Biophysics. — *The effect of radiation on light emission by luminous bacteria.* I. By A. J. KLUYVER, G. J. M. VAN DER KERK and A. VAN DER BURG.

(Communicated at the meeting of September 26, 1942.)

1. *Introduction.*

In the course of their study of the action of carbon monoxide on respiration and light emission by *Photobacterium phosphoreum* VAN SCHOUWENBURG and VAN DER BURG [1]) made the unpublished observation that luminescence was markedly reduced by irradiation of the bacteria with blue light, an effect which proved to be reversible within a relatively short period of time. These observations were quite unexpected, since HARVEY [2]) had already long ago attempted to establish the same effect, but had been unable to do so. This negative result was somewhat surprising, because shortly before HARVEY [3]) had succeeded in proving that the luminescence of the ostracod crustacean *Cypridina hilgendorfii* was unmistakably — and at least partially in a reversible way — inhibited by illumination from a carbon arc of 15.000 foot candles, after removal of all deleterious ultraviolet rays.

It is difficult to decide why the result obtained by VAN SCHOUWENBURG and VAN DER BURG differs from that of HARVEY. It seems possible that a difference between the types of bacteria used in both investigations must be deemed responsible, although HARVEY reports to have been working with *Bacterium phosphorescens*, a specific name usually considered to be synonymous with *Photobacterium phosphoreum* Beijerinck. It is also not excluded that the intensity of the light applied in HARVEY's experiments has been inferior to that used in the Utrecht investigation, but HARVEY's statement that the illumination in the region of the bacteria surpassed that of sunlight at noon in summer does neither add to the probability of this suggestion.

Anyhow, in VAN SCHOUWENBURG and VAN DER BURG's experiments the irradiation effect showed quite clearly, in so far as use was made of light originating from the blue region of the spectrum. On the other hand no effect whatever was observed, when the same experiment was made using yellow light at about the same intensity.

Obviously blue light effects a photochemical conversion of some compound intimately connected with the light emission process as normally occurring in the luminous bacteria, whilst yellow light fails to do so. Leaving apart for the moment the possibility that some photosensitizer would take part in the radiation effect, these observations seemed to open the possibility to determine in an indirect way the absorption spectrum of one of the components of the light emitting system in luminous bacteria. Hereto it would be only necessary to repeat the irradiation experiments with monochromatic light of different wave-lengths, and to determine the amount of inhibition of the bacterial luminescence taking into account the quantum intensity of the light applied in the various cases.

Now it has to be remembered that the outlook for a successful isolation of one or more of the components of the light emitting system from luminous bacteria is still far from bright. Such an isolation could only be attempted with a reasonable amount of success, if one should succeed in separating the light emission process from the intact bacterial cell. It is well known, however, that until now luminous bacteria have withstood all efforts

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1) K. L. VAN SCHOUWENBURG and A. VAN DER BURG, Enzymologia **9**, 34 (1940).

2) E. N. HARVEY, Journ. Gen. Physiol. **7**, 687 (1925).

3) E. N. HARVEY, Journ. Gen. Physiol. **7**, 679 (1925).

made in this direction [1]). For this reason the indirect determination of the absorption spectrum of one of the compounds involved in the light emission by luminous bacteria seemed to be of real importance. One might surmise that the establishment of such a spectrum would yield valuable indications as to the chemical nature of the compound in question. In this respect the successful outcome of WARBURG's well-known attempt to identify the nature of his "Atmungsferment", whilst applying the same principle, was most encouraging.

In the following paragraphs we shall give a preliminary report of the results obtained in an investigation carried out along the line indicated above [2]).

2. *Derivation of the absorption coefficient from the degree of luminescence inhibition.*

It is evident that a relatively high value of the absorption coefficient β_λ of the light absorbing compound for light of the wave-length λ will lead to a high degree of luminescence inhibition. Nevertheless, the interdependence between β_λ and the intensity of the light emitted by the bacteria before and immediately after the irradiation is not at once clear. In order to obtain an insight into this relation it is unavoidable to make some additional assumptions.

We shall, therefore, assume that the reduction of the said intensity from Y_0 to Y in consequence of the irradiation will be caused by the photochemical conversion of some compound M, the concentration of which will be reduced from m_0 to m, and that moreover the relation

$$\frac{Y_0}{Y} = \frac{m_0}{m} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

will hold.

It is now necessary to inquire into the question how the reduction of m_0 to m will depend on the irradiation applied.

According to EINSTEIN's law of photochemical conversion we must conclude that the velocity of the conversion $\frac{dm}{dt}$ will be proportional to the number of light quanta absorbed per unit of time (A) or:

$$\frac{dm}{dt} = -\varphi A. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

In this equation φ is a constant, usually designated as photochemical yield, since it represents the ratio between the number of molecules converted and the number of quanta absorbed.

Now it is clear that for A the following formula will hold:

$$A = \gamma I_q m \beta_\lambda \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

in which γ is a constant and I_q the intensity of the irradiating light (expressed in quanta per cm^2 $\times$ sec.) at the spot of the absorbing system.

Hence we have:

$$\frac{dm}{dt} = -\varphi \gamma I_q m \beta_\lambda \text{ or } m = m_0 \times e^{-\varphi \gamma \beta_\lambda I_q t}. \quad . \quad . \quad . \quad . \quad (4)$$

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[1]) We refer here only to the fairly recent publication by I. M. KORR, (Biol. Bull. 68, 347, 1935), and we can add that fresh attempts made by one of us (V. D. K.) according to new principles also have been quite in vain.

[2]) For full details the reader is referred to the doctorate thesis by G. J. M. VAN DER KERK (now in press).

This means that

$$ln \frac{m_0}{m} = \varphi \gamma I_q \beta_\lambda t. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

With a view to equation (1) we may also write:

$$ln \frac{Y_0}{Y} = \varphi \gamma I_q \beta_\lambda t$$

or

$$\beta_\lambda = \frac{ln \frac{Y_0}{Y}}{I_q t} \times \frac{1}{\varphi \gamma}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

It ensues from this equation that in each irradiation experiment the expression $ln \frac{Y_0}{Y}$, *i.e.*, the natural logarithm of the quotient of the experimentally established intensities of the bacterial light before and after the irradiation — for which expression we introduce here the term photochemical effect (W) — is one of the factors determining the absorption coefficient. Divided by the product of I_q and the time of irradiation t we obtain the photochemical effect per quantum — the specific photochemical effect: W_λ — which is a direct measure for the absorption coefficient.

This means that the curve representing the interrelation between W_λ and λ — the so-called inactivation spectrum — is at the same time the absorption spectrum of the light absorbing compound M, expressed in a relative measure.

It should be kept in mind, that the I_q used in the foregoing formulae is the intensity of the irradiating light at the spot of the absorbing system and, therefore, certainly not equal to the experimentally determined intensity of the light as thrown on the bacterial cells. However, on ground of special observations we have good reason to accept that there is a close proportionality between both quantities in the whole range of wave-lengths applied in our experiments. This implies, of course, that by introducing the experimentally determined intensities in our calculations of W_λ the relative values remain unchanged and the same holds for the finally obtained inactivation spectrum, since this is also expressed only in a relative measure.

3. *Methods and apparatus employed.*

We shall refrain from giving here a description of the earlier part of our investigation in which aerated suspensions of *Ph. phosphoreum* were submitted to irradiation.

In the final experiments we have found it advantageous to make use of homogeneous plate cultures of the said species. A Philips water-cooled super-high tension mercury lamp S. P. 500 was used as a light source. Apart from the mercury lines this lamp emits a strong continuous radiation both in the visible and the near ultraviolet regions of the spectrum. The light of this lamp passed either a quartz or a glass spectrograph, and the spectrum thus obtained was projected on the plate culture of *Ph. phosphoreum*. It was found that under these conditions a radiation time of a few minutes sufficed to bring about a well visible inhibition effect in the irradiated zone of the otherwise uniformly luminous bacterial culture. Moreover, it could be observed that the intensity of the inhibition differed quite markedly in the various parts of the spectrum, partly depending on the differences in intensity of the various wave-lengths in the spectrum, partly owing to the different sensitivity of the bacteria towards light of various wave-lengths.

It now proved possible, to register this effect by directly photographing the culture in its own light, exposition times being restricted to 30—45 seconds.

Fig. 1 shows a reproduction of such photographs. Under *B* the uniformly luminous zone of a plate before the irradiation is represented, whilst under *A* the same zone immediately after the irradiation is reproduced. For *C* and *D* we must refer to Chapter 5.

It will not need elucidation that this result opened the possibility to apply the well-known photographic method described in the monograph "Objektive Spektralphotometrie"

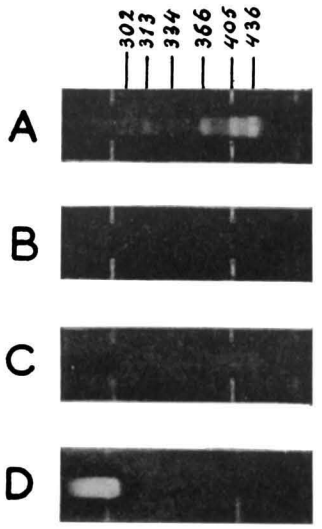

Fig. 1. Photographs of luminous bacteria in their own light.
A. Immediately after irradiation.
B. Before irradiation.
C. Two hours after irradiation.
D. Six hours after irradiation.

by ORNSTEIN, MOLL and BURGER [1]) for the determination of the intensity of the light emitted by the bacteria before and after the irradiation.

In this preliminary communication we shall confine ourselves to a few remarks regarding the experimental procedures finally adopted [2]).

a. The bacterial cultures. In order to avoid possible complications caused by scattering of the light in the medium carrying the bacterial layer, it proved essential to use a quite transparent gelatin medium, well clarified with egg albumen. Agar media were much less satisfactory in this respect, and for this reason *Ph. phosphoreum* being a gelatin non-liquefying species proved to be a much more favourable object than the gelatin liquefying species *Ph. Fischeri* and *Ph. splendidum.* In order to obtain uniformly luminescent cultures, which could easily be brought before the spectrograph we proceeded as follows. In a Petri dish which was kept horizontally some melted agar solution (2%), containing 3% NaCl was poured. After solidification a microscope slide was put on the surface of the agar gel, and hereupon a layer of about 1 cm of a nutritive gelatin medium (1% peptone Poulenc, 3% NaCl, 12% gelatin) was brought into the dish. When the gelatin had also solidified, its surface was evenly inoculated with a thick suspension of a young and vigorous culture of *Ph. phosphoreum,* previously freed from bacterial clumps by filtering through filter paper (Schleicher and Schüll Nr. 595). After incubation for 18 to 20 hours at a temperature of about 16° C. such plates showed an optimal and uniform luminescence which remained constant for at least several hours. Now the slide with the adhering gelatin layer was cut out of the plate, and after modelling the gelatin block, the slide together with the luminescent block were transferred to the apparatus used for the photographing, and afterwards to the special device employed in the irradiation experiment. Apart from the facilitation in the handling of the culture the procedure described

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[1]) L. S. ORNSTEIN, J. W. MOLL und H. C. BURGER, Objektive Spektralphotometrie. Sammlung Vieweg. Berlin (1932).

[2]) For full details the reader is again referred to the doctorate thesis by G. J. M. VAN DER KERK, to be published shortly.

guaranteed an uniform thickness of the gelatin layer which is essential, because the full length of the spectrum had to be projected quite sharply on the bacterial layer.

b. The irradiation of the bacterial cultures. Since the preliminary experiments had learnt that the inactivation spectrum was partly situated in the ultraviolet, partly in the visible range of the lamp spectrum, it was necessary to use both a FUESS quartz and a ditto glass spectrograph. A schematic representation of the apparatus used is given in Fig. 2.

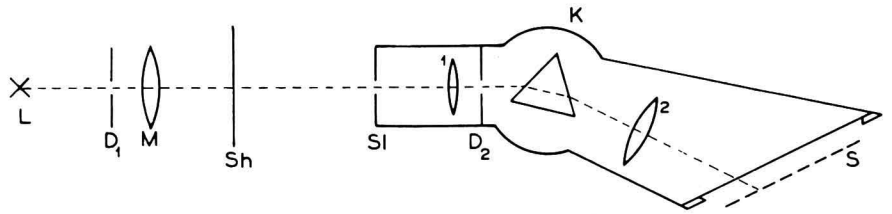

Fig. 2. Schematic representation of the apparatus used for the irradiation of the bacterial cultures (for explanation cf. the text).

In this figure L is the light source — the high tension mercury lamp already referred to —, D is a diaphragm, and M a quartz lens which gives an image of L on the slit Sl (width 0.3 mm) of the quartz spectrograph K. A sharp spectrum was obtained in the plane indicated with S, at a short distance of the backside of the spectrograph. The intensity of the spectrum as a whole could be regulated with the iris diaphragm D_2; the time of irradiation could sharply be regulated with the aid of the shutter Sh placed before the spectrograph. For the accurate and quick adjustment of the spectrum on the bacterial layer a special apparatus was built which will not be here described in detail. It may suffice to state that the slide with the luminescent gelatin block could be inserted into a frame which by means of a micrometer screw could be moved in such a way that the luminescent front of the block could at once brought in the correct position. This position had been determined beforehand making use of a specially constructed device for the detection of the plane in which the spectrum was sharp. Owing to this procedure an irradiation of the bacteria preceding the experiment was prevented.

c, The photographic determination of the intensity of the bacterial light and the computation of the photochemical effect. With a lens an image (1 : 1) of the luminescent culture was thrown on a photographic plate (Ilford Special Rapid, extra sensitive), which plate then was developed under standardized conditions. In each series of experiments the correct time of exposure was first established. This time appeared to vary between 30 and 45 seconds.

The intensities of the light emitted by the bacteria at corresponding spots before and after the irradiation were determined in the usual way, *i.e.* by photometric registration of the degree of blackening — or density — of the photographic plate by means of a galvanometer, subsequent measurement of the photograms, and by the conversion of the procentual transmissions in relative intensities with the aid of specially determined "density curves". For a detailed description of this procedure we may refer to the monograph by ORNSTEIN et al., cited above.

The curves in the micro-photograms always had a somewhat serrate character, partly caused by the grains in the photographic plate, partly by slight variations in the luminescence of the bacterial culture. These systematic irregularities in the measurements could be practically eliminated by taking care that the photometric determination of the density before and after the irradiation related to exactly the same spots of the cultures. In order to remove also as far as possible more incidental irregularities always three photometric determinations were made at different heights: one 0.3 mm below, one 0.3 mm above, and one exactly in the middle of the spectrum. From the values thus obtained an average was calculated.

In Fig. 3 the three lines indicated with b_1, b_2 and b_3 mark the spots where photometric determinations were made; h being the height of the irradiated zone. Finally both on the plates obtained before and after the illumination always a fourth determination was made at the spot indicated with *a*, *i.e.* outside the irradiated zone. Small deviations in the values

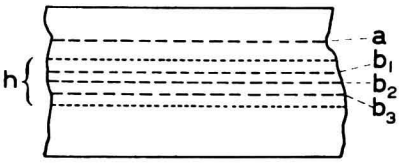

Fig. 3. Scheme indicating the spots where photometric registration was made (for explanation cf. the text).

of *a* for both plates must be due to changes in the luminescence caused by other factors than the irradiation (*e.g.* small changes in temperature, in moistness of the bacteria etc.). A procentual correction of all values obtained after the irradiation was applied on the basis of the deviations thus established.

From the intensity before irradiation (Y_0) and the corrected intensity after irradiation (Y) the photochemical effect could easily be computed with the aid of the formula

$$W = ln \frac{Y_0}{Y}.$$

d. The determination of the energy distribution in the spectrum used for the irradiation and the computation of the specific photochemical effect. The intensity of the light throughout the spectrum used for the irradiation was sufficiently high to permit a direct measurement of the intensities at the various spots with the aid of a vacuum-thermo-element after MOLL together with a sensitive galvanometer in series. The thermo-element consisted of a manganine-constantan strip 1 μ thick, 0.1 mm broad and 7 mm long, and was covered with carbon-black. The window in the vacuum tube was made of quartz with a view to the measurement of the ultraviolet part of the spectrum. A diaphragm on the quartz window had a height inferior to that of the spectrum, since the latter declines to the side of the short wave-lengths, and not the total energy of the spectrum, but the "energy density" had to be determined. The spectrum was sharply projected on the strip and the thermo-element could be moved with the aid of a micrometer screw in such a way that the strip passed horizontally through the spectrum. By this procedure the spectral energy distribution could be accurately determined and by taking into account the dispersion curve, we were able to read the wave-length corresponding with each position of the micrometer screw. The galvanometer readings were converted into absolute intensities in the spectrum expressed in erg per cm^2 $\times$ sec. by comparing these readings with those of a combination of a large surface thermopile after MOLL and a galvanometer, standardized in absolute measures, under conditions of equality of incident light.

From the values for I thus obtained the intensity I_q in quanta per cm^2 $\times$ sec. was calculated with the aid of the formula:

$$I_q = \frac{\lambda}{hc} \times I$$

in which λ = wave-length in cm
c = the velocity of the light (3×10^{10} cm per sec.)
h = PLANCK's constant (6.55×10^{-27} erg $\times$ sec.).

From the photochemical effect W, obtained in the way described under *c*, the specific photochemical effect W_λ could now easily be computed by dividing W by the product of I_q and the time of irradiation t in sec.

4. *Experimental results.*

We must refrain here from giving a full survey of all experimental results obtained in several extensive series of experiments. By way of example we reproduce in Fig. 4 a complete curve representing the relation between the photochemical effect W and wave-length, as obtained in one of the experiments.

It is clear that this relation depends primarily on the energy distribution in the spectrum applied in the irradiation experiment, and we give, therefore, in Fig. 5 a curve representing

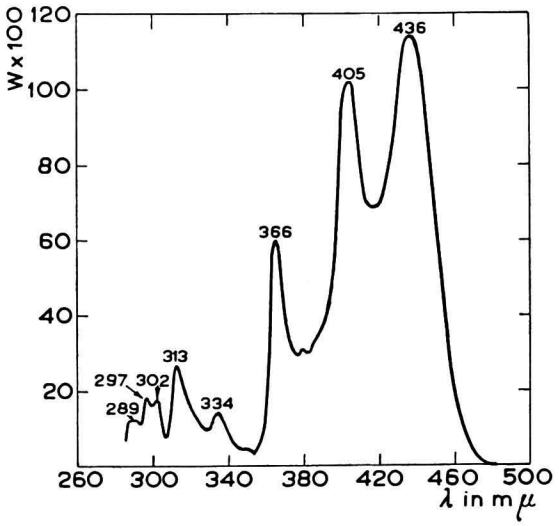

Fig. 4. The interrelation between photochemical effect (W) and wave-length.

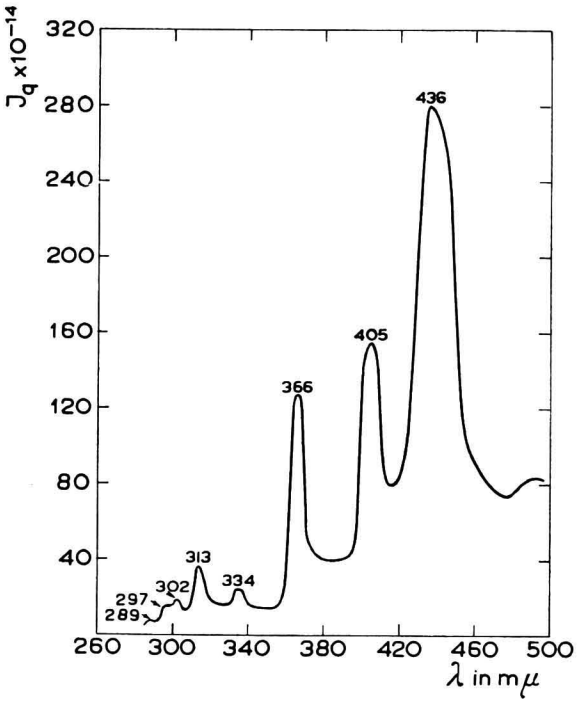

Fig. 5. The interrelation between the wave-length and the intensity of incident light used in the irradiation experiment.

this distribution. In this curve the intensity of the various wave-lengths is again expressed in quanta per $cm^2 \times$ sec.

A comparison of the curves in both figures shows that at first sight they have a certain amount of resemblance, in so far as wave-lengths which represent places of high intensity in the spectrum also have a marked photochemical effect. A closer inspection shows, however, that there is no proportionality whatever, the discrepancies, of course, being caused by the differences in specific photochemical effect of the various wave-lengths.

The final determination of the photochemical inactivation spectrum was based on the following series of experiments:

a. A series with the quartz spectrograph, in which the intensity of the irradiated light was varied, but the time of irradiation was kept constant (2 min.).

TABLE I.

Summary of the experimental data concerning the specific photochemical effect, on the average of which the final inactivation spectrum was based.

Wave-length in mμ	Specific photochemical effect: $W_\lambda \times 10^{18}$ (separate determinations)	$W_\lambda \times 10^{18}$ (Average)	Mean error ε
490	—0.03 , 0 , 0 , 0.04 , 0 — 0 , 0 , 0 , 0	0	—
478	—0 , 0 , 0 , 0	0	—
468	—0.17 , 0.03 , 0.02 , 0.18 — 0.04 , 0.07	0.04	$\pm 0.01^3$
460	—0.18 , 0.18^5 , 0.21 — 0.19 , 0.21 — 0.05	0.17	$\pm 0.02^3$
451	—0.45 , 0.50 , 0.35 — 0.28 , 0.29	0.37	± 0.05
445	—0.48^5 , 0.44^5	0.46^5	$(\pm 0.02^5)$
436	—0.58 , 0.60 — 0.51 , 0.71 , 0.61 — 0.75	0.63	± 0.04
427	—0.56^5 , 0.59 , 0.61 — 0.79 , 0.75^5	0.66	$\pm 0.05^5$
424	—0.61 , 0.58 , 0.64 — 0.82^5 , 0.75^5	0.68	$\pm 0.05^4$
422	—0.64^5 , 0.67^5 , 0.70 — 0.81^5 , 0.71	0.71	$\pm 0.02^7$
418^5	—0.71 , 0.76^5 , 0.74 — 0.78 , 0.61 , 0.85 — 0.81 , 0.74	0.75	$\pm 0.02^4$
415	—0.77 , 0.79 , 0.76 — 0.85^5 , 0.77^5	0.79	$\pm 0.01^6$
413	—0.78 . 0.82 , 0.76 — 0.84^5 , 0.78 , 0.88^5 — 0.91	0.82^5	$\pm 0.02^4$
405	—0.80 , 0.86 — 0.75^5 , 0.68 , 0.91 , 0.91 — 0.96 , 0.88^5	0.84^5	$\pm 0.03^5$
390	—0.75 , 0.81 , 0.69 — 0.81 , 0.95 , 0.88 — 0.49^5 , 0.51	0.74	$\pm 0.06^0$
386	—0.70 , 0.70 , 0.69 — 0.74 , 0.80 , 0.85 — 0.48^5 , 0.46 , 0.50	0.66	$\pm 0.05^2$
379^5	—0.64 , 0.54 , 0.58^5 — 0.67^5 , 0.71 , 0.76^5 — 0.44 , 0.42^5 , 0.46	0.58	$\pm 0.04^6$
376	—0.52 , 0.50 , 0.61 — 0.54 , 0.63 , 0.62 — 0.41^5 , 0.40^5 , 0.43	0.52	$\pm 0.03^2$
366	—0.40 , 0.45 , 0.48 — 0.44 , 0.51 — 0.33	0.43^5	$\pm 0.02^5$
355	—0.37 — 0.45 , 0.33 — 0.31 , 0.32	0.35^5	$\pm 0.02^7$
350	—0.28 — 0.44^5 , 0.35 — 0.27 , 0.32	0.33	$\pm 0.03^4$
345	—0.34 — 0.43 , 0.41 — 0.27 , 0.30	0.35	$\pm 0.03^5$
334	—0.44 , 0.49 , 0.45 — 0.48^5 , 0.42 , 0.55 — 0.34 , 0.35 , 0.36	0.43	$\pm 0.02^5$
328	—0.50 , 0.52 — 0.60 , 0.47^5 , 0.57 — 0.43 , 0.43 , 0.44	0.49^5	$\pm 0.02^4$
313	—0.60 , 0 61^5 , 0.52 — 0.52 , 0.60 — 0.45^5 , 0.53 , 0.49	0.54	$\pm 0.02^2$
306^5	—0.70 — 0.60 , 0.49 , 0.76 — 0.51 , 0.53 , 0.51	0.58^5	$\pm 0.04^4$
302	—0.74 , 0.77 — 0.65 , 0.87 — 0.69^5 , 0.69^5	0.74	$\pm 0.03^2$
297	—0.90 , 0.92 — 0.84 , 1.19 — 0.92 , 1.01 , 1.22	1.00	$\pm 0.06^1$
292	—1.55 , 1.60 — 1.05 , 1.00 , 1.38 — 1.44 , 1.44	1.35	$\pm 0.09^5$
289	—1.51 , 1.29 — 1.15 , 1.23 , 1.50 — 1.59 , 1.59	1.41	$\pm 0.08^1$
280	—1.20 — 0.82 , 0.66 — 1.03 . 1.27 , 1.63	1.10	$\pm 0.14^8$
275	—0.98 — 0.72^5 , 0.58 — 1.02 , 1.21^5 , 1.25	0.96	$\pm 0.11^5$
270	—0.75 — 1.08 , 1.12^5	0.98	$(\pm 0.14^0)$

Remark: The values of the specific photochemical effect obtained in different series of experiments are separated by —.

b. Two series with the quartz spectrograph, in which with constant intensity the time of irradiation was varied.

c. Two series with the glass spectrograph, in which the intensity of the irradiated light was varied, but the time of irradiation was kept constant (2 min.).

For the experimental results obtained we must chiefly refer to the doctorate thesis of G. J. M. VAN DER KERK.

It may suffice here to state that both in the experiments with intensity variation and in those with time variation for the various wave-lengths investigated proportionality between the photochemical effect W and either of these factors, or in other words an approximately constant value for the specific photochemical effect W_λ, was obtained, at least in so far as the value of W ($\times$ 100) did not much surpass 50. It is clear that this result justifies the assumptions on which the computation of W and W_λ is based.

It is true that the values obtained show a certain amount of spreading caused by the inevitable imperfections of the method (incomplete homogenity of the bacterial cultures etc.). Nevertheless, the fact that for the more essential wave-lengths a relatively large number of determinations was made, enabled us to calculate for these wave-lengths an average value, the probable error of which apparently remains within reasonable limits.

In Table I we have collected all values for the specific photochemical effect on which the final determination of the inactivation spectrum was based. Finally in Fig. 6 the inactivation spectrum itself is given.

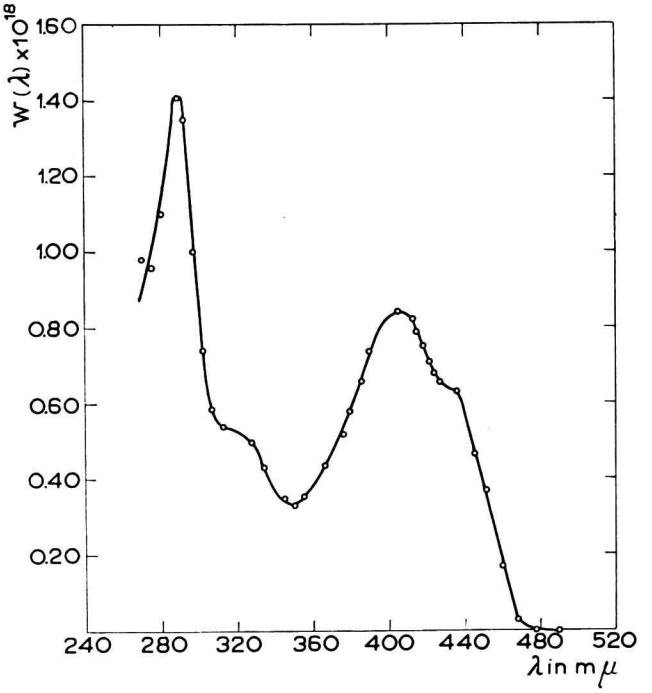

Fig. 6. The inactivation spectrum of *Photobacterium phosphoreum.*

We wish here to recall that in Chapter 2 it was argued that this inactivation spectrum at the same time represents the absorption spectrum — in a relative measure — of the compound absorbing the radiation which inhibits the light emission by the bacteria. As will be seen the curve obtained is quite fluent and especally the linking-up between the parts determined with the quartz and the glass spectrograph is quite smooth. The spectrum shows two distinct bands at 290 m μ and 405 to 410 m μ respectively, and moreover there are indications for two secondary maxima at about 320 and 430 m μ.