ACTION OF ULTRAVIOLET LIGHT ON SPORES AND VEGETATIVE FORMS OF B. MEGATHERIUM SP.

By FERDINAND HERČÍK

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Accepted for publication, June 30, 1936)

These experiments have been made to determine the survival ratios of vegetative forms and spores of *B. megatherium sp.* under the influence of monochromatic ultraviolet light. As such they are an extension of previous irradiation studies made in this laboratory.

Technique

A strain of *B. megatherium sp.* has been used which, besides being an exceptionally good spore-former, produces vegetative forms that hold together only weakly in chains. Microscopic examination demonstrated that these chains could be broken up by moderate shaking to furnish the suspension of single organisms essential to good quantitative studies. The bacteria were cultivated at 25°C. and stock cultures for spores were incubated at 37°C. for 2 months in sealed tubes. The experimental methods for irradiation were similar to those previously described,[1] the bacteria being irradiated after spreading on an agar surface. To do this 1 cc. of a 24 hour old broth culture was diluted with 10 cc. of nutrient broth and left 60 minutes in 26°C. This fresh culture was then diluted ten times with saline, shaken mechanically for 10 minutes, and spread in 0.5 cc. portions on nutrient agar, poured 24 hours previously into 7.5 cm. Petri dishes. This bacterial suspension was left on the agar for 3 minutes, drained for 10 minutes at the room temperature, and put immediately in the ice box.

The old cultures containing spores were placed for 10 minutes in a 60°C. water bath before dilution, it having been found that this time and temperature were sufficient to kill all vegetative forms. The suspension was then diluted tenfold with saline, shaken for 10 minutes, and spread in the manner just described.

After irradiation the plates were put in a thermostat at 14°C. This low incubation temperature was needed to give single colonies. At higher temperatures long chains of bacteria are formed which, spreading widely over the agar, give a confluent growth before the colonies can be counted.

The arrangement for irradiation was the same as that previously used.[1] The source was a powerful quartz mercury arc; the monochromator was a large one,

[1] Wyckoff, Ralph W. G., *J. Gen. Physiol.*, 1931–32, **15**, 351.

having 6 inch fused quartz lenses and prisms. The current for the lamp, which was operated at either 100 or 119 volts, was drawn from a 200 volt storage battery of large capacity. Irradiations were made only after the arc had been running for at least 2 hours. Its output was controlled by an attached quartz sodium photoelectric cell. Single spectral lines were selected and the monochromator was adjusted till the irradiation slit, 29 x 3 mm., was uniformly filled with light of one wave length. The sharp edges of the slit were used to mark the irradiated and standard areas on the surface of the agar, several areas being irradiated on a single plate. Before and after each experiment the energy flux at the point of irradiation was measured by a thermocouple calibrated with a carbon lamp standardized by the U. S. Bureau of Standards. Survival ratios were determined from counts after incubation of the number of colonies on the irradiated and control areas.

TABLE I

Survival Ratio of Vegetative Forms of B. megatherium sp.

Time	Wave length		Time	Wave length
	2536 Å	2803 Å		3132 Å
sec.			*min.*	
5	0.873	0.819	1	0.947
10	0.800	0.683	2	0.689
20	0.729	0.586	3	0.650
30	0.692	0.455	4	0.582
40	0.609	0.371	5	0.597
60	0.346	0.232	6	0.476
80	—	0.130	8	0.317
Energy incident per mm.^{2} per sec.....	2.9 ergs	5.5 ergs		64.0 ergs

EXPERIMENTAL RESULTS

The vegetative forms and spores of *B. megatherium* were irradiated by light of the wave lengths 2536 Å, 2803 Å, and 3132 Å. The results are tabulated in Tables I and II and graphically represented by Figs. 1 and 2. The survival ratios are averages of many counts, the mean number of control colonies for every point being 1580. Large counts are essential, especially for small survival ratios after prolonged irradiations, when the variations in the number of survivors influence the ratio very strongly.

TABLE II

Survival Ratio of Spores of B. megatherium sp.

Time	Wave length	
	2536 Å	2803 Å
sec.		
30	0.814	0.689
40	—	0.773
60	0.700	0.546
90	0.397	0.304
120	—	0.306
180	0.296	0.076
240	0.209	0.051
Energy incident per mm.² per sec.....	2.6 ergs	5.7 ergs

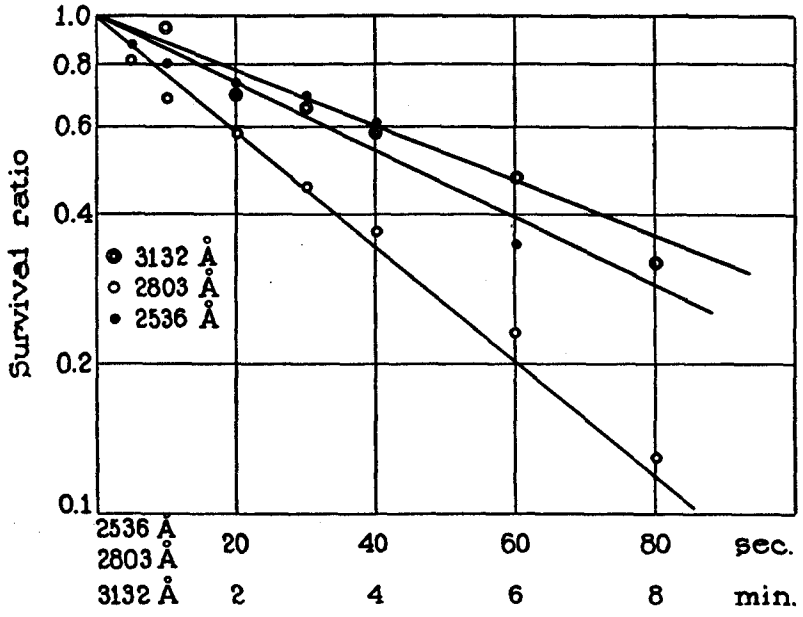

FIG. 1. Survival ratio of the vegetative forms of *B. megatherium sp.* irradiated with ultraviolet light of wave lengths 2536 Å, 2803 Å, and 3132 Å.

From the figures, it is evident that within the limits of experimental error (which amounts to *ca.* 10 per cent) the results on both vegetative forms and spores are semilogarithmically linear. This conclu-

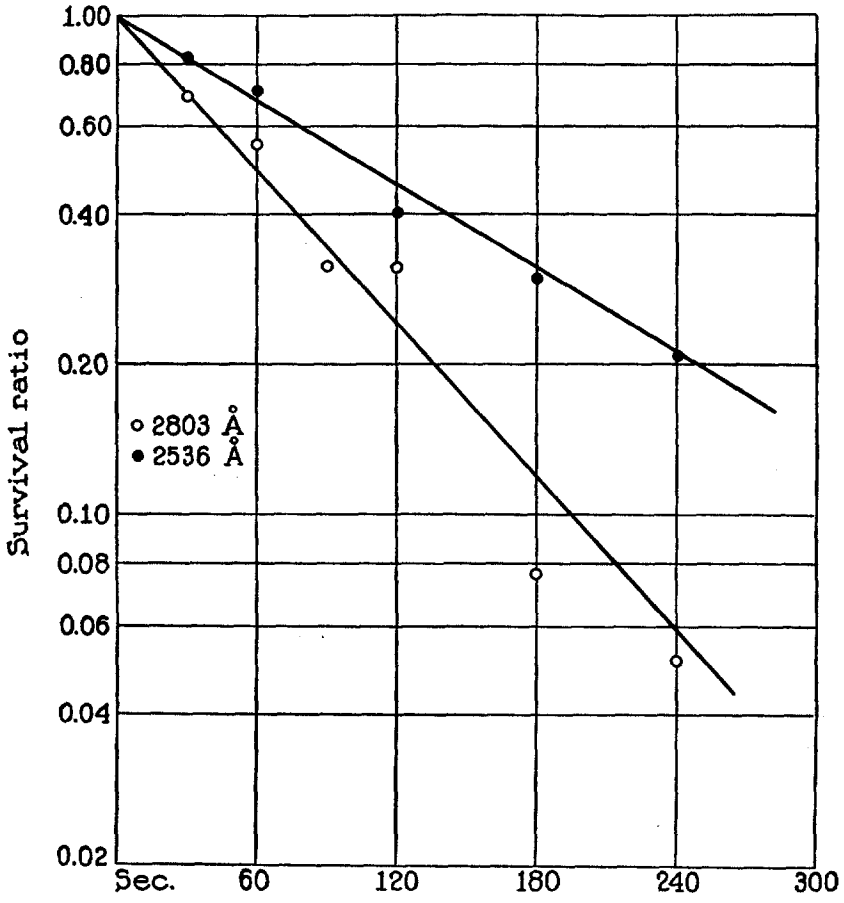

FIG. 2. Survival ratio of the spores of *B. megatherium* irradiated with ultraviolet light of wave lengths 2536 Å and 2803 Å.

sion, though agreeing with the data on most other bacteria, conflicts with some recent experiments[2] in which it was found that the vegetative forms and spores of *B. subtilis* and the spores of *B. megatherium*

[2] Duggar, B. M., and Hollaender, A., *J. Bact.*, 1934, **27**, 241.

sp. have the survival ratios following a multiple hit curve. These results showed a wide range of variation, however, and it seems probable that single cell suspensions were not obtained.

Incident energies necessary for 50 per cent killing (Table III) were calculated by multiplying the incident energies per second by the time of irradiation giving the survival ratio 0.5. Spores evidently are destroyed by about twice as much energy as is required for the vegetative forms. Sensitivities are of the same order of magnitude as those prevailing with *B. coli* except that the 3132 Å energy is much greater for *B. megatherium.* It would be interesting to determine from spectrophotometric studies whether this was due to differences in absorption spectrum.

TABLE III

Energies for 50 Per Cent Killing of Bacteria

Wave length	*B. coli*[1]		*B. megatherium*		
			Vegetative forms		Spores
	Incident energy	Energy absorbed per bacterium	Incident energy	Energy absorbed per bacterium	Incident energy
	*ergs/mm.*²	*ergs*	*ergs/mm.*²	*ergs*	*ergs/mm.*²
2536 Å	200	2.75×10^{-5}	113	6.2×10^{-5}	273
2803 Å	240	2.50×10^{-5}	149	5.9×10^{-5}	342
3132 Å	5200	10.9×10^{-5}	21,150	18×10^{-4}	—

By making the assumption, which may or may not be true, that the spectra of *B. megatherium* and *B. coli*[3] are the same, an estimate can be formed of the energy absorbed per bacterium for 50 per cent death. The results, computed on the basis of measurements indicating that the average bacillus irradiated is a rod 2.2 μ long and 0.9 μ in diameter, are listed in Table III. Ultraviolet microphotography[4] points to a very different absorption for spores; therefore no effort has been made to calculate the energy absorbed in them.

SUMMARY

Spores and vegetative forms of a strain of *B. megatherium* were irradiated by ultraviolet light of the wave lengths 2536 Å, 2803 Å,

[3] Gates, F. L., *J. Gen. Physiol.*, 1930–31, **14**, 31.

[4] Wyckoff, Ralph W. G.. and Ter Louw, A. L., *J. Exp. Med.*, 1931, **54**, 449.

and 3132 Å. The killing rate of both bacteria and spores is exponential, in agreement with irradiation results on other bacteria. Twice as much incident energy is needed to kill the spores as the vegetative forms (50 per cent death).

The absorbed energy per bacterium for 50 per cent killing has been calculated on the assumption that the absorption of the vegetative cells is the same as that of colon bacilli. These results are compared with previous measurements on other bacteria.

I am indebted to Dr. R. W. G. Wyckoff for his kind interest in this work.