70,235	51,268	45,728	46,385	46,573
II	Initial.	152	152	152	152	152	152	152
	24	9,484	12,113	13,333	6,854	9,390	6,948	9,108
	48	24,131	26,948	28,451	17,840	18,028	20,563	17,746
	72	49,014	55,681	64,319	54,648	49,484	48,075	46,662
III	Initial.	194	194	194	194	194	194	194
	24	13,052	10,047	12,300	10,329	8,545	7,700	8,545
	48	23,850	24,789	29,890	23,944	21,972	211	20,940
	72	57,840	20,563	63,005	47,700	56,432	57,934	52,300

Note: Data in the table indicate the number of organisms in 1 cc. by thousands.

Table III indicates that no appreciable difference was noted among these flasks indicating that the influence was due to the action of rays.

Next the possible influence of visible rays was investigated by eliminating the visible rays by the use of reliable Hanovia light filter. The flasks were prepared same as before and were placed at 15 cm. distance and the quantity of ultra-violet rays was determined by molybdic acid method and found to be aH 0.49—0.52 and the temperature during the experiment was 24°C. and 29°C. The following results were obtained :

Table IV.

Change in the Number of Cells.

Experiment No.	Hours.	Control.	Length of exposure.				
			1 min.	2 min.	5 min.	10 min.	30 min.
I	Initial.	43	43	43	43	43	43
	24	4,883	4,788	7,981	4,319	3,441	4,319
	48	8,357	8,451	9,465	8,826	5,352	6,009
	72	27,512	33,333	33,146	21,408	16,432	18,122
II	Initial.	19	19	19	19	19	19
	24	2,233	2,166	2,775	1,873	1,309	1,105
	48	3,271	3,452	4,557	2,459	2,233	1,918
	72	6,904	7,287	8,753	6,340	6,430	4,738
III	Initial.	12	12	12	12	12	12
	24	2,504	3,046	3,046	2,594	1,489	1,918
	48	5,121	5,798	7,242	5,076	4,038	4,219
	72	6,204	8,167	8,235	5,031	5,279	5,595

Note: Data in the table indicate the number of organisms in 1 cc. by thousands.

The results given in Table IV indicate that 2 minutes exposure gave the best growth in all cases, indicating that the influence is entirely due to the action of ultra-violet rays.

Thus the preceding experiments proved that the ultra-violet rays stimulate the growth of Azotobacter.

II. Influence of Ultra-violet Rays on the Physico-chemical Properties of Culture Medium :

It was shown in the previous experiment that the stimulation is not due to the change in the medium caused by the action of rays. However it is widely known that in the presence of free oxygen, organic substance are oxidized

markedly by the action of ultra-violet rays. DUCLAUX[8] noted the oxidation of carbohydrate by the sunlight. Again CALABEK[9] reported that the swelling properties of agar becomes less by the action of ultra-violet rays. Consequently an attempt was made to ascertain if any physico-chemical change may occur in the culture medium when it is exposed to the ultra-violet rays, and the following experiment was undertaken: 50 cc. of the medium were placed in Erlenmeyer flask as usual and exposed to the rays for different intervals and examined the medium as to its electrical conductivity, P_H, osmotic pressure, viscosity and surface tension, as noted in Table 5.

Table V.

Influence on the Physical Properties of Medium.

Length of exposure.	Exp't No.	Physical properties.				
		Electrical conductivity. (10^{-3} mho.)	P_H	Osmotic pressure. (atmospheric.)	Viscosity.	Surface tension. (dynes/sq. cm.)
Control.		8.72114	6.78	1.6577	1.04852	80.42974
1 min.	I	8.67647	6.83	1.6577	1.04430	80.42974
	II	8.72114	6.79	1.6698	1.03797	80.52134
	Average	8.69881	6.81	1.66375	1.041135	80.47554
10 min.	I	8.53272	6.91	1.7424	1.03110	80.42974
	II	8.56869	6.88	1.6940	1.03532	80.42974
	Average	8.55071	6.895	1.7182	1.03321	80.42974
30 min.	I	8.55914	6.94	1.8392	1.04304	80.52134
	II	8.56869	6.93	1.8634	1.04522	80.42974
	Average	8.56391	6.935	1.8513	1.04413	80.47554

Table V indicates that the electrical conductivity and hydrogen ion concentration decreased gradually while the osmotic pressure increased. No appreciable change in viscosity and surface tension took place so that it seems that the action of ultra-violet rays is effective on the electrolysed substance, which may indicate the reduction.

In order to see the subsequent influence on the lethal action by long exposure, the following experiment was undertaken:

The culture medium was placed in quartz tubes, and some of them were exposed to the rays previous to the inoculation, and the others were exposed after the inoculation, and the results obtained were as follows:

Table VI.

Influence on the Cell Number by exposing before and after the Inoculation.

Experiment No.	Hours.	Control.	Manner of exposure.			
			Before inoculation.		After inoculation.	
			5 min.	10 min.	5 min.	10 min.
I	Initial.	14,366	14,366	14,366	14,366	14,366
	24	3,756	3,850	3,662	70	94
	48	11,549	10,141	10,516	94	23
	72	32,958	33,333	28,075	47	164
II	Initial.	17,277	17,277	17,277	17,277	17,277
	24	3,756	3,756	3,192	305	329
	48	12,958	13,333	12,864	258	217
	72	26,526	29,390	21,502	282	141
III	Initial.	17,653	17,653	17,653	17,653	17,653
	24	8,451	6,385	8,197	188	163
	48	14,272	16,150	14,291	94	94
	72	17,371	16,808	16,995	258	103

Note: Data in the table indicate the number of cells by thousands.

As the results in Table VI indicate, the pre-exposure of the medium has no influence of any significance on the results and obtained almost the identical results as in the control. In those exposed after the inoculation, the bacterial number decreased markedly after 5—10 minutes. From these results it may be stated that the physico-chemical change which might have occured during the experiment has little influence on the lethal action on bacteria.

III. Stimulating Action and Method of Exposure:

If the organisms are exposed continuously to the stimulation, often the stimulation action ceases to be effective, so that the ultra-violet rays applied continuously and the other intermittently for different intervals during 24 hours, as noted below and obtained the results shown in Table VII and Fig. IV—VI.

Table VII.

Influence of Continuous and Intermittent Exposure.

Experiment No.	Hours.	Control.	Manner of exposure.											
			Continuous.						Intermittent.					
			5 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.	5 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.
I	Initial.	111	111	111	111	111	111	111	111	111	111	111	111	111
	5	446	446	469	587	457	423	587	493	446	634	540	493	399
	24	16,056	16,244	16,526	18,685	14,366	10,423	12,207	14,554	14,366	16,244	15,211	12,207	10,704
	48	33,333	38,122	48,920	52,864	38,028	28,169	26,009	38,216	40,939	40,939	37,277	23,380	29,202
	72	40,939	46,385	65,164	79,437	45,822	38,028	37,277	60,188	59,061	48,075	40,939	38,967	41,878
	120	105,164	108,356	120,751	142,911	88,639	80,000	28,216	100,939	127,136	93,427	79,343	62,629	71,080
II	Initial.	166	166	166	166	166	166	166	166	166	166	166	166	166
	5	423	376	423	563	282	305	305	423	493	587	305	516	446
	24	15,211	17,465	20,845	19,061	10,798	9,671	7,512	14,554	14,836	18,028	10,423	12,488	8,732
	48	44,507	47,981	44,225	55,211	38,967	20,845	24,601	45,540	48,515	49,484	27,136	29,390	17,371
	72	59,061	62,347	74,460	82,318	65,916	49,953	51,268	65,164	65,634	53,803	37,371	34,930	45,634
	120	83,850	102,535	103,099	108,357	87,042	61,315	61,409	88,639	90,517	85,071	77,747	63,662	60,188
III	Initial.	136	136	136	136	136	136	136	136	136	136	136	136	136
	5	798	915	915	1,033	587	587	563	845	823	804	493	399	423
	24	8,197	8,732	12,207	14,085	9,484	10,141	9,263	9,484	12,207	13,831	7,042	8,639	7,014
	48	26,237	33,709	37,653	53,897	23,380	20,376	19,061	25,164	26,852	23,474	22,066	15,681	12,207
	72	45,721	50,704	56,244	60,798	40,563	32,301	30,704	46,385	44,319	44,413	40,845	33,521	24,601
	120	75,691	81,972	81,972	88,639	76,244	43,286	38,967	76,808	79,718	65,540	48,075	33,991	28,639

Note: Data in the table indicate the number of cells by thousands.

Figure IV.

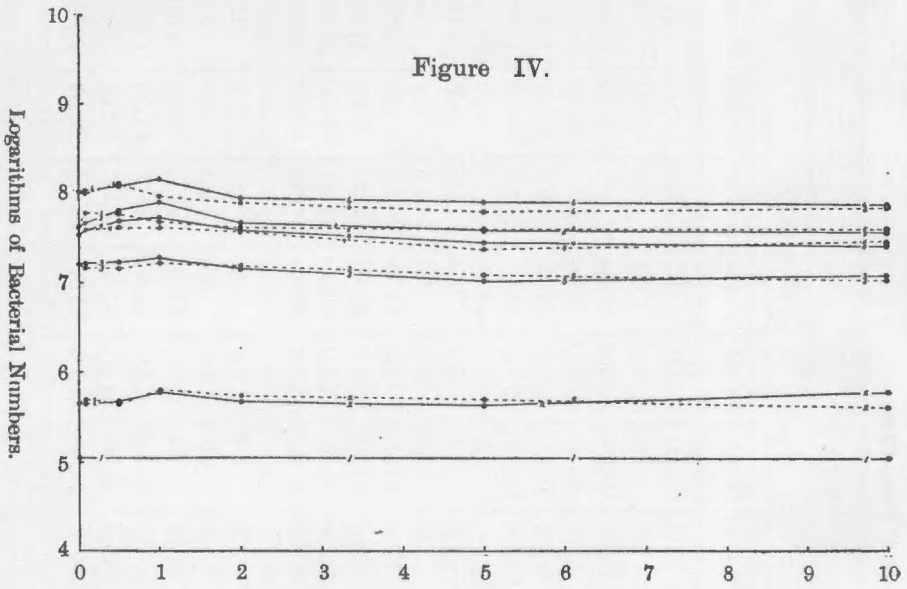


Figure V.

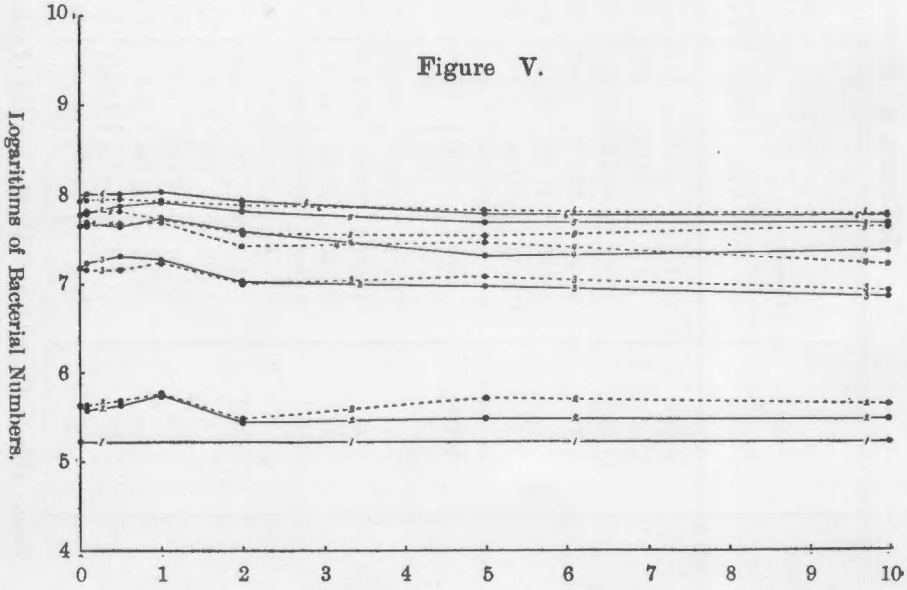
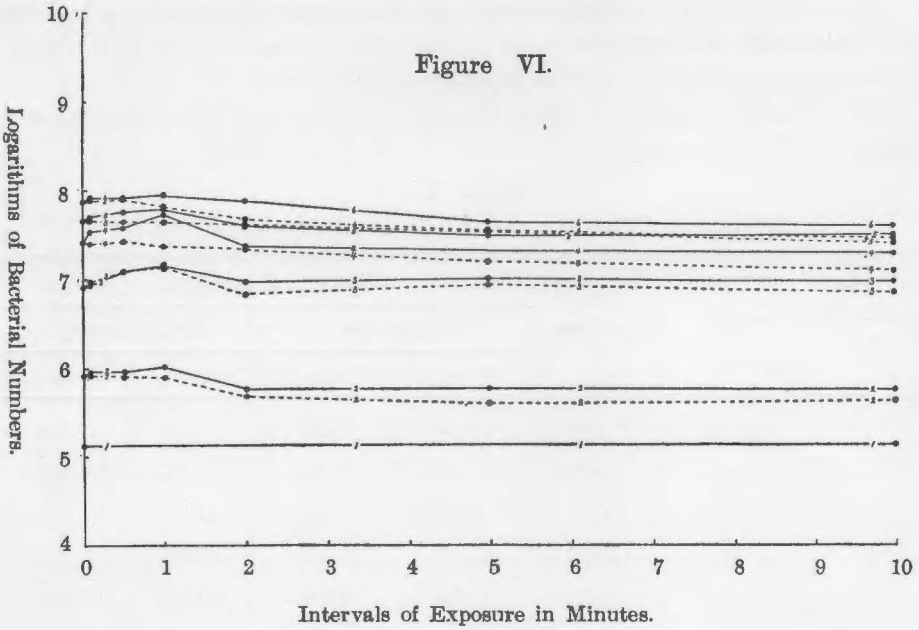


Figure VI.



Explanations of Graph IV, V and VI:

Graph IV, V and VI represent the results of corresponding experimental number, and the numerical figure in the graphs indicate the number of hours as noted previously. The solid line shows the continuous exposure and the broken, the intermittent.

Table VII indicates that no marked difference was noted during the first 24 hours but on repeating exposure, the number of bacteria became highest on shorter exposure after 5 days. One minute was most effective in the continuous exposure while 5 or 35 seconds in the intermittent series, and longer exposure made no difference. From these results, it is better to expose just once than repeating it. The comparative data of one and two minutes continuous, twice and four times 30 seconds exposure are noted in Table VIII.

Table VIII.

Influence of Continuous and Intermittent Exposure.

Exposure. / Exp't No.	1 minute.		2 minutes.	
	Continuous.	Intermittent.	Continuous.	Intermittent.
I	52,864	40,939	88,639	127,136
II	55,211	48,545	87,042	90,517
III	53,897	26,852	76,244	79,718

Note: Data in the table indicate the number of cells in 1 cc. by thousands.

As the above data indicate that in the continuous exposure more bacteria were found than in the intermittent in case of one minute but in two minutes exposure, the intermittent exposure stimulated much better.

By using the quarts test tubes, the similar experiment was repeated and obtained the following results.

Table IX.

Influence of Continuous and Intermittent Exposure.

Experiment No.	Hours.	Control.	Manner of exposure.			
			Continuous.		Intermittent.	
			5 sec.	30 sec.	5 sec.	30 sec.
I	Initial.	111	111	111	111	111
	5	376	423	164	376	235
	24	3,944	5,446	376	4,254	376
	48	10,423	16,338	6,103	12,770	728
	72	16,714	20,470	8,826	21,033	1,502
	120	32,019	41,090	16,995	36,244	2,535
	168	62,535	64,507	35,024	60,460	17,089
II	Initial.	136	136	136	136	136
	5	446	446	423	376	235
	24	3,850	4,319	1,596	4,441	399
	48	14,366	15,211	4,038	9,859	4,038
	72	29,718	31,455	15,962	30,704	4,225
	120	37,277	45,822	15,024	35,399	7,324
	168	48,028	60,845	33,991	49,577	23,850

Note: Data in the table indicate the number of cells in 1 cc. by thousands.

Table IX indicates again that no better stimulation influence was observed by the intermittent exposure.

Summary:

In this investigation, the stimulation influence of ultra-violet rays on Azotobacter chroococcum was undertaken by using Hanovia mercury lamp as the source of rays.

1.) In Erlenmeyer flasks of hard glass, the number of bacteria was greatest on one minute exposure and longer exposure caused depression. The change of P_H was greatest at one minute exposure and tended to become acidic.

2.) The stimulation observed in this investigation was solely due to the action of ultra-violet rays and the heat rays and physico-chemical change in the culture medium had little influence.

3.) By exposing the medium to ultra-violet rays, the electrical conductivity, hydrogen ion concentration and osmotic pressure were changed more than the viscosity and surface tension, which indicates that reduction took place although the reaction could not be considered as an important factor in connection with the growth of organism.

4.) The continuous exposure under the experimental condition had greater influence than the intermittent exposure although the time of effective exposure was shortened to 5—30 seconds in the latter.

References:

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